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Prolactin receptor expression by splenocytes from rats in various hormonal states

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Abstract. Prolactin (PRL) is mitogenic for lymphocytes *in vitro*, but the responsiveness of lymphocytes depends on the *in vivo* hormonal status of the rats from which the cells were obtained. Lymphocytes from ovariectomized (OVX) rats, but not from rats in oestrus or from male rats, respond to prolactin; administration of oestradiol to OVX rats diminishes the response. In order to determine if a correlation exists between lymphocyte responsiveness to prolactin and levels of cell surface prolactin receptors (PRL-R) expression, the percentage of splenocytes and each splenocyte subpopulation expressing surface PRL-R from rats of various hormonal states (OVX, oestradiol-injected OVX, oestrus and male) was analysed by single-colour and dual-colour flow cytometric analysis. We found that approximately 20% of splenocytes expressed surface PRL-R regardless of hormonal states ($n=16$). The majority (85%) of PRL-R positive splenocytes were B lymphocytes whereas 11.1% and 4.8% of splenocytes expressing the PRL-R were CD4 positive T-helper (T_H) and CD8 positive T-cytotoxic (T_C) lymphocytes, respectively. B lymphocytes also stained more brightly than T lymphocytes. This distribution of PRL-R expression did not show significant alterations on total splenocytes or T_H and T_C lymphocytes during various hormonal stages. However, the percentage of PRL-R-positive B lymphocytes increased markedly in OVX rats (twofold), compared to rats at oestrus. In summary, no correlation was found between the responsiveness to prolactin as a mitogen and levels of PRL-R expression by lymphocytes from rats at different hormonal states. This result suggests that sex steroid hormones may control prolactin responsiveness of lymphocytes by affecting the signal transduction pathway through PRL-R rather than by altering the level of the cell surface receptor expression.

Prolactin (PRL) is a member of a family of polypeptide hormones including growth hormone and placental lactogen which probably arose by duplication of an ancestral gene (Niall *et al.* 1971). PRL affects a variety of physiological processes in vertebrates such as reproduction, lactation, growth and morphogenesis, metabolism, behaviour and immunoregulation (Nicoll & Bern 1972).

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The immunoregulatory function of PRL has been supported by substantial evidence. PRL can affect the immune system *in vivo* and cells of the immune system *in vitro*. *In vivo*, PRL has been reported either to enhance or suppress cellular or humoral immune responses. For example, in adult rats, hypophysectomy or bromocriptine treatment caused a lowered production of antibody and of adjuvant-induced arthritis (Nagy & Berzci 1978, Bernton *et al.* 1988). Administration of PRL restored these responses. In addition, *in vivo* administration of PRL appears to regulate DNA synthesis and expression of the c-myc proto-oncogene in lymphoid tissue of hypophysectomized rats (Berczi *et al.* 1991).

Evidence from *in vitro* studies indicates that PRL can affect lymphoid cells directly. A rat lymphoma cell line, Nb2, depends on PRL for proliferation (Tanaka *et al.* 1980). PRL induces the transcription of growth related genes: c-myc, ODC, hsp-70 and β-actin in Nb2 cells (Yu-Lee 1990). PRL also can regulate interferon regulatory factor (IRF-1) expression in lymphocytes (Stevens & Yu-Lee 1992). More recent studies indicated that PRL is mitogenic for splenocytes from ovariectomized (OVX) rats (Mukherjee *et al.* 1990; Viselli & Mastro 1991). Cells from females in oestrus, from OVX rats injected with oestradiol, or from males did not proliferate in response to PRL. However, cells from all groups gave similar responses to a polyclonal T-cell mitogen, Con A. From these studies, PRL appears to behave like a classical T-cell mitogen in that it causes IL-2 production and IL-2R expression.

Prolactin exerts its biological effects by binding to its receptor. Prolactin receptor (PRL-R) is encoded by a single gene on chromosome 5. PRL-R cDNA from rat liver and ovary, rabbit mammary gland, human breast, liver tumour cell lines (review by Kelly *et al.* 1993) and mouse mammary gland (Moore & Oka 1993) have been cloned and sequenced. Two forms of PRL-R have been identified in normal rat tissues: long and short forms. The short form differs from the long form in length and sequence of the last 30 amino acids in the C-terminal region (Boutin *et al.* 1988, Edery *et al.* 1989; Shirota *et al.* 1990). The significance of the existence of two forms of the PRL-R is not known. In addition to the short and the long form, a mutant intermediate form of PRL-R has been found in Nb2 cell line (Ali *et al.* 1991). This form of PRL-R is missing 198 amino acid region in cytoplasmic domain.

