

Volume 13, issue 1, March 2002 | Articles originaux

Estradiol and raloxifene decrease the formation of multinucleate cells in human bone marrow cultures

Abstract

Estrogen (E2) deficiency is responsible for increased bone turnover in the postmenopausal period, and it can be prevented by estrogen replacement therapy. The way estrogen acts on bone cells is not fully understood. Human bone marrow cell cultures may be a reliable model for studying the action of steroids on osteoclastogenesis in vitro. We examine the effects of estradiol and Raloxifene, a selective estrogen receptor modulator, on human primary bone marrow cells cultured for 15 days. 17beta-estradiol and Raloxifene significantly

decreased the number of tartrate-resistant acid phosphatase multinucleate cells from osteoclast precursors on day 15. Estrogen receptor alpha (ER-alpha) mRNA was present in bone marrow mononuclear cells cultured for 5 days, but there was no estrogen receptor beta (ER-beta) mRNA, suggesting that this effect was mediated by ER-alpha. 15-day cultures no longer contained ER-alpha mRNA, suggesting that estrogen acts on early events of osteoclast differentiation. Finally, 10-8 M 17beta-estradiol has no effect on the release of IL-6 and IL-6-sr into the medium of marrow mononuclear cells cultured for 5 or 15 days. Osteoclast apoptosis was not affected by estradiol or Raloxifene after 15 days of culture under our conditions. In conclusion, we have shown that both estradiol and Raloxifene inhibit osteoclast differentiation in human bone marrow mononuclear cultures. The biological effect that can mimic in vivo differentiation could be mediated through ER-alpha.

Full text

INTRODUCTION

The increased bone resorption that occurs in postmenopausal women is due to a lack of estrogen (E2), and can be prevented by estrogen replacement therapy [1]. The mechanism by which estrogen acts on bone is not fully understood, but it involves inhibition of the proliferation and differentiation of osteoclast precursors [2]. It has been proposed that the effects of estrogen on osteoclast differentiation are mediated by interactions between osteoblasts/stromal cells and osteoclast precursors, through cytokines [3, 4]. There is evidence that the action of estrogen is mediated by IL-6 and IL-6 soluble receptors, as shown in animal models and in murine cell cultures [5, 6], but this remains controversial in humans [7-9]. Several reports indicate that many cytokines and membrane-associated factors regulate the effects of estrogen [2, 10, 11]. Although the beneficial effect of estrogen on bone *in vivo* has been well documented, its adverse effects on reproductive tissues have led to the development of a new class of drugs: the selective estrogen receptor modulators (SERM) [12-14]. These SERMs bind to estrogen receptors and have tissue-selective, estrogen-like effects [15, 16]. Raloxifene acts as an estrogen agonist on bone of postmenopausal women [16]. Contrasting with the *in vivo* data, the mechanism by which SERMs act on bone is unclear. Most of the studies done to assess the effect of estrogen have been performed *ex vivo* using bone marrow cells obtained from ovariectomized mice. Human bone marrow cell cultures have

rarely been used, although they would be more relevant for studies on human osteoporosis. We have therefore used primary cultures of human bone marrow mononuclear cells (BMMC) to assess the effect of estradiol and Raloxifene on the differentiation of osteoclast precursors.

Both osteoblasts and stromal cells possess estrogen receptor ER-alpha and ER-beta [17], but isolated rabbit osteoclasts have only ER-alpha and no ER-beta [18]. We still do not know which of these isoforms mediates the antiresorptive effect of estradiol. We have verified the presence of both receptors in human BMMC and evaluated the effect of estradiol on the release of cytokines, and osteoclast apoptosis. These studies with human bone marrow cell cultures indicate that estradiol and Raloxifene inhibit osteoclast differentiation in a dose-dependent fashion, with an expression of ER-alpha at 5 days of culture, but no ER-beta. Estradiol has no effect on cytokine release or multinucleate cell apoptosis.

