Prolactin down-regulates CD4⁺CD25hiCD127low/— regulatory T cell function in humans


Abstract

Among its many functions, prolactin (PRL) participates in immune responses and promotes the activation, differentiation and proliferation of T cells. However, the mechanisms by which PRL regulates regulatory T (Treg) cells are still unknown. Our goal was to determine whether PRL plays a role in Treg function. We measured the expression of PRL and its receptor in Treg and effector T (Teff) cells from 15 healthy individuals. We also evaluated the functional activity of Treg cells by examining proliferation and cytokine secretion in cells activated with anti-CD3/CD28 in the presence or absence of PRL. We report that Treg cells constitutively expressed PRL receptor, whereas Teff cells required stimulation with anti-CD3/CD28 to induce PRL receptor expression. Expression of PRL was constitutive in both populations. We found that the addition of PRL inhibited the suppressor effect (proliferation) mediated by Treg cells in vitro, reducing suppression from 37.4 to 13% when PRL was added to co-cultures of Treg and Teff cells (P<0.05). Cultures treated with PRL favoured a Th1 cytokine profile, with increased production of TNF and IFNγ. We report for the first time that PRL receptor expression was constitutive in Treg cells but not in Teff cells, which require stimulation to induce PRL receptor expression. PRL inhibited the suppressive function of Treg cells, apparently through the induced secretion of Th1 cytokines.

Journal of Molecular Endocrinology (2012) 48, 77–85

Introduction

Prolactin (PRL) is a lactogenic hormone that is synthesised and secreted by the pituitary gland. It has been associated with more than 300 different functions, with its functionality depending on the type of cells that express PRL receptor (Kelley et al. 2007). The PRL receptor belongs to the haematopoietic receptor superfamily; interaction between PRL and its receptor activates the Jak–Stat pathway, which is also commonly activated during cytokine signalling (Bole-Feyesot et al. 1998, Kelley et al. 2007). PRL can also be synthesised in an extra-pituitary way by B and T cells from the immune system, which also express PRL receptor (Bole-Feyesot et al. 1998). During an immune response, PRL promotes the proliferation and differentiation of T cells (Carreño et al. 2005) and influences the expression of CD69 and CD154 in CD4⁺T cells (Chávez-Rueda et al. 2005, 2007). In addition, when CD8⁺T cells are co-incubated with PRL and phytohaemagglutinin, CD69 expression increases (Takizawa et al. 2005). In CD4⁺T cell cultures activated with phorbol myristate acetate and incubated with an anti-PRL antibody, reduced secretion of IL2 and IFNγ is observed, indicating a role for PRL in the regulation of cytokine secretion (Chávez-Rueda et al. 2005). These results demonstrate the importance of PRL in the immune system. In B cells, PRL is involved in the induction of tolerance (Saha et al. 2009). We hypothesised that PRL could participate in the regulation of the immune system via regulatory T (Treg) cells.

Treg cells help maintain peripheral tolerance and exert a suppressive effect on the activation, proliferation and function of other cells in the immune system (Le & Chao 2007). There are two types of Treg cells: natural Treg cells, which are generated in the thymus, and inducible Treg cells, which are generated in the periphery; both exhibit the same CD4⁺CD25⁺CD127low/—FoxP3⁺ phenotype (Liu et al. 2006, Seddiki et al. 2006, Le & Chao 2007). Treg cells are unable to proliferate or secrete IL2 after their TCR is stimulated; instead, they exert an inhibitory effect on CD4⁺CD25⁻CD127⁺ conventional or effector T (Teff) cells. Treg cells inhibit Teff cell proliferation and IL2 production (Thornton & Shevach 1998) through a contact-dependent mechanism and/or the secretion of...
anti-inflammatory cytokines, including TGFβ (Nakamura et al. 2001) and IL10 (Asseman et al. 1999). It has been proposed that Treg cells prevent the development of autoimmune diseases that have been associated with high levels of PRL (De Bellis et al. 2005), including systemic lupus erythematosus (Scheinecker et al. 2010), type 1 diabetes (Salomon et al. 2003), inflammatory bowel disease (Maloy et al. 2003, Mottet et al. 2003).

