Endocrine (1995) 3, 775-780

Effect of 17β-oestradiol on expression of IL-2, IL-6 and IFN-γ mRNA in human tonsillar cells

Marianthi Evagelatou & John Farrant

MRC Immunodeficiency Research Group, Department of Clinical Immunology, Royal Free Hospital School of Medicine, London NW3 2PF, UK

Oestradiol (E_2) alters lymphocyte function *in vitro* including T cell DNA synthesis and B cell immunoglobulin production in human tonsillar, splenic and peripheral blood cells. We have investigated whether one mechanism for this effect is that E_2 modifies the expression of IL-2, IL-6 and IFN-γ mRNA in human tonsillar mononuclear cells. Without E_2, addition of PHA (1 μg ml^{-1}) for 10 h increased the expression of IL-2 and IL-6 mRNA but had no significant effect on IFN-γ mRNA. In separated T cells after 24 h incubation, E_2 (7×10^{-8} M) increased only the IFN-γ mRNA levels. However, when E_2 was present in PHA-stimulated T cell cultures, mRNA levels from all cytokines were suppressed. E_2 decreased IL-2 mRNA levels in the T cell preparation after 24 h culture. For IL-6, E_2 decreased mRNA both in mononuclear cells and T cells after 10 h incubation. For IFN-γ, E_2 decreased mRNA levels in the mononuclear cell preparation after 24 h culture. Stimulation of the T cell preparation with PHA after 24 h incubation with E_2 decreased the IFN-γ mRNA levels compared to the cultures incubated with E_2 only. One part of the action of E_2 may be through a block in the up-regulation of the mRNA of IL-2, IL-6 and IFN-γ in activated cells.

Keywords: 17β-oestradiol; IL-2 mRNA; IL-6 mRNA; IFN-γ mRNA; tonsillar cells; human

Introduction

Several studies have shown direct effects of oestrogens on cytokine transcription and production in cells of lymphoid origin but the exact pathway of action is still unclear. The effects of these steroid hormones seem to be cytokine and tissue specific and sometimes appear contradictory. For example, 17β-oestradiol (E_2) increases IL-1 (α and β) gene expression in activated human monocytic leukaemia cell lines (Shanker *et al.*, 1994) yet decreases IL-1β mRNA levels in stimulated human peripheral blood monocytes (Lake-Polan *et al.*, 1989). Moreover, E_2 increases IL-5 mRNA expression in murine T cell lines (Wang *et al.*, 1993).

Dehydroepiandrosterone (DHEA), an intermediate in sex steroid synthesis, enhances IL-2 mRNA expression in PHA-activated human blood T lymphocytes whereas vitamin D_3 and glucocorticoids down-regulate IL-2 production (Suzuki *et al.*, 1991). Other steroid hormones (progesterone and testosterone) have been reported to stimulate IL-2 mRNA in murine T cell lines (Wang *et al.*, 1993).

Steroid hormones also affect production of the pleiotropic cytokine IL-6. E_2 strongly suppresses IL-6 expression in a variety of cell types. IL-6 is lowest during the follicular phase when E_2 is highest and oestrogen-modulated production of IL-6 can contribute to systemic alterations during the menstrual cycle ie. body temperature, plasma protein composition (Tabibzadeh *et al.*, 1989). Girasole *et al.* (1992) have shown that IL-6 production and IL-6 mRNA levels are inhibited by E_2 (10^{-8} M) in IL-1 and TNF-α stimulated normal human bone-derived cells and murine bone marrow-derived stromal cells. When murine spleen macrophages were treated overnight with E_2 prior to LPS stimulation, E_2 decreased the LPS-induced IL-6, IL-1α and TNF-α production both at mRNA and protein levels (Deshpande *et al.*, 1994). By contrast, pharmacological doses of E_2 ($> 10^{-6}$ M) enhanced IL-6 and IL-1 production by normal human PBMC (Li *et al.*, 1993).

There are few reports on the effects of steroid hormones on IFN-γ. E_2 (10^{-9} M) was shown to increase the activity of IFN-γ promoter and IFN-γ mRNA expression in Con A-stimulated murine spleen cells (Fox *et al.*, 1991).

We have previously shown that E_2 has significant effects on immunoglobulin production by resting or stimulated tonsillar, splenic and peripheral blood B and T lymphocytes and on DNA synthesis by resting or stimulated tonsillar, splenic and peripheral blood T cells *in vitro* (Evagelatou & Farrant, 1994; Evagelatou *et al.*, 1994). Furthermore, we have studied the effects of E_2 on the expression of the oestrogen receptor (ER) mRNA in mononuclear tonsillar lymphocytes and the data suggest that E_2 significantly decreases the ER mRNA levels after 10 h of incubation with E_2 (Evagelatou & Farrant, 1995).