PATIENTS AND METHODS

Bone marrow mononuclear cell cultures with estradiol and Raloxifene

Bone marrow was collected from the femoral diaphysis of patients who underwent surgery for hip arthrosis. Mononuclear cells were isolated on a Ficoll-Hypaque gradient and plated out at 10^6 cells/ml, in 8-well plastic chambers (Lab-Tek, Nunc, France). All mononucleated cells were allowed to adhere for 24 hours and then were washed. Only adherent mononucleate cells (BMMC) were cultured for 15 days in phenol red-free DMEM (Gibco BRL, Cergy-Pontoise, France), containing 10% horse serum and 10^{-8} M $1.25(OH)_2$ vitamin D3.

In the first set of experiments, we assessed the inhibition of osteoclast differentiation and looked for a dose-effect for estradiol. Adherent BMMC from 18 patients were incubated with vehicle (controls) or with increasing doses of 17beta-estradiol (10^{-8}, 10^{-9}, 10^{-10} and 10^{-11} M). In these experiments, 10^{-8} M 17alpha-estradiol as a negative control in 17 samples, and calcitonin was used as a positive control in 14 samples, as calcitonin is known to inhibit osteoclast differentiation.

In the second set of experiments, adherent BMMC were cultured under the same conditions as above but with Raloxifene (n = 13) at concentrations of 10^{-8},

10^{-9} and 10^{-10} M. Raloxifene was a gift from Dr. S. Liu-Leage (Laboratoire Lilly France). All cultures were performed in duplicate wells, medium being changed every 5 days. RNA was extracted from five samples of untreated BMMC grown for 5 and 15 days.

Multinucleate TRAP cells from adherent BMMC cultures

Tartrate-resistant acid phosphatase (TRAP) activity was detected by enzyme histochemistry. Briefly, at the end of the culture, cells were fixed in 10% glutaraldehyde and stained for acid phosphatase using naphthol ASTR phosphate (Sigma, France) as substrate, in the presence of 50 mM tartrate. The reaction product was stained with hexazotized pararosaline (Sigma, France) and counterstained with Toluidine blue. $TRAP^+$ cells containing three or more nuclei were counted as multinucleate cells, in duplicate, in 8 random fields (0.47 mm^2 each) and expressed as cells per mm^2.

*ER-*alpha *and ER-*beta *mRNA*

Total RNA was extracted using RNA Now (Biogentex, Montigny-le-Bretonneux, France) to evaluate ER-alpha and ER-beta. The large subunit of RNA polymerase II was used as control. cDNA was made in 10 mul with 225 ng total RNA using the following protocol: 1) RNAs were denatured for 30 sec at 95° C in 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM DTT, 250 ng oligo(dT15) primer (Promega), 2) once the temperature reached 42° C, 3 mM $MgCl_2$ was added and samples were incubated for 40 min at 42° C, 3) 5 mM dXTP (Pharmacia) and 100 units of Moloney murine leukemia virus reverse transcriptase (Gibco-BRL) were added and samples were incubated for 40 min at 42° C, 4) reverse transcriptase was inhibited by heating to 95° C for 5 min. PCR amplifications were performed using 0.5 mul (control) or 4 mul (ER-alpha and ER-beta) of the reverse transcription mixture in 30 mul of (20 mM Tris-HCl (pH 8.4), 50 mM KCl, 5 mM dXTP, 2.5 mM $MgCl_2$, 2 mg/ml acetylated BSA, 1 mM DTT, 100 ng of each sense and anti-sense primer and 1 unit of Taq DNA polymerase (Gibco-BRL)) for 19 (control) or 27 (Er-alpha and Er-beta) cycles (30 sec at 95° C, 2 min 30 sec at the primer annealing temperature (see below), and 3 min at 74° C). For labelling the products, 10 mul of the PCR mixture was further amplified for 5 cycles in a 15 mul reaction mixture containing 5 ng of the nested primer radiolabeled as described [19]. Then, 4 mul were loaded onto a 6% polyacrylamide denaturing gel.