Therefore, we decided to investigate the participation of PRL in Treg cell function. Our results indicate that Treg cells constitutively express PRL receptor, whereas in Teff cells, PRL receptor expression requires stimulation. We observed that when PRL is added to co-cultures of Treg and Teff cells, it reverses the Treg suppressive effect on Teff cells and promotes a Th1 cytokine profile.

Materials and methods

Study group

The ethics committee on Human Research of the Instituto Mexicano del Seguro Social (IMSS) approved this study, and it was conducted according to the Declaration of Helsinki with the informed consent of all participants. The samples studied were obtained from 15 healthy women of reproductive age (25–45 years) without menstrual disorders and with normal levels of serum PRL (<20 ng/ml). The samples were obtained from the cubital vein between 0800 and 1100 h after overnight fasting or a light morning meal consisting of fruit and tea.

Prolactin

The human PRL used in this study was kindly provided by Dr A F Parlow, from the National Hormone and Pituitary Program (NHPP, Harbor-UCLA Medical Center, Los Angeles, CA, USA).

Antibodies

The following antibodies were used: mouse anti-human CD4-APC (OKT4), CD25-PE-Cy5 (BC96), CD127-FITC (eBioRDR5), FoxP3-PE (PCH101) and CD25-APC (BC96), all from eBioscience (San Diego, CA, USA); mouse anti-human CD28 (37407) from R&D Systems (Minneapolis, MN, USA); mouse anti-human CD3 (HIT3a) from BD Biosciences (San Jose, CA, USA); mouse anti-PRL receptor (ECD, 1A2B1) from Invitrogen and biotin rat anti-mouse IgG2b (R12-3) from BD Pharmingen (San Jose, CA, USA). The biotinylated secondary antibody was detected with streptavidin–phycoerythrin–Cy5.5 from BD Biosciences (Mountain View, CA, USA).

CD4+ T cell purification and sorting of Treg and Teff populations

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples by density centrifugation using Lymphoprep (Axis Shield, Oslo, Norway). CD4+ T cells were isolated from PBMCs using a CD4+ T cell isolation kit II (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. Enriched CD4+ T cells were stained with anti-CD4, -CD127 and -CD25 for 30 min at 4°C. CD4+CD25+CD127+ (Teff) cells and CD4+CD25hiCD127lo/- (Treg) cells were sorted using a FACSAria (BD Biosciences) supported by BD Biosciences FACSDiva software. The purity of the sorted cells ranged between 96 and 98%.

Cell culture and proliferation assays

Cells were cultured in AIM-V liquid medium (Gibco BRL) supplemented with 50 units/ml penicillin and 50 µg/ml streptomycin (Gibco). Treg cells (CD4+CD25hiCD127lo/-) were plated at 1·0×10⁴ cells/well in 96-well U-bottom plates (Nunc, Roskilde, Denmark) in the presence or absence of 2·0×10⁴ Teff cells (CD4+CD25−CD127+) and cultured in synthetic serum-free medium (AIM-V, Gibco). We determined the optimum ratio of Teff:Treg cells using a dose–response curve with the following ratios: 1:0·5, 1:1, 1:2 and 1:4. The suppressive effect was observed with all ratios tested. We decided to use a ratio of 1:0·5 Teff:Treg because, given the percentage of circulating Treg cells, it would be feasible to obtain enough cells to perform all the experiments.