The aim of the present study was to investigate whether one possible mechanism to explain the functional data was through alterations of the cytokine mRNA profile. We assessed whether E_2 induced changes in the levels of mRNA of IL-2, IL-6 and IFN-γ in tonsillar cells during incubation with E_2 in the presence or absence of the T-cell mitogen phytohaemagglutinin (PHA). The hypothesis that oestrogens alter cytokine levels can show a new aspect in the cause and treatment of autoimmune diseases as well as possible effects of intake of oral contraceptives and hormonal replacement-therapy (HRT).

Results

Establishment of technique for measuring cytokine mRNA

Previous experiments have shown that β-actin was not affected by the presence of E_2 in culture and was a suitable internal control to normalise the transfer of RNA to the slot blots (Evagelatou & Farrant, 1995). Two dilutions of approximately 30 and 15 μg of total RNA from PHA-stimulated tonsillar cultures were blotted onto a nylon membrane and hybridised with IL-2, IL-6, IFN-γ and β-actin cDNA probes sequentially (Figure 1). The β-actin and cytokine mRNA levels calculated after densitometric scanning of the slot blots were in arbitrary densitometric units 44.1 and 20.2 for β-actin, 62.4 and 26.4 for IL-2, 26.1 and 12.0 for IL-6 and 28.3 and 13.4 for IFN-γ mRNA respectively. For subsequent experiments, 20 μg of total RNA were loaded onto the slot blots.

Effects of E_2 on cytokine mRNA levels in tonsillar mononuclear cells

The mononuclear cell preparations obtained from the Ficoll interface consisted mainly of $CD19^+$ (B cells), $CD3^+$ (T cells)

Correspondence: M. Evagelatou
Received 2 May 1995; accepted 3 August 1995

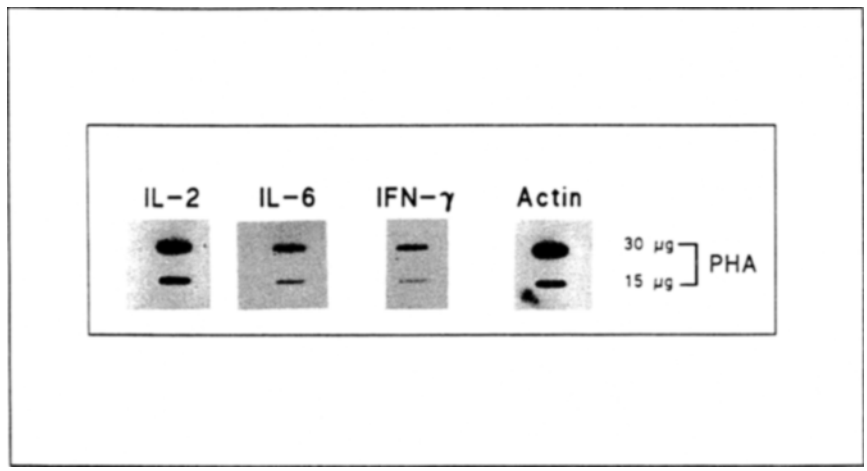

Figure 1 Slot blot analysis of IL-2, IL-6, IFN-γ and β-actin mRNA levels in tonsillar cells from a representative experiment. Two dilutions of total RNA (30 and 15 μg) from PHA-stimulated cultures were blotted in order to test the accuracy of the technique. The actin was used as an internal marker of the RNA amount deposited on each slot

and a small proportion of $CD14^+$ (monocytes) cells. Mononuclear cells from eight different tonsils were used for the 10 h cultures and six different tonsils for the 24 h cultures.

IL-2

Addition of E_2 (7×10^{-8} M) at the start of cultures of resting tonsillar mononuclear cells had no effect on the mean IL-2 mRNA levels after 10 or 24 h culture (Figure 2a). However, stimulation of the cultures with PHA (1 μg/ml, in the absence of E_2) for 10 h significantly increased ($P<0.05$) the mean IL-2 mRNA expression to double that of the control (unstimulated 10 h culture) levels. By contrast, after 24 h culture with PHA, the IL-2 mRNA levels in the cells had returned to the unstimulated levels. Whenever E_2 was present in the PHA-stimulated cultures for 10 or 24 h, there was no rise in the mean IL-2 mRNA expression in comparison to the unstimulated samples. These data suggest that E_2 is able to block the significant rise in IL-2 mRNA observed after 10 h culture with PHA.

IL-6

The presence of E_2 (7×10^{-8} M) in 10 or 24 h cultures of resting tonsillar cells had no effect on the mean IL-6 mRNA level (Figure 2b). Stimulation of the cultures with PHA (1 μg/ml, in the absence of E_2) for 10 h significantly increased ($P<0.05$) the mean IL-6 mRNA expression compared to the control levels. PHA-stimulation for 24 h did not alter the IL-6 mRNA levels in the cells. The presence of E_2 in the 10 h PHA-stimulated cultures significantly decreased ($P<0.05$) the mean IL-6 mRNA expression as compared to the levels from 10 h cultures stimulated with PHA alone. Again, this suggests that E_2 can block the PHA-driven increase in cytokine mRNA. E_2 had no significant effect on the mean IL-6 mRNA expression in the 24 h PHA-stimulated cultures.

