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Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations

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Abstract

Estrogens have been shown to modulate immune responses. Several studies have demonstrated the capacity of T cells, B cells, and monocytes to respond to estrogens and estrogen receptor (ER) expression in these cell types has been reported. However, little is known regarding the relative expression in these cells of ERα and the more recently identified ERβ. In the present study, results of quantitative TaqMan™ RT-PCR analyses indicate that ERs are differentially expressed in PBMC subsets. $CD4^+$ T cells express relatively high levels of ERα mRNA compared with ERβ, whereas B cells express high levels of ERβ mRNA but low levels of ERα. Peripheral blood $CD8^+$ T cells and monocytes express low but comparable levels of both ERs. This quantitative analysis of ER expression in distinct PBMC subsets may provide a basis for dissecting the mechanisms of immune modulation by estrogens and identifying therapeutic targets for the treatment of inflammatory and immunologic disorders.
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1. Introduction

Both clinical and experimental observations suggest that pregnancy can alter clinical symptoms of a number of autoimmune diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), and the alterations observed have been attributed to the effect of female sex hormones, such as estrogen, on immune responses [1]. For example, clinical symptoms improve during pregnancy in females with MS and RA, when plasma estrogen levels are high, but are often exacerbated post-partum when estrogen levels decrease [2–5]. In contrast, SLE is exacerbated during pregnancy and ameliorated post-partum [6]. Treatment with estrogens has yielded similar results where the administration of estrogen results in suppression of disease in humans and in animal models of MS, but exacerbation of disease in murine SLE [7–16].

It is thought that the disease-modulating effects of estrogens and pregnancy on autoimmune disease is due to a Th2 environment associated with increased hormone production during pregnancy [17]. A predominant Th2 response induced in pregnancy would inhibit autoreactive Th1 responses involved in the pathology of MS and RA [1,18]. In contrast, it would potentially promote pathogenic autoantibody production in SLE and therefore exacerbate disease. The disparate effects of estrogens on cell-mediated versus humoral immune responses also have been demonstrated in MRL lpr/lpr mice [18]. Estrogen enhanced B cell activation and anti-double stranded DNA Ab production leading to immune complex-mediated glomerulonephritis in these animals, but T cell-mediated renal vasculitis and sialadenitis were suppressed. It has also been demonstrated that in vitro estrogen treatment results in the suppression of human T cell activation

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but stimulation of Ab production by human B cells [19–23]. Together, these observations suggest that immune cells have the capacity to respond to estrogen, with differential effects of estrogens on T cells versus B cells.

Estrogen mediates many of its effects through two estrogen receptor (ER) isoforms, ERα and ERβ, which are members of the nuclear receptor superfamily of genes (reviewed in [24]). Upon binding of ligand to the estrogen receptor, dimerization occurs resulting in the recruitment of essential cofactors necessary for transcriptional activation or repression of target genes. The actions mediated by estrogen in a particular cell may be determined by several factors, including cellular expression of corepressor and coactivator proteins, as well as the relative expression ratio of ERα to ERβ [24].

To examine one particular mechanism by which immune cells are differentially affected by estrogen, we compared ERα and ERβ expression in immune cell subsets. Since the relatively recent identification of ERβ, the presence of both ERα and ERβ mRNA in human peripheral blood lymphocytes and monocytes has been reported, however, quantitative analyses of ER expression have not been performed [25–27]. The work described here provides a comparison of the relative expression of ERα and ERβ mRNA levels within specific PBMC subsets, including $CD4^+$ and $CD8^+$ T cells, B cells and monocytes. ER expression patterns in resting and activated cell populations were also examined. This quantitative analysis of ER expression in distinct PBMC subsets provides a useful tool in understanding the responsiveness of these various cell types to estrogen and provides a basis for dissecting the mechanisms underlying immune modulation by estrogens.

2. Materials and methods

2.1. Blood donors

Peripheral blood was obtained from healthy donors by venipuncture. All procedures performed were with the approval of an Institutional Review Board. Donors included males and pre-menopausal females between the ages of 18 and 45 years. Post-menopausal female donors included women up to the age of 70 years. Subjects were excluded if they were using hormonal contraception, hormone replacement therapy, had taken anti-inflammatory medication 7 days prior to blood donation, had been diagnosed with any autoimmune disease, or had an acute illness at the time of donation.

2.2. Peripheral blood mononuclear cell isolation

Blood was collected from donors and PBMCs were isolated by Ficoll–Hypaque® (Sigma, St. Louis, MO) density cushion as per manufacturer's instructions. The $CD4^+$, $CD8^+$, $CD14^+$ (monocytes) and $CD19^+$ (B cells) populations were isolated from PBMCs by positive selection using Dynabeads® (Dynal Biotech, Lake Success, NY) as per manufacturer's instructions. Samples of each population were taken to confirm the purity of isolated populations by flow cytometric analysis. All purified populations were stained with the following Abs: CyChrome-labeled anti-CD3, FITC-labeled anti-CD4, PE-labeled anti-CD8, PE-labeled anti-CD14 and FITC-labeled anti-CD19 (BD Biosciences, San Diego, CA). Three-color analysis was performed using a FACS Calibur™ (Becton Dickinson, San Jose, CA) flow cytometer. Data acquisition and analysis was performed using CellQuest™ software. Cell populations were determined to be >90% pure. The remaining cells were resuspended in TRIzol® (Invitrogen, Carlsbad, CA) for RNA isolation.

2.3. Lymphocyte activation

PBMCs from pre-menopausal female donors were isolated using Ficoll–Hypaque® as above. $CD4^+$ and $CD8^+$ T cells were activated by incubating PBMCs for 3 days in the presence of 1 μg/ml Phytohemaglutinin-P (PHA) (Sigma) in RPMI-complete medium (RPMI containing 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen)). $CD4^+$ and $CD8^+$ T cells were isolated using Dynabeads® as described above.

2.4. $CD4^+$ T helper cell differentiation

PBMCs from five pre-menopausal female donors were isolated by Ficoll–Hypaque® as above. $CD8^+$ T cells were depleted from the PBMCs using Dynabeads® and the remaining cells were stimulated under neutral, Th1-, or Th2-polarizing conditions as described by Rogge et al. [28]. Briefly, $CD8^+$-depleted PBMC were cultured for 3 days in PHA alone (neutral conditions), PHA with IL-12 (2 ng/ml) and anti-IL-4 Ab (200 ng/ml, BD Pharmingen) (Th1), or PHA with IL-4 (200 U/ml) and anti-IL-12 Ab (2 μg/ml, BD Pharmingen) (Th2). Cells were then washed and expanded in IL-2 (10 IU/ml) for 4 days. Cells were resuspended in TRIzol® for RNA isolation and receptor expression determination.

2.5. Activation of monocyte-derived macrophages (MDM)

PBMCs were isolated from three males and three pre-menopausal females. MDM were obtained as previously described [29]. Briefly, PBMCs were plated for 2 h, and non-adherent cells were removed by washing. The adherent cells were incubated for 4 days, and then stimulated with IFN-γ (100 U/ml) for three additional days. Cells were harvested and resuspended in TRIzol® for RNA isolation and receptor expression determination.

2.6. Estrogen receptor analysis by quantitative TaqMan™ real-time RT-PCR

RNA was isolated from purified PBMC populations using TRIzol® reagent (Invitrogen) as per manufacturer's recommended protocol. RNA was resuspended in 85 μl RNase-free

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