Based on a dose–response curve, we decided to use 2·5 µg/ml soluble anti-CD3 and 1 µg/ml soluble anti-CD28 antibodies to specifically activate T cells in the presence or absence of 50 ng/ml human PRL (NHPP). The concentrations of antibodies and PRL were also determined based on dose–response curves. Cells were cultured for 5 days, and 1 µCi [³H]thymidine (Hartmann Analytical, Braunschweig, Germany) was added 18 h before harvesting. Thymidine incorporation was determined using a liquid scintillation analyser (Packard 1900 TR, Meriden, CT, USA), and the suppression of proliferation was calculated as a percentage. All conditions had previously been standardised and optimised.

Cytokine detection

Cell culture supernatants were collected on day 2, and Th1 (TNF, IFNγ and IL2), Th2 (IL4, IL5 and IL10) and Th17 (IL17A) cytokine levels were measured using a commercially available BD Cytometric Bead Array (CBA); TGFβ was measured by ELISA (R&D Systems).
Real-time PCR assay
Total RNA was extracted from purified Treg and Teff cells using TRIzol Reagent (Invitrogen) according to the manufacturer’s instructions. The RNA concentration was determined using u.v. spectrophotometry. To generate cDNA, 1 μg of total RNA was used with SuperScript II reverse transcriptase (Invitrogen). The PRL receptor, PRL, and β-actin were then amplified by real-time PCR using a LightCycler TaqMan Master kit (Roche Diagnostic) and hydrolysis probes and primers designed by Roche Diagnostic; all reactions were performed according to the manufacturer’s specifications. The following primers and probes were used: probe number 18 from the Universal Probe Library for PRL determination, forward primer AAA GGA TCG GCC ATG GAA AG and reverse primer GCA CAG GAG CAG GGT TTG AC; probe number 8 from the Universal Probe Library for PRL receptor determination, forward primer CTT TCC ACA TGA ACC CTG AAG and reverse primer GCC ATG GAA AG and reverse primer GCA GAT GCC ACA TTT TCC TT and probe number 64 from the Universal Probe Library for β-actin determination, forward primer CCA ACC GCG AGA AGATGA and reverse primer CCA GAG GCC TAC AGG GAT AG. The reactions were carried out in a final volume of 10 μl using a LightCycler 1.5 instrument (Roche Diagnostic). The PCR conditions were as follows: 10 min at 95 °C, followed by 45 cycles of 10 s at 95 °C, 30 s at 59 °C and 1 s at 72 °C, followed by 1 cycle of 30 s at 40 °C. The samples were normalised to β-actin gene expression. The relative expression of PRL and its receptor were calculated using the 2ΔCt formula.

Cell surface staining and flow cytometry
Treg and Teff cells were incubated with fluorescently labelled antibodies (anti-CD4, -CD25, -CD127 and -PRL receptor) for 20 min at 4 °C in staining buffer (PBS with 0.5% BSA and 0.01% sodium azide). The cells were then washed and fixed in 2% paraformaldehyde (Sigma–Aldrich). Data were acquired using a FACS Aria flow cytometer (BD Biosciences) and analysed with FlowJo software (Tree Star, Ashland, OR, USA).

Detection of PRL in the culture supernatants
PRL concentrations in culture supernatants from Teff and Treg cells were measured using the Nb2 lymphoma cell assay. The cells were kept at 37 °C in RPMI-1640 medium supplemented with 10% FBS, 2 mM glutamine, 50 units/ml penicillin and 50 μg/ml streptomycin. The cells were arrested in the early G1 phase of the cell cycle by pre-incubation (24 h) in medium with 1% FBS. Afterwards, the cells were distributed in flat-bottom 96-well plates at a density of 2×10^5 cells/well, and the culture supernatants from Teff and Treg cells (1×10^5 cells/well) stimulated with anti-CD3/CD28 were added to reach a final volume of 200 μl, without FBS addition. After 72 h at 37 °C with 5% CO₂, the presence of viable cells was assessed by measuring incorporated [3H]thymidine.