IFN-γ

E_2 (7×10^{-8} M) added at the start of the 10 or 24 h cultures of resting tonsillar mononuclear cells had no effect on the mean IFN-γ mRNA levels (Figure 2c). The presence of PHA (1 μg/ml) in the tonsillar cell cultures in the absence of E_2 did not alter significantly the IFN-γ mRNA levels in the cells after 10 or 24 h in comparison to the unstimulated levels. However, the presence of E_2 in the 24 h PHA-stimulated cultures significantly decreased ($P<0.05$) the mean IFN-γ mRNA expression compared to the levels from cultures with PHA alone, again suggesting an inhibitory action of the hormone. E_2 had no significant effect on the mean IFN-γ mRNA expression of the 10 h PHA-stimulated cultures.

Effects of E_2 on cytokine mRNA levels in separated tonsillar T cells

Separated tonsillar T cell preparations contained more than 95% of $CD3^+$ cells and less than 2% of $CD19^+$ cells. For both 10 and 24 h cultures, T cells were separated from three different tonsils.

IL-2

The presence of E_2 (7×10^{-8} M) in unstimulated T cell tonsillar preparations cultured for 10 or 24 h, had no significant effect on the mean IL-2 mRNA levels (Figure 3a). Stimulation of the T cells with 1 μg/ml of PHA, significantly increased ($P<0.05$) the mean IL-2 mRNA expression after 10 h culture to almost four times the control levels. The presence of E_2 in the 24 h PHA-stimulated cultures significantly decreased ($P<0.05$) the mean IL-2 mRNA expression compared to the levels from T cell cultures stimulated with PHA alone. By contrast, addition of E_2 in the 10 h PHA-stimulated T cell cultures lowered the mean expression in comparison with PHA alone, but the effect was not significant. These results were analysed by Student's *t*-test.

IL-6

The effects of E_2 on tonsillar T cell preparations were the same as the effects on the unseparated mononuclear cell cultures (Figure 3b). Addition of E_2 (7×10^{-8} M) to the 10 or 24 h cultures of resting tonsillar T cells had no effect on the mean IL-6 mRNA level. Stimulation of T cell cultures with PHA 1 μg/ml (in the absence of E_2) for 10 h significantly increased ($P<0.01$) the mean IL-6 mRNA expression compared to the control levels, but PHA-stimulation for 24 h did not alter the IL-6 mRNA levels in the cells. The presence of E_2 in the 10 h PHA-stimulated cultures significantly decreased ($P<0.01$) the mean IL-6 mRNA expression compared to the levels from T cell cultures stimulated with PHA alone. However, E_2 had no effect on the mean IL-6 mRNA expression of the 24 h PHA-stimulated cultures. These results were analysed by Student's *t*-test.