The nucleotide sequences (5' to 3') of the primers were as follows (the annealing temperature used for each primer is indicated in parentheses):

Control sense, CCACCTACAGTCTCACAAGC (56° C);

control anti-sense, GTTCTAACAGCACAAGTGGAG (56° C);

control nested GTCACCCCCAGCCCAGCTCCGTT (68° C);

Amplicon length: 87 pb

Er-alpha sense, TCATCCTCTCCCACATCAGGC (56° C);

Er-alpha anti-sense, GGAGCTCTCAGACTGTGGCA (56° C);

Er-alpha nested, TATGACCTGCTGCTGGAGATGCTGG (68° C);

Amplicon length: 187 pb

Er-beta sense, GAATATCTCTGTGTCAAGGC (56° C);

Er-beta anti-sense, ATGTTGAGCAGATGTTCCCAT (56° C);

Er-beta nested, CGATGCTTTGGTTTGGGTGATTGCC (68° C).

Amplicon length: 135 pb

Samples of total RNA from SaOS and MCF-7 cells were used as positive controls, omitting the reverse transcription step was used as a negative control.

Cytokine production

The supernatants of BMMC cultures treated with estradiol were collected on days 5 and 15, for measurement of IL-6 and IL-6sr. The IL-6 and IL-6sr in the medium was measured using commercial radioimmunoassay kits (Medgenix, Belgium). These assays use coated tube separation and an oligoclonal system, in which several specific monoclonal antibodies are directed against distinct

epitope epitopes of IL-6. The sensitivity of the assay was 5-6 pg/ml. The intra- and interassay coefficients of variation for IL-6 were 6.7 and 8.3%. Results at day 5 and 15 are expressed in ng/ml.

IL-6sr was measured by immunoassay (Quantikine hIL6Sr, RD systems, detection 3.5 pg/ml). Inter and intra-assay reproducibility was below 10%. Results at day 5 and 15 are expressed in pg/ml.

SERM effects on apoptosis of multinucleate cells from human BMMC

Apoptosis was assessed in BMMC cultures treated with 10^{-8} M 17beta-estradiol (n = 8) and Raloxifene (n = 6). BMMC were plated at 10^6 cells/ml onto plastic 8-well chambers (Lab-Tek), cultured for 15 days in phenol red-free DMEM with or without 10% horse serum, and medium was changed every 5 days. Cells were fixed with 4% paraformaldehyde on day 15. Apoptosis was evaluated by the TdT-mediated dUTP-biotin nick end labelling method (TUNEL, Dako, France). Labeled cells with 3 or more nuclei and condensed cytoplasm were counted. The percentage of multinucleate apoptotic cells per total multinucleate cells in each well was calculated.

Statistical analysis

Data are expressed as means ± SD. Statistical analyses were performed by ANOVA, followed by the Fisher PLSD test and Friedman test. Differences were considered as significant when the p value was below 0.05 (Statistica Software, Abacus, USA).

RESULTS

*Effects of 17*beta*-estradiol and Raloxifene on osteoclast precursors from BMMC cultures*

Cultures incubated with 10^{-8} M and 10^{-9} M 17beta-estradiol contained significantly fewer TRAP multinucleate cells (6.49 ± 2.07 and 8.54 ± 2.41/mm^2) than did control cultures (10.81 ± 2.35, $p < 0.01$), suggesting that 17beta-estradiol inhibits the formation of TRAP multinucleate cells. Cultures incubated with lower doses of 17beta-estradiol (10^{-10} and 10^{-11}M) and the 17alpha-estradiol negative controls were similar to controls (9.83 ± 2.5/mm^2). The effect of 10^{-8} M

17beta-estradiol (6.49 ± 2.07 cells/mm^2) was similar to that of calcitonin (5.41 ± 0.98 cells/mm^2), indicating a decreased osteoclast differentiation (Figure 1). Incubation with 10^{-8} M and 10^{-9} M Raloxifene caused a significant decrease in the number of $TRAP^+$ multinucleate cells (5.28 ± 1.09 and 5.47 ± 1.05/mm^2) compared to controls (7.69 ± 1.78, n = 13, $p < 0.01$ and $p < 0.05$), but lower doses were without effect.