Statistical analysis
Statistical analyses were performed by the statistical software package SPSS, version 15.0 (SPSS, Inc., Chicago, IL, USA). The experimental data were analysed using standard statistical tests (e.g. mean value, s.d., Student’s t-test and ANOVA), and the results were expressed as the mean±s.d. The threshold for significance was set at P<0.05.

Results
CD4⁺ T cells were isolated by negative selection from healthy women PBMCs with purity >90%. The cells were then stained with fluorochrome-conjugated monoclonal antibodies, and Treg (CD4⁺CD25hi CD127low/−) and Teff (CD4⁺CD25−CD127hi) cells were sorted using a FACS Aria; a purity higher than 98% was obtained for each population. In addition, FoxP3 expression analysis showed that only the sorted Treg cell population was FoxP3⁺ (Fig. 1).

When the relative mRNA expression of PRL receptor was examined in unfractionated CD4⁺ T cells, we found it to be constitutively expressed; this cell population was used as a control to calculate the relative expression of PRL receptor mRNA in purified cell subsets. Next, PRL receptor mRNA expression was examined in the sorted Treg and Teff cell populations. We found that while Treg
cells constitutively expressed PRL receptor mRNA (0.8 ± 0.4), PRL receptor mRNA expression was not detected in Teff cells (Fig. 2A). We also evaluated expression at the protein level by flow cytometry, and again, we detected PRL receptor expression in Treg cells (3.49 ± 0.80·75 mean fluorescence intensity (MFI)) but not in Teff cells (Fig. 2B and C). To investigate whether PRL receptor mRNA expression in Teff cells could be induced, cells were stimulated with anti-CD3/CD28 antibodies for different periods of time before the mRNA expression was analysed. Teff cells up-regulated the expression of PRL receptor mRNA as early as 2 h after stimulation (0.77 ± 0.55); this expression increased at 4 h post-stimulation (2.3 ± 0.41). However, at 6 h (1.83 ± 0.28) after stimulation, PRL receptor mRNA expression decreased without the difference observed being statistically significant (Fig. 2D). At the protein level, we found that Teff cells up-regulated the expression of the PRL receptor 2 h after stimulation (505 ± 17 MFI); this expression was maintained throughout the time course (Fig. 2E). Together, these results suggest that PRL receptor mRNA expression in Teff cells is inducible. As mentioned, Treg cells constitutively expressed PRL receptor mRNA; this gene was overexpressed at 2 h post-stimulation (3.9 ± 1.39) and tended to be down-regulated at 4 and 6 h after stimulation (2.9 ± 1.31 and 0.76 ± 0.15, respectively; Fig. 2F). We also observed an increase in the expression of the protein at 2 h post-stimulation (781 ± 104·5 MFI), and these levels were maintained at 4–6 h (698·7 ± 175·6 and 625·7 ± 226·7 MFI; Fig. 2G).

Using the same approach, we found that, unlike PRL receptor, PRL mRNA was constitutively expressed in all cell populations examined (Treg and Teff cells). PRL mRNA expression in Teff cells was significantly higher compared with Treg cells (10.52 ± 3.61 vs 4.71 ± 0.68, respectively, P < 0.05; Fig. 3A). We also stimulated Teff and Treg cells with anti-CD3/CD28 at different times to evaluate the expression of this gene, and we found that PRL mRNA remained constant over time in Teff and Treg...
PRL mRNA in Teff cells stimulated with anti-CD3 (2.5 μg/ml) and anti-CD28 (1 μg/ml). The addition of PRL to this culture resulted in a slight increase in cellular proliferation (81.32 ± 6.81 × 10³ c.p.m.; P<0.05). However, when PRL was added to the Treg:Teff co-culture, we only observed a 13% decrease in proliferation (59.0 ± 8.8 × 10³ vs 67.0 ± 10 × 10³ c.p.m.), suggesting that PRL restored the proliferative capacity of the Teff cells. Furthermore, if we compare the proliferation of Teff cells alone against the co-culture (Teff:Treg cells) when both cultures were treated with PRL, no significant differences were found; therefore, the Treg cells had no suppressive effect in the co-culture with PRL.