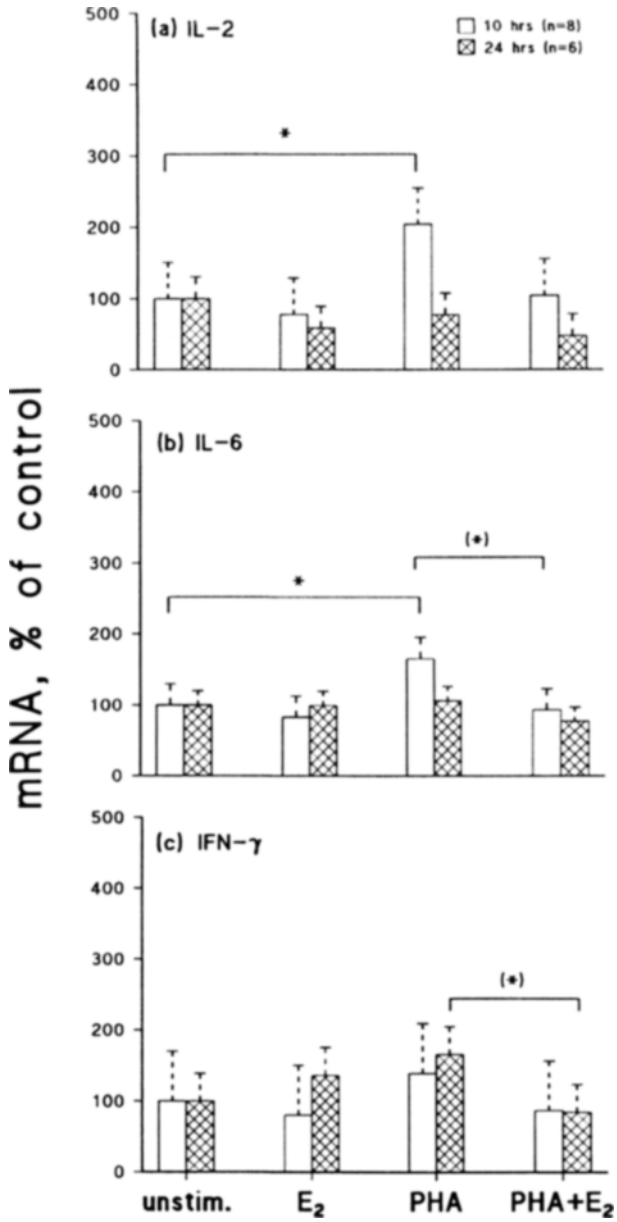

Figure 2 Mean mRNA levels of: (**a**) IL-2, (**b**) IL-6 and (**c**) IFN-γ in tonsillar mononuclear cell preparations after stimulation with or without PHA in the absence or presence of E_2 (7×10^{-8} M). At each time point, 10 h (□) or 24 h (▩), the mean effects of E_2 and/or PHA are normalised (100%) to the level of each cytokine mRNA for cells cultured without E_2 or PHA at the same time. Data (including the replicate data for the unstimulated samples) were analysed by Genstat-ANOVA and the dashed error bars represent the standard errors of the difference (s.e.d.) between the mean values for each condition. [*represents a significant increase ($P<0.05$) at each time point between a treatment mean and the normalised unstimulated control (without E_2 or PHA) at the same time, but (*) in brackets represents a significant decrease between the indicated treatment means ($P<0.05$)]

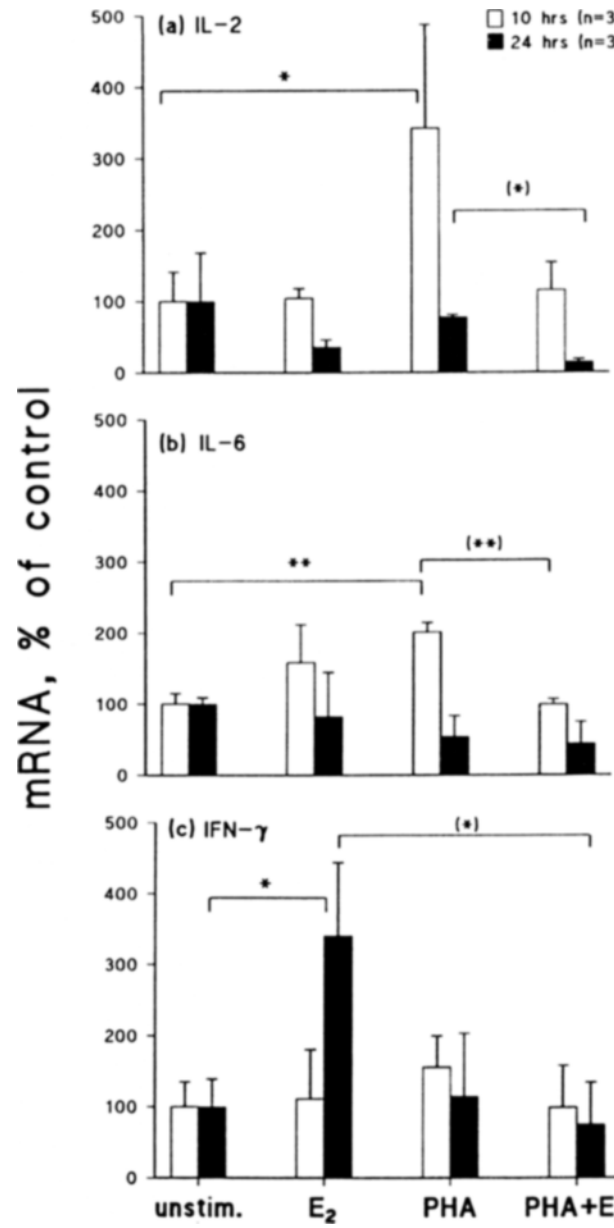

Figure 3 Mean mRNA levels of: (**a**) IL-2, (**b**) IL-6 and (**c**) IFN-γ in tonsillar T cell preparations after stimulation with or without PHA in the absence or presence of E_2 (7×10^{-8} M). At each time point, 10 h (□) or 24 h (■), the mean effects of E_2 and/or PHA are normalised to the level of each cytokine mRNA for cells cultured without E_2 or PHA at the same time. Because of the lower number of donors compared to Figure 2, data in this Figure were analysed by Student's *t*-test alone and the solid error bars represent the SEM for each condition. [* and ** represent significant increases ($P<0.05$ or $P<0.01$ respectively) at each time point between a treatment mean and the normalised unstimulated control (without E_2 or PHA) at the same time, and (*) or (**) represent significant decreases between the indicated treatment means ($P<0.05$ or $P<0.01$ respectively)]