*ER-*alpha *and ER-*beta *expression in BMMC cultures*

The ER-alpha and ER-beta mRNA in BMMC after 5 and 15 days of culture was first assayed by classical RT-PCR with oligonucleotides. Using this method, neither ER-alpha nor ER-beta mRNA was found in any cells on day 5 or day 15 (data not shown). We then used the more sensitive, nested RT-PCR, checking the specificity by MCF-7 and and SaOS cells as positive controls. mRNAs encoding the ubiquitous large subunit of RNA polymerase II (RNA pol II) was detected in extracts of MCF-7, SaOS and bone marrow cells after 19 PCR amplification cycles. mRNA encoding ER-alpha was also detected in MCF-7 cells after 19 PCR amplification cycles, but mRNA encoding ER-beta was only detected in SaOS cells after at least 27 PCR amplification cycles (Figure 2A). mRNA encoding ER-alpha was detected in primary cultures of BMMC after 27 PCR amplification cycles, suggesting that only a subpopulation of cells contained this messenger, or that the concentration of ER-alpha mRNA was low (Figure 2B). ER-alpha mRNA was detected in all samples cultured for 5 days (5 samples). The ER-alpha mRNA was no longer found in most cells (1/5) cultured for 15 days, suggesting that the estrogen-dependent reduction of $TRAP^+$ multinucleate cells occurs early in the differentiation of osteoclasts (Figure 2B). In contrast, ER-beta mRNA was never detected in cells cultured for 5 or for 15 days (Figure 2B), even after 35 PCR amplification cycles.

*Effect of 17*beta*-estradiol on the release of IL-6 and IL-6rs into the culture medium of human BMMC cultures*

The release of IL-6 and IL-6sr into the culture medium varied widely from one culture to another after both 5 and 15 days in culture. 10^{-8} M 17beta-estradiol seemed to have no effect on the release of IL-6 (Table 1). The release of IL-6sr was also unaffected by 17beta-estradiol. However, the medium concentrations of IL-6 and IL-6sr were higher on day 5 than on day 15 ($p < 0.001$).

Effect of estrogen and Raloxifene on apoptosis of multinucleate cells from human BMMC cultures

We compared the effects of 17beta-estradiol and Raloxifene on apoptosis. Only TRAP multinucleate cells, displaying both criteria for apoptosis (immunostaining and condensed cytoplasm) were counted (Figure 3). Neither 17beta-estradiol nor Raloxifene, at the concentration of 10^{-8} M, had any effect on multinucleate cell apoptosis.

DISCUSSION

It is widely accepted that osteoclasts are derived from hematopoetic precursors, and that differentiation is induced in co-culture with stromal cells [20].

Culturing human BMMC that contain mostly monocytes and stromal cells, we were previously able to obtain mulinucleate cells which displayed the phenotypic patterns of osteoclasts such as the presence of TRAP [21]. At the end of culture, cells responded to calcitonin by an increase in cAMP, indicating that they were differentiating to an osteoclast lineage. Indeed, the number of the multinucleate $TRAP^+$ cells correlated with the number of osteoclasts in the adjacent bone.

Estradiol decreases bone resorption *in vivo*, but there are few reports of the action of estradiol on osteoclast formation *in vitro*. Most results have been obtained from *ex vivo* studies on mice [5, 22] but there is only one published set of data on the differentiation of osteoclast precursors in bone marrow cultures incubated with estradiol. In the present study, estradiol and Raloxifene inhibited the formation of multinucleate $TRAP^+$ cells in human bone marrow cultures, confirming the results of Flanagan with estradiol [23]. Raloxifene decreases *in vivo* bone resorption in ovariectomized rats [24] and postmenopausal women [16], and therefore, the *in vitro* effect we observed parallels the *in vivo* action on bone resorption. However, it is noteworthy that the *in vitro* effect is weaker than the *in vivo* effect, indicating other systemic actions of estradiol. We found that estradiol and raloxifene decrease the number of multinucleate $TRAP^+$ cells to the same extent. This is in contrast with *in vivo* effect where estradiol is more powerful than Raloxifene. This discrepancy may also be due to other systemic effects.