We examined the presence of Th1 and Th2 cytokines in supernatants from cells cultured under the following conditions: Teff cells stimulated with anti-CD3/CD28 in the presence or absence of PRL; Treg cells stimulated in the presence or absence of PRL; and Treg:Teff (0.5:1) co-cultures of stimulated cells in the presence or absence of PRL. We did not detect IL4, IL6, IL10, IL17A or TGFβ in any of the conditions tested (data not shown). Although we did detect IL2, the differences between the conditions were not statistically significant. Figure 5A shows the concentration of each cytokine.

Teff cells stimulated with anti-CD3/CD28 in the absence or presence of PRL secreted IFNγ (31.25 ± 2.93 pg/ml and 36.9 ± 10-0 pg/ml, respectively), but the difference between the conditions was not statistically significant. In contrast, in the Treg:Teff co-cultures, IFNγ production was reduced 24.48% compared with stimulated Teff cells cultured alone (23.6 ± 0.85 pg/ml and 31.25 ± 2.93 pg/ml; P<0.05). The concentration of IFNγ produced in the Treg:Teff cells co-culture increased in the presence of PRL (51.4 ± 14.84 pg/ml), as shown in Fig. 5B.

The TNF concentration was similar in the absence or presence of PRL (6.7 ± 1.3 vs 6.48 ± 1.48 pg/ml). The TNF concentration decreased when stimulated Teff cells were co-incubated with the Treg cells (4.4 ± 0.7 pg/ml) and increased in the presence of PRL (6.7 ± 0.3 pg/ml). The difference between the TNF concentrations in co-cultures treated with or without PRL was statistically significant (P<0.05), as shown in Fig. 5C. These results suggest that PRL inhibits the suppressive function of Treg cells through the induction of Th1 cytokines.

**Discussion**

Several studies have demonstrated that PRL participates in immune responses by interacting with its receptor, which is expressed by a wide variety of immune cells (Bole-Feyt et al. 1998, Kelley et al. 2007). PRL influences the proliferation and activation of T cells and stimulates the secretion of cytokines.
Figure 4 T_{reg} cells function in the presence or absence of PRL. T_{reg} (CD4^{+}CD25^{hi}CD127^{low/−}) and T_{eff} (CD4^{+}CD25^{−}CD127^{+}) cell populations purified from PBMCs from healthy women were stimulated with anti-CD3 (2.5 μg/ml) and anti-CD28 (1 μg/ml) in the presence or absence of PRL (50 ng/ml). Cellular proliferation was determined on the fifth day of culture by measuring the incorporation of [3H]thymidine and reported as c.p.m. The mean values ± s.d. from 15 independent assays performed in triplicate are shown. *P<0.05.

(Chávez-Rueda et al. 2005, Takizawa et al. 2005, Tomio et al. 2008). In this study, we demonstrated for the first time that T_{reg} cells (CD4^{+}CD25^{hi}CD127^{low/−}) constitutively expressed PRL receptor, whereas resting T_{eff} cells (CD4^{+}CD25^{−}CD127^{+}) did not. However, stimulation of T_{eff} cells with anti-CD3/CD28 induced PRL receptor expression, which increased over time. These results introduce the possibility of using PRL receptor expression as an activation marker for T_{eff} cells. The difference in PRL receptor expression between T_{eff} and T_{reg} cells suggests a different role for PRL in these populations. It appears that PRL can signal at any time in T_{reg} cells and therefore could be involved in early activation events in these cells. In contrast, T_{eff} cells require activation to express PRL receptor; thus, PRL in T_{eff} cells most likely acts to increase their proliferation, as has been reported in studies with PBMCs, T and B cells (Chávez-Rueda et al. 2005, Xu et al. 2010). However, it is also possible that PRL could be involved in the activation of T_{eff} cells.