IFN-γ

Unlike the data with mononuclear cell cultures, addition of E_2 (7×10^{-8} M) alone in unstimulated T cell preparations cultured for 24 h, significantly increased ($P<0.05$) the mean IFN-γ mRNA expression (Figure 3c). E_2 alone had no effect in the 10 h unstimulated T cell cultures. Stimulation of the T cells with 1 μg/ml of PHA, also had no effect on the mean IFN-γ mRNA expression in either 10 or 24 h cultures. The presence of E_2 in the 24 h PHA-stimulated T cell cultures significantly decreased ($P<0.05$) the mean IFN-γ mRNA expression compared to the levels from the unstimulated T cell cultures in the presence of E_2 alone. By contrast, the presence of E_2 in the 10 h PHA-stimulated T cell cultures had no significant effect. These results were analysed by Student's *t*-test.

Discussion

Our previous studies demonstrated that E_2 has direct effects on the differentiation and proliferation of human lym-

phocytes including tonsillar cells (Evagelatou & Farrant, 1994; Evagelatou *et al.*, 1994) as well as on the expression of ER mRNA (Evagelatou & Farrant, 1995). A dose of E_2 of 20 ng/ml was effective in this previous published work and therefore we used this concentration to study the action of E_2 on the cytokine mRNA expression.

The close interaction of B and T cells for the immune response is well established. Initial activation of resting B cells requires signals (including cytokines) delivered by T cells. IL-2, IL-6 and IFN-γ are some of the cytokines secreted by lymphoid cells including T cells and play an essential role in B and T cell activation.

The present data suggest that E_2 can alter the mRNA expression of IL-2, IL-6 and IFN-γ in human lymphocytes. Principally, E_2 was able to block the increase in IL-2, IL-6 and IFN-γ mRNA levels in tonsillar cells induced on PHA stimulation. However, in addition, E_2 alone increased IFN-γ mRNA expression of the resting tonsillar T cells after 24 h incubation.

IL-2 is produced primarily by $CD4^+$ T cells but also by other cell types such as $CD8^+$ T cells, NK cells, B cells and accessory cells. IL-2 is produced following stimulation with class I MHC alloantigens or non-physiological polyclonal stimulants (ie. PHA). Usually, freshly isolated resting T cells do not contain IL-2 mRNA or IL-2. However, a small proportion of resting lymphocytes may have been primed *in vivo* to express some IL-2 mRNA (Ruscetti, 1990). In line with these observations, very low levels of IL-2 mRNA have been detected in unstimulated tonsillar cells taken from donors on antibiotic therapy to subdue tonsillar infection. In normal human lymphocytes, 4 h stimulation with PHA induces IL-2 mRNA accumulation with a peak at 12 h, after which the levels decline (Ruscetti, 1990). Two suppressor mechanisms are thought to control expression of the human IL-2 gene (Kaempfer *et al.*, 1987). One mechanism, independent of continued protein synthesis, shuts off the transcription of the IL-2 gene. The second mechanism seems to act post-transcriptionally and causes about 95% of the nuclear IL-2 mRNA precursors to be degraded. The present data on human tonsillar cells show a significant increase in the IL-2 mRNA levels after PHA stimulation for 10 h. After 24 h cultures with PHA, the IL-2 mRNA levels have already declined.

Observations from the present studies have shown that E_2 influences IL-2 mRNA in PHA-stimulated human tonsillar lymphocytes. Studies from other groups have reported that different steroid hormones regulate IL-2 gene transcription and production *in vivo* and *in vitro* in the human and murine systems. For example, DHEA, an adrenal steroid, at concentrations of 10^{-9} M increased IL-2 mRNA expression in PHA-stimulated human T cells after 20 h in culture (Suzuki *et al.*, 1991). However, the present study is the first to show effects with E_2.

IL-6 induces differentiation and proliferation of B cells and is mainly produced by stimulated monocytes (Tabibzadeh *et al.*, 1989). Under certain conditions normal T cells can also produce IL-6 upon activation, yet in unstimulated T cell cultures IL-6 mRNA was not detected (Sehgal, 1992). IL-6 mRNA levels however, were detectable after 3 h stimulation with IL-1 or TNF (tumour necrosis factor) and the levels increased up to 10 h (Girasole *et al.*, 1992). In the present data with tonsillar cells, very low background IL-6 mRNA levels have been found, which could be due to some prior *in vivo* stimulation or activation by n-SRBC used to separate B and T lymphocytes. PHA was found to increase the IL-6 mRNA expression significantly in both the tonsillar mononuclear preparation and in the T cell enriched preparation after 10 h in culture. Previous observations on the effects of oestrogens have shown that these hormones suppress IL-6 mRNA and IL-6 production in a variety of cell types from humans and mice (Tabibzadeh *et al.*, 1989, Girasole *et al.*, 1992, Sehgal, 1992, Deshpande *et al.*, 1994). Data from the current project confirm these studies and show that E_2 (7×10^{-8} M) inhibits IL-6 mRNA expression in PHA-stimulated tonsillar cells after 10 h in culture. However, there is one study by Li *et al.* (1993) where E_2 enhanced IL-6 production in human peripheral blood mononuclear cells but the hormone concentrations used were supraphysiological ($\geqslant 10^{-6}$ M).