Although both estrogen receptors alpha and beta are present in bone, their roles in the hyperresorption caused by a lack of estrogen are not clear. Both ER-alpha or ER-beta knock-out mice lose bone after ovariectomy [25, 26]. We therefore evaluated the synthesis of ER-alpha or ER-beta in our cultures. We looked for the estrogen receptors after 5 and 15 days in culture because we postulated that receptor synthesis might change during cell differentiation, as observed in tumor cells [27]. All cultures contained ER-alpha mRNA (5/5) on day 5, and the absence of ER-beta strongly suggests that estradiol acts on osteoclast differentiation *via* ER-alpha. Classical PCR revealed no mRNA for either receptor, but nested RT-PCR did. This suggests that there were very few copies of ER-alpha mRNA in these cultures. ER-beta was still not detected in cells cultured for 15 days, and there was ER-alpha mRNA in only 1/5 samples, indicating that ER-alpha synthesis decreases with time of culture. As our cultures contained a mixed population of hematopoietic and stromal cells, no conclusion can be drawn about synthesis in any one type of cell. However, these results show that estrogen acts through ER-alpha and not through ER-beta.

The mechanisms by which estradiol and Raloxifene inhibit bone resorption are not clear, but cytokine release and apoptosis are candidates. The effect of estradiol on cytokine production remains controversial. There are differing findings about the effect of estradiol on IL-6 production by bone cells [5-8]. These contradictory results may be due to differences in the species used. The inhibition of the development of multinucleate cells by estradiol in our human culture system was not associated with changes in IL-6 and IL-6sr in the supernatant. The concentrations of both IL-6 and IL-6sr were higher on day 5 than on day 15, suggesting that the main source of cytokines was hematopoietic cells or undifferentiated precursors. Nevertheless, IL-6 and IL-6sr do not appear to be regulated in this human model, confirming other results [7, 8].

Others have used the TUNEL method to show that estrogen causes osteoclast apoptosis in rabbit osteoclasts [18], human osteocytes [28] and murine osteoclasts [29]. To our knowledge, estradiol has not yet been shown to have a direct effect on osteoclast apoptosis in humans. We found that neither estradiol nor Raloxifene clearly induce apoptosis in human osteoclast precursors. Our negative data do not exclude definitely an effect on apoptosis, because of the wide individual variations in one hand, and a possible effect on mature osteoclasts on the other.

CONCLUSION

In conclusion, we have shown that estradiol and Raloxifene inhibit osteoclast differentiation of human bone marrow mononuclear cells, in a dose-dependent manner. This biological effect, mimicking *in vivo* differentiation, could be mediated through ER-alpha in human bone marrow cells.

Acknowledgements. The authors are indebted to the members of the orthopedic surgery department (Pr L. Sedel) for providing human bone marrow and Valérie Maurin and Armelle Guignan for their technical support for the cytokine measurements.

Dr. A.-C. Ramalho is the recipient of a grant from the Conselho Regional de Desenvolvimento Cientifico e Tecnologico of Brasil (CNPq).