Lymphocytes have previously been shown to constitutively express the gene for PRL (Montgomery 2001). Our results showed that both T_{eff} and T_{reg} cells constitutively expressed PRL mRNA; this is different from PRL receptor expression, which is only constitutively expressed in T_{reg} cells. In addition, we showed that PRL mRNA expression did not increase in T_{eff} or T_{reg} cells when they were activated with anti-CD3/CD28; these results differed from a previously published report in which the authors observed an increase in PRL mRNA expression in T cells (Gerlo et al. 2006). This difference could be accounted for in several ways. For instance, different activation pathways were used; we stimulated T cells with anti-CD3/CD28, whereas they used cAMP. They also conducted their studies using a mixed population of T cells, including both CD4^{+} and CD8^{+} cells, whereas we used purified CD4^{+} T_{reg} and T_{eff} cells. Despite these differences, we agree with their finding that T cells express PRL mRNA. Moreover, we found that CD4^{+} T_{eff} cells expressed more PRL mRNA than T_{reg} cells, although the protein level detected in culture supernatants was similar in both subsets. Therefore, both T_{eff} and T_{reg} cells could be the source of PRL in CD4^{+} T cells. These results suggest that the PRL secreted by T_{eff} or T_{reg} cells may act in an autocrine manner to form a feedback loop in the cells that produced it. However, any possible autocrine effect is dependent on the expression of PRL receptor in T_{eff} and T_{reg} cells, which varies depending on the activation status and time post-stimulation.

It is well known that T_{reg} cells function to regulate the response of T_{eff} cells by mediating a decrease in T_{eff} cell proliferation; our in vitro findings are consistent with this phenomenon (Sakaguchi et al. 2009). Knowing that T_{reg} cells constitutively express PRL receptor and that T_{eff} cells up-regulate PRL receptor expression following stimulation, we investigated the effect of PRL on cell proliferation.

We observed a tendency towards increased T_{eff} cell proliferation after co-incubation with PRL and anti-CD3/CD28; these results are consistent with a previously observed additive effect of PRL in PBMCs co-incubated with a mitogen (Chávez-Rueda et al. 2005). However, this effect was not observed in T_{reg} cells because T_{reg} cells do not proliferate (Annunziato et al. 2002). When T_{eff} and T_{reg} cells were co-incubated, our results confirmed previous observations of a decrease in T_{eff} cell proliferation (Sakaguchi et al. 2009). Interestingly, when PRL was added to the T_{reg}:T_{eff} cell co-cultures, the suppressive effect exerted by the T_{reg} cells was weaker (comparing the proliferation of the co-cultured cells with the proliferation of T_{eff} cells activated in the presence or absence of PRL). Our results suggest that PRL down-regulates T_{reg} cell function because the addition of PRL to the T_{reg}:T_{eff} co-culture restored the proliferative capacity of the T_{eff} cells, and the levels of proliferation were comparable to those of T_{eff} cells alone. If we compared the proliferation of T_{eff} cells with the co-culture (T_{reg}:T_{eff}) when both had been treated with PRL, no statistically significant difference was found, which indicates that no suppression occurred in the co-culture with PRL. Moreover, we found a statistically significant difference between the co-cultures in the presence and absence of PRL. This reinforces our hypothesis that PRL...
down-regulates T<sub>reg</sub> cell function to prevent their suppressive activity. One possible explanation is that PRL could interact with the T<sub>reg</sub> cells, which constitutively express PRL receptor, to modify their regulatory function (Sojka et al. 2008). Given that T<sub>reg</sub> cells may suppress the response of T<sub>eff</sub> cells through cell–cell contact and/or the secretion of cytokines, particularly IL10 or TGFβ (Annunziato et al. 2002, Buckner 2010), we examined the role of PRL in T<sub>eff</sub> regulation by measuring the secretion of different cytokines associated with Th1, Th2 and Th17 CD4<sup>+</sup>T cell profiles. Our results show that the secretion of IFNγ and TNF increased when PRL was added to the T<sub>reg</sub>:T<sub>eff</sub> co-cultures and that this increase correlated with an increase in cellular proliferation. These results suggest that PRL favours a Th1 cytokine profile, thus inhibiting the suppressive function of T<sub>reg</sub>. This hypothesis is further supported by reports that increased concentrations of TNF decreased the regulatory function of T<sub>reg</sub> cells (Valencia et al. 2006, Nagar et al. 2010). IFNγ also inhibits the function of T<sub>reg</sub> cells in collagen-induced arthritis murine models (Kelchtermans et al. 2005, Wood & Sawitzki 2006). Interestingly, IFNγ inhibits the generation of T<sub>reg</sub> cells in vivo (Caretto et al. 2010), and PRL is important for IFNγ secretion (Chávez-Rueda et al. 2005).