IFN-γ is produced by T and NK cells but recent studies by Dayton *et al.* (1992) have found a constitutive expression of IFN-γ in many human B cell lines. IFN-γ costimulates proliferation and differentiation of activated B cells and may be involved in class switching of the human B cells to IgE (Butch *et al.*, 1993). It also induces macrophages to secrete IL-1 which is important in T cell growth regulation, antigen presentation and inflammatory responses (Gray & Goeddel, 1987). Unstimulated PBL and Jurkat T cells do not have IFN-γ mRNA. Again, in tonsillar preparations very low background IFN-γ mRNA levels were observed. Butch *et al.* (1993) have also detected some IFN-γ mRNA in freshly isolated tonsillar T cells. In the present studies, stimulation of tonsillar cells with PHA for 10 or 24 h failed to induce any significant increase in IFN-γ mRNA expression.

The effects of E_2 on resting and pre-activated lymphocytes reveal different responses. Observations from the current project have suggested that E_2 can increase significantly IFN-γ mRNA expression in human resting tonsillar T cells after 24 h culture. These are in agreement with the studies by Fox *et al.* (1991) which showed a significant increase in IFN-γ mRNA levels in murine splenic cells after addition of E_2. However, PHA activation of lymphocytes prevents E_2 from exerting its stimulatory effects on IFN-γ mRNA. When PHA-stimulated mononuclear tonsillar cell preparations were incubated with E_2 for 24 h, the IFN-γ mRNA level was decreased compared to the control levels of PHA-alone stimulated cultures.

Lymphocytes from women taking oral contraceptives or hormonal replacement therapy after menopause or hysterectomy or even males with prostatic cancer receiving oestrogen therapy, showed significantly reduced responses to mitogens like PHA (Grossman, 1984; Lee Nelson & Steinberg, 1987). Our observations provide a possible mechanism for the oestrogen action. Our data could also be useful in the understanding of autoimmunity and its treatment, as previous studies have shown that administration of oestrogen-containing oral contraceptives to patients with SLE was associated with exacerbation of the disease whereas withdrawal of the pill resulted in the amelioration of the disease (Ansar Ahmed *et al.*, 1985).

In general, the effects of E_2 that we report are not great but are significant. Small effects may be biologically important and it is interesting that the mainly enhancing effects on lymphocyte function that we have previously reported (Evagelatou & Farrant, 1994; Evagelatou *et al.*, 1994; Evagelatou & Farrant, 1995) are accompanied by an inhibitory effect of E_2 on the expression of some cytokine mRNA as shown in the present data.

In conclusion, the actions of E_2 that we have previously reported in T cell-dependent immunoglobulin production and mitogen-driven T cell DNA synthesis in human tonsillar cells, can be mediated, at least partially, through regulation of the expression of ER and various cytokine mRNA. The present data shows that E_2 is able to block the mitogen-driven increases in mRNA of IL-2 and IL-6 in tonsillar mononuclear or T cell preparations. The same effect is seen for IFN-γ mRNA in the mononuclear cell preparations, yet interestingly, in T cells E_2 alone increases the mRNA but only in resting cells. The stimulatory effects of E_2 on immunoglobulin production and T cell DNA synthesis may thus involve the inhibition of negative cytokine signals. Further studies are needed to see whether the reduction by E_2 of cytokine mRNA levels in activated cells is due to regulation at a transcriptional, post-transcriptional or even translational level.

Materials and methods

Cell preparations

Human tonsils obtained after routine operations for chronic tonsillitis were used as the source of lymphocytes as previously described (Evagelatou & Farrant, 1994). The donors were eight pre-pubertal children, six boys aged 5–13 years and two girls aged 3 and 6 years. In brief, after the tonsils were teased into phenol red-free RPMI-1640 medium (GIBCO BRL), the cell suspension was passed through a fine cell strainer to remove remnants of tissue and then layered onto Ficoll-Paque (Pharmacia, UK) gradient. The mononuclear cell preparation obtained from the interface consisted mainly of $CD19^+$ (B cells), $CD3^+$ (T cells) and a very small proportion of $CD14^+$ (monocytes). In some experiments, mononuclear cells were rosetted overnight with neuraminidase-treated sheep erythrocytes (n-SRBC) to separate T cells (Evagelatou & Farrant, 1994). The T cells were recovered by lysing the treated sheep erythrocytes with ammonium chloride lysis fluid. By flow cytometry, the T cell preparation contained more than 95% of $CD3^+$ cells.