Accepted on 2/12/01

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Figures

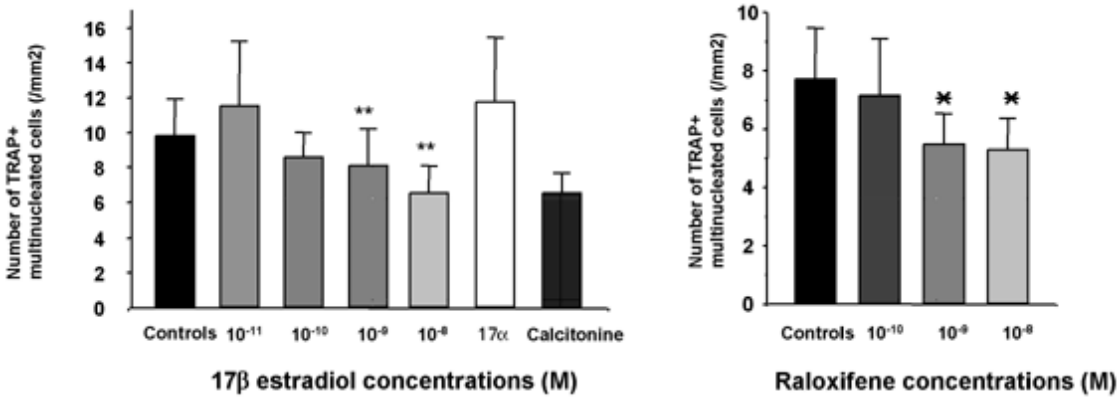


Figure 1

Dose-dependent decrease in multinucleate cell formation in human bone marrow cultures. Human bone marrow was collected at the femoral neck. Mononuclear cells were isolated using Ficoll-Hypaque, and after 24 hours-adherence, adherent mononuclear cells were cultured for 15 days with increasing doses of 17beta-estradiol, 17alpha-estradiol or vehicle (left panel) or in the presence of increasing doses of Raloxifene (right panel). The number of TRAP multinucleate cells were measured at the end of the experiments. *: different from controls $p < 0.05$; **: different from controls $p < 0.01$.

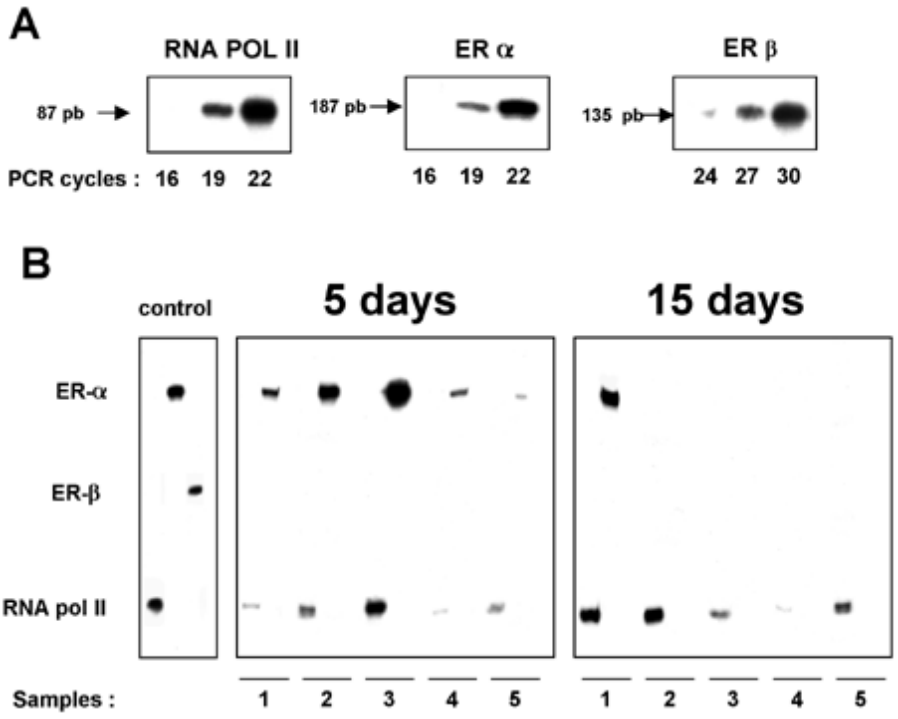


Figure 2

Expression of ER-alpha and ER-beta in human bone marrow cells. Panel **A:** cycle-effect of nested RT-PCR amplification for signals for RNA polymerase II, ER-alpha and ER-beta. Panel **B:** RNA polymerase II, ER-alpha and ER-beta nested RT-PCR products are respectively analysed in 5 samples after 5 and 15 days of BMMC culture. Total RNA from SaOS and MCF-7 were used as positive controls. Numbers below each figures correspond to individual samples (lane 1 to 5). Signals of ER-alpha were present in all samples after 5 days of culture, and only in one sample after 15 days of culture. ER-beta was not found at any time point.