The key findings of this study were as follows: a) PRL receptor was constitutively expressed in T<sub>reg</sub> cells but not in T<sub>eff</sub> cells, which required stimulation for expression; b) PRL was secreted by both T<sub>eff</sub> and T<sub>reg</sub> cells; c) the suppressive effect of T<sub>reg</sub> cells in co-cultures was reduced in the presence of PRL; and d) Th1 cytokines, which have previously been shown to have an inhibitor effect on T<sub>reg</sub> cells, were produced after PRL treatment. Based on these results, it is possible that T<sub>reg</sub> and T<sub>eff</sub> cells respond to PRL with different kinetics during the T cell activation process, favouring the secretion of Th1 cytokines and thus inhibiting the activity of T<sub>reg</sub> cells.

Our results may explain the relationship between hyperprolactinaemia and disease activity in lupus (Bianco et al. 1999, Jara et al. 2008, 2009, Nociti et al. 2010) because, by down-regulating the suppressive activity of T<sub>reg</sub> cells, PRL could favour the activation of T<sub>eff</sub> cells and thus perpetuate the disease. This hypothesis is supported by the fact that a functional deficiency has been shown in T<sub>reg</sub> cells (Crispin et al. 2003, Dejaco et al. 2006, Mudd et al. 2006), and there is evidence to suggest that this deficiency is not an inherent defect in the T<sub>reg</sub> cells of patients with lupus but is the result of changes in other mechanisms (Tower et al. 2011), which could involve PRL. Together, with the fact that PRL has been shown to modulate the immune response (Chávez-Rueda et al. 2005, 2007, De Bellis et al. 2005), these findings suggest that PRL could modulate the regulatory function of T<sub>reg</sub> cells through its receptor to favour a Th1 response and activate T<sub>eff</sub> cells. Such activation may then influence the clinical course of patients with lupus.
Based on the results described here, we suggest that PRL inhibits the function of Treg cells by inducing a Th1 cytokine profile. However, further research into the effects of PRL and its mechanism of action will be required.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This study was supported in part by Consejo Nacional de Ciencias y Tecnología (CONACYT-113815) and Fondo de Investigación en Salud, IMSS (FIS-2005-3603-0046).

Acknowledgements

We thank Dr A F Parlow from the National Hormone and Pituitary Program, Harbor-UCLA Medical Center for the donation of human PRL (hPRL). We are grateful to Dr Ezequiel Fuentes Panañá for providing critical review and comments on the manuscript.

References


Caretto D, Katzman SD, Villarino AV, Gallo E & Abbas AK 2010 Cutting edge: the Th1 response inhibits the generation of peripheral regulatory T cells. Journal of Immunology 184 30–34. (doi:10.4049/ jimmunol.0903412)


Le NT & Chao N 2007 Regulating regulatory T cells. Bone Marrow Transplantation 39 1–9. (doi:10.1038/sj.bmt.1705529)


Received in final form 14 October 2011
Accepted 6 December 2011
Made available online as an Accepted Preprint 7 December 2011