Cell cultures

About 10^7–10^8 cells obtained either from the Ficoll interface (mononuclear cells) or after ammonium chloride lysis of n-SRBC in rosettes (T cells) were cultured at 37°C for 6–24 h in round bottom tubes (Nunc, UK) in 5% CO_2 in air. The medium was phenol red-free RPMI-1640 supplemented with penicillin/streptomycin (100 U/ml) and L-glutamine (2 mM). At the end of the culture the cell suspension was centrifuged, the medium removed and the cells washed twice with ice-cold phosphate-buffered saline (PBS). The pellet was snap-frozen in liquid nitrogen and stored at −70°C until use.

Reagents

Oestradiol (17β-E_2, Sigma, UK) supplied as a lyophilised and γ-ray irradiated powder, was reconstituted in 1 ml of sterile absolute ethanol followed by 49 ml of RPMI making a stock solution of 20 μg/ml and aliquoted before storage at −70°C. Dilutions of E_2 were made with media immediately before use at a final concentration of 20 ng/ml (7×10^{-8} M). Phytohaemagglutinin (PHA, Wellcome, UK) was obtained in freeze-dried form, reconstituted in sterile water, stored at −70°C and used at a final concentration of 1 μg/ml.

Isolation and characterisation of mRNA

RNA isolation was done by guanidinium thiocyanate extraction followed by overnight ultra-centrifugation in caesium chloride as described by Maniatis (Sambrook *et al.*, 1989). The amount of RNA was quantified by spectrophotometric determination of absorbance at 260 nm. The purity of the RNA preparation was checked using the ratio OD_{260}:OD_{280}.

RNA slot blots

RNA samples were prepared according to the procedure described by Mason *et al.* (1993). Briefly, the RNA was resuspended with deionised formamide, 37% formaldehyde and 20 × SSC and denatured at 68°C for 15 min. The samples were applied onto a nylon membrane (Hybond N, Amersham) using a Minifold II slot blot apparatus (Schleicher & Schuell). The membrane was placed on a filter paper, baked in a UV stratalinker 2400 (Stratagene, UK), then rinsed in 3 × SSC and wrapped in transparent film (Saran Wrap) until hybridisation.

cDNA probes

The 1.465 Kb insert of the human IFN-γ cDNA from pAT153 was kindly provided by the UK Human Genome Mapping Project (HGMP), Clinical Research Centre, Harrow and was isolated by HindIII-SalI digestion. The 0.9 Kb insert of human IL-6 cDNA from pUC9 was also provided by HGMP and was isolated by HindIII-EcoRI digestion (Bowcock *et al.*, 1988). The 0.5 Kb insert of the human IL-2 cDNA from pBluescript II KS was isolated after BamHI digestion by Margaret North in our Research group. Finally, the 0.8 Kb insert of β-actin cDNA isolated by PstI digestion was generously provided by John Cheshire (MRC Molecular Rheumatology, Clinical Centre, Harrow, UK). After digestion with restriction enzymes, the cDNA probes were run on agarose gel with appropriate molecular weight DNA ladder and checked for specificity for each cytokine. DNA contaminants and agarose gel were removed from the above clones with the Geneclean II Kit (BIO 101 Inc., USA).

Hybridisation of the slot blots with cDNA probes

Using a Megaprime DNA labelling kit (Amersham, UK), the probes were labelled with [α-^{32}P]dCTP (3000 Ci/mmol, NEN-Dupont UK Ltd) and sequentially hybridised onto the membrane at 42°C overnight. The hybridisation solution (10 ml) contained de-ionised formamide (5 ml), 50 × Denhardt's solution (0.1 ml), denatured salmon sperm DNA (0.1 ml), Dextran (1 g), 10% SDS (1 ml), 20 × SSC (2.5 ml) and distilled water (1.3 ml). The membrane was washed with 2 × SSC + 1% SDS before it was autoradiographed onto an X-ray film (X-AR5, Kodak Scientific) with an intensifying screen at −70°C. The RNA samples were quantified by scanning densitometry of the autoradiographs using a dual-wavelength flying-spot scanner and software (Shimadzu CS9000, V.A. Howe & Co, UK). For data analysis, the results of cytokine mRNA were expressed as ratios to the β-actin mRNA content. In the Figures, the mean ratios are normalised to that of the unstimulated control for each different condition.

Data analysis

Analysis of variance (ANOVA) of the cytokine/actin ratio data on mRNA expression was performed using a computerised statistical programme (Genstat 5, NAG Ltd, Oxford, UK) to assess the treatment effects within different tonsils. The variation due to tonsils from different donors was used as a block within the program. This approach removes the variance due to donor variation and allowed the calculation of the standard error of the difference (s.e.d., dashed errorbars on the Figures) between treatment means (histograms in the Figures). In order to compare populations with normally distributed variance, separate ANOVA analyses of the effects of E_2 were done for each time of culture. Significant differences between the treatments were detected using the s.e.d., with the residual variance and degrees of freedom from the ANOVA in Student's *t*-test. For the T cell preparation data where the number of different donors was low, the results were analysed by Student's *t*-test alone and the error bars on these figures represent the standard error of the mean (SEM).