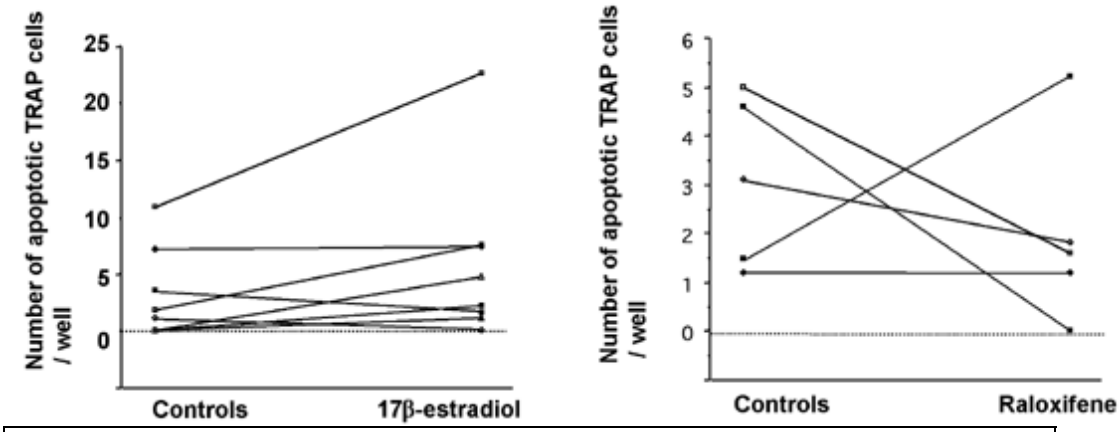


Figure 3

Changes in apoptotic TRAP multinucleate cells after 17beta-estradiol and Raloxifene addition. Bone marrow mononuclear cells were cultured for 15 days in the presence of vehicle (controls) or 17beta-estradiol (left graph) or Raloxifene (right graph). At day 15 of culture, apoptotic cells were counted in duplicate wells using the TUNEL method as described in the methods section. Individual changes after 17beta-estradiol or Raloxifene addition are presented. No significant changes were observed.

Table 1
IL-6 and IL-6sr levels in the supernatants at days 5 and 15 of culture

	IL-6 ng/ml		IL-6sr pg/ml	
	day 5	day 15	day 5	day 15
Controls	13.16 ± 20.34	1.91 ± 2.23	477 ± 346	618 ± 402
17β-estradiol	14.69 ± 22.31	2.24 ± 3.04	502 ± 395	628 ± 358
17α-estradiol	10.01 ± 15.25	1.82 ± 2.05	511 ± 398	627 ± 415

Mononuclear cells from human bone marrow cells were cultured for 15 days in the presence of vehicle (controls), 10^{-8} M 17β-estradiol or 10^{-8} M 17α-estradiol, which was used as negative control. Medium was changed twice a week. Cytokine levels were measured in the supernatants. IL-6 is expressed as ng/ml and IL-6sr as pg/ml.
No statistical difference was observed for IL-6 or IL-6sr levels between controls, 17β-estradiol or 10^{-8} M 17α-estradiol.

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IL-10, IL-19, IL-20, IL-22, mda-7, AK155.

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NK cells, dendritic cells, cell-to-cell interaction, activation, proliferation.

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dendritic cell, nonobese diabetic mice, autoimmunity, insulin-dependent diabetes mellitus.

Estradiol and raloxifene decrease the formation of multinucleate cells in human bone marrow cultures

Ana Claudia Ramalho, Philippe Couttet, Claude Baudoin, Caroline Morieux, Anne-Marie Graulet, Marie-Christine de Vernejoul, Martine E. Cohen-Solal
osteoclast, estrogen receptor, SERM, cytokines, apoptosis.

Interleukin-10 and cAMP-elevating agents cooperate to induce suppressor of cytokine signaling-3 via a protein kinase A-independent signal

Sara Gasperini, Luca Crepaldi, Federica Calzetti, Luana Gatto, Chiara Berlato, Flavia Bazzoni, Akihiko Yoshimura, Marco A. Cassatella
SOCS-3, PGE, IL-10, PBMC, neutrophils, PKA.

Different patterns of interleukin-1alpha and interleukin-1beta expression in organs of normal young and old mice

Moshe Hacham, Shmuel Argov, Rosalyn M. White, Shraga Segal, Ron N. Apte
IL-1alpha, IL-1beta, organ cytokine expression, young/old age, homeostasis.

The combination of the interleukin-1alpha (IL-1alpha-889) genotype and the interleukin-10 (IL-10 ATA) haplotype is associated with increased interleukin-10 (IL-10) plasma levels in healthy individuals

Sanna Kilpinen, Heini Huhtala, Mikko Hurme
interleukin-1, interleukin-10, gene polymorphism, plasma.

The interleukin-6 G(-174)C polymorphism and the ex vivo interleukin-6 response to endotoxin in severely injured blunt trauma patients

Michael Heesen, Udo Obertacke, F. Ulrich Schade, Brunhilde Bloemeke, Matthias Majetschak
interleukin-6, endotoxin, genetic polymorphism, polymerase chain reaction, whole blood, critical illness.

Comparison of leptin- and interleukin-6-regulated expression of the rPAP gene family: evidence for differential co-regulatory signals

Daniël Broekaert, Sven Eyckerman, Delphine Lavens, Annick Verhee, Wim Waelput, Joël Vandekerckhove, Jan Tavernier
forskolin, IL-6, leptin, rPAP/Reg gene family, signal transduction.

Mice lacking alpha2-macroglobulin show an increased host defense against Gram-negative bacterial sepsis, but are more susceptible to endotoxic shock

Tino Hochepied, Fred Van Leuven, Claude Libert
TNF, shock, acute phase, inflammation, alpha2-macroglobulin.

Tumor necrosis factor-alpha mRNA stability in human peripheral blood cells after lipopolysaccharide stimulation

Lucile Baseggio, Carole Charlot, Jacques Bienvenu, Pascale Felman, Gilles Salles
tumor necrosis factor, mRNA stability, lipopolysaccharide, tristetraprolin.

Caspase-1 levels in biological fluids from patients with multiple sclerosis and from patients with other neurological and non-neurological diseases

Diego Franciotta, Gianvito Martino, Elisabetta Zardini, Roberto Furlan, Roberto Bergamaschi, Maira Gironi, Alessandra Bergami, Giovanna Angelini, Fabrizio De Benedetti, Patrizia Pignatti, Gianna Moscato, Vittorio Cosi
asthma, cerebrospinal fluid, juvenile idiopathic arthritis, sputum, synovial fluid, THP-1 cells.

Divergent effects of tumor necrosis factor-alpha and lymphotoxin-alpha on lethal endotoxemia and infection with live Salmonella typhimurium in mice

Edi Dharmana, Monique Keuter, Mihai G. Netea, Ineke C.M.M. Verschueren, Bart Jan Kullberg
tumor necrosis factor, lymphotoxin, salmonella typhimunium, knock-out mice.

Th1/Th2 cytokine balance and nitric oxide in cerebrospinal fluid and serum from patients with multiple sclerosis

María del Carmen Rodríguez-Sáinz, Silvia Sánchez-Ramón, Clara de Andrés, Margarita Rodríguez-Mahou, M Angeles Muñoz-Fernández
cytokine, nitric oxide, multiple sclerosis, cerebrospinal fluid, oligoclonal bands, Igs.

Retinoids, cisplatin and interferon-alpha in recurrent or metastatic cervical squamous cell carcinoma: clinical results of 2 phase II trials

Anne-Chantal Braud, Laurence Gonzague, François Bertucci, Dominique Genre, Jacques Camerlo, Gwenaelle Gravis, Anthony Goncalves, Vincent Moutardier, Frédéric Viret, Dominique Maraninchi, Patrice Viens
all-trans-retinoic acid, 13 Cis retinoic acid, interferon-alpha, cervical squamous cell carcinoma.

Circulating macrophage colony stimulating factor as a marker of tumour progression

Raymond S. McDermott, Laurent Deneux, Véronique Mosseri, Jacques Védrenne, Krishna Clough, Alain Fourquet, José Rodriguez, Jean-Marc Cosset, Xavier Sastre, Philippe Beuzeboc, Pierre Pouillart, Susy M. Scholl
CSF-1, tumour progression, metastasis, de-differentiation.

The effect of interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) on granulocyte macrophage-colony stimulating factor (GM-CSF) production by neuronal precursor cells

J. Benjamin Dame, Nasser Chegini, Robert D. Christensen, Sandra E. Juul
IL-1beta, TNF-alpha, GM-CSF, neuron, mRNA, protein.

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