Acknowledgements
We would like to thank Mr Graeme Penny (Comparative Biology Group, Clinical Research Centre, Harrow) for his valuable assistance on the techniques used and Dr Mark Stoll (Glycoconjugates Dept, Clinical Research Centre, Harrow) for his help with the densitometry. We also thank Mr John Cheshire, Ms Margaret North and HGMP (Clinical Research Centre, Harrow) for kindly providing us with the cDNA probes. Marianthi Evagelatou was holding an MRC-PhD studentship.

References

Ansar Ahmed, S., Penhale, W.J. & Talal, N. (1985). *Am. J. Pathol.*, **121,** 531–551.

Bowcock, A.M., Kidd, J.R., Lathrop, M., Daneshvar, L., May, L.T., Ray, A., Sehgal, P.B., Kidd, K.K. & Cavalli-Sforza, L.L. (1988). *Genomics*, **3,** 8–16.

Butch, A.W., Chung, G.H., Hoffmann, J.W. & Nahm, M.H. (1993). *J. Immunol.*, **150,** 39–47.

Dayton, M.A., Knobloch, T.J. & Benjamin, D. (1992). *Cytokine*, **4,** 454–460.

Deshpande, R.R., Michael, S.D. & Chang, M.Y. [abstract] (1994). *FASEB J.* **8,** 484.

Evagelatou, M. & Farrant, J. (1994). *J. Steroid Biochem. Molec. Biol.*, **48,** 171–177.

Evagelatou, M., Webster, A.D.B. & Farrant, J. (1994). *Clin. Exp. Immunol.*, **98,** 203–209.

Evagelatou, M. & Farrant, J. (1995). *J. Mol. Endocrinol.*, **14,** 13–19.

Fox, H.S., Bond, B.L. & Parslow, T.G. (1991). *J. Immunol.*, **146,** 4362–4367.

Girasole, G., Jilka, R.L., Passeri, G., Boswell, S., Boder, G., Williams, D.C. & Manolagas, S.C. (1992). *J. Clin. Investigations*, **89,** 883–891.

Gray, P.W. & Goeddel, D.V. (1987). In: *Lymphokines: Molecular cloning and analysis.* (Webb D.R. and Goeddel D.V., eds), Academic Press Inc., pp. 151–162.

Grossman, C.J. (1984). *Endocrine Reviews*, **5,** 435–455.

Kaempfer, R., Efrat, S. & Marsh, S. (1987). In: *Lymphokines: Molecular cloning and analysis.* (Webb D.R. and Goeddel D.V., eds), Academic Press Inc., pp. 59–72.

Lake-Polan, M., Loukides, J., Nelson, P., Carding, S., Diamond M., Walsh, A. & Bottomly, K. (1989). *J. Clin. Endocrinol. Metab.*, **69,** 1200–1206.

Lee Nelson, J. & Steinberg, A.D. (1987). In: *Hormones and Immunity*, (Berczi I. and Kovacs K., eds), MTP Press Ltd., pp. 93–119.

Li, Z.G., Davis, V.A. & Brooks, P.M. (1993). *Clin. Exp. Rheumatol.*, **11,** 157–162.

Mason, P.J., Enver, T., Wilkinson, D. & Williams, J.G. (1993). In: *Nucleic Acids Hybridization, a practical approach*, (2nd ed.), ed. Hames and Glover, IRL Press, pp. 5–63.

Ruscetti, F.W. (1990). In: *Immunophysiology: The role of cells and cytokines in Immunity & Inflammation*, (Oppenheim J.J. & Shevach E.M., eds), Oxford University Press, pp. 46–66.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). In: *Molecular Cloning, A Laboratory Manual*, (2nd ed). Cold Spring Harbor Laboratory Press.

Sehgal, P.B. (1992). *Research in Immunology*, **143,** 724–734.

Shanker, G., Sorci-Thomas, M., Register, T.C. & Adams, M.R. (1994). In: *Lymphokine and Cytokine Research*, vol 13(1), M.A. Liebert Inc. pp. 1–7.

Suzuki, T., Suzuki, N., Daynes, R.A. & Engelman, E.G. (1991). *Clin. Immunol. Immunopathol.*, **61,** 202–211.

Tabibzadeh, S.S., Santhanam, U., Sehgal, P.B. & May, L.T. (1989). *J. Immunol.*, **142,** 3134–3139.

Wang, Y., Campbell, H.D. & Young, I.G. (1993). *J. Steroid Biochem. Molec. Biol.*, **44,** 203–210.