Because PRL is a potent mitogen for splenocytes from OVX rats, we believe that there is a difference in the splenocytes from animals in different hormonal states (OVX, OVX rats injected with oestradiol and oestrus, and male rats). One difference may be at the cellular level, i.e. the lymphocyte subpopulation composition may be different or the lymphocytes may express more or different receptors. Therefore, the aim of this study was to investigate how lymphocyte subpopulations and the expression of cell surface PRL-R on lymphocytes change during different hormonal states. Cells treated with antibodies against PRL-R and lymphoid specific surface markers were analysed by flow cytometry.

MATERIALS AND METHODS

Animals

Fisher F344 rats (Charles River, Wilmington, MA) were housed in a room maintained at 20–22°C on a 14 h light, 10 h dark schedule with free access to food and water. Female rats were ovariectomized (OVX) at 43 days of age. After 14 days, OVX rats were sacrificed or injected with 17-β-oestradiol in 0.1 ml sesame oil. Two days and 1 day prior to sacrifice, animals were administered 0.5 μg and 50 μg oestradiol, respectively. Intact females with 4 day regular cycle were sacrificed on the day of oestrus. All oestrus females and male rats were sacrificed between 57 and 75 days of age.

Lymphocyte preparation

Rats were sacrificed by CO_2 asphyxiation. Spleens were removed asceptically, placed in dishes in sterile Dulbecco's phosphate buffered saline (PBS), pH 7.2, and gently crushed to release the lymphocytes from the tissue. Cells were passed through fine wire mesh to remove residual connective tissue and briefly suspended in Tris ammonium chloride, pH 7.2, in order to lyse erythrocytes. Cells were washed in weak, buffered acid (25 mM sodium acetate in PBS, pH 4) to free any ligands from their cell surface receptor, thereby ensuring that epitopes recognized by antibodies were free from obstruction. Cells were counted using a haemocytometer; viability was determined by trypan-blue exclusion. Viability was >85%.

Antibodies and staining of splenocytes

Mouse monoclonal antibodies (IgG1 isotype) used to stain for specific rat leukocyte antigens were obtained from Bioproducts for Science (Indianapolis, IN). Anti-rat CD4, anti-rat CD8, anti-rat CD5, anti-kappa light chain and anti-IL-2R α-chain were used at 1/400, 1/800, 1/400, 1/800 and 1/400 final dilutions, respectively, following previously published procedures (Güneş & Mastro 1996). All leukocyte surface antigen antibodies were detected by flow cytometry as log integrated green fluorescence (LIGFL) by using rat anti-mouse IgG conjugated to FITC (Jackson Immuno Research Laboratories Inc., West Grove, PA) at a dilution of 1/100. Surface PRL-R were detected using the polyclonal antisera (at 1/50 dilution) to the first 13 amino acids in N terminus of rat PRL-R (Hooper *et al.* 1993), a gift from Dr Kurt Ebner (University of Kansas, Medical Center). Cells expressing PRL-R were detected as log integrated yellow fluorescence (LIYFL) by using goat anti-rabbit IgG conjugated to PE (Caltag Laboratories, San Francisco, CA) at a dilution of 1/100. Control antibodies were used to determine background fluorescence and non-specific binding of antibodies. Mouse IgG specific for bovine CD8, an isotype control with irrelevant specificity served as a control for leukocyte surface antigens. Cells exhibited 1% to 3% non-immune staining. Cells incubated with normal rabbit serum (NRS), which served as a control for PRL-R antibody, exhibited 2% to 5% non-immune staining. These background values were substructed from experimental values.

Freshly isolated splenocytes were plated at 1×10^6 cells/well in 96-well v-bottom plates. In dual staining experiments, 50 μl of anti-rat leukocyte surface antigen antibody and 50 μl of anti-PRL-R antibody were added concurrently to the wells. Cells were incubated at 4°C for 25 min and washed twice with PBS (pH 7.2) containing 2% calf serum, 1% goat serum, and 0.01% azide. Next, 100 μl appropriate secondary antibodies conjugated to either FITC or PE were added and incubated with the cells for 25 min in the dark. Cells were washed twice, placed in 100 μl of fixative (1% paraformaldehyde dissolved in PBS) and stored up to 5 days at 4% in the dark before the flow cytometric analysis. Prior to analysis, cells were washed three times to remove residual fixative.

Flow cytometric analysis

Flow cytometric analysis were carried out as described by Viselli & Mastro (1993). Briefly, 10000 cells per sample were analysed in duplicate by an EPICS 753 Flow Cytometer (Coulter Electronics, Hialeah, FL) equipped with two argon ion lasers. The filters used were a 457–504 nm laser blocking filter and a 550 nm long pass dichronic filter with a 525 nm band pass filter for FITC fluorescence and 575 nm band pass filter for PE fluorescence. The laser was tuned to 488 nm at 100 mW of power. During data collection, forward angle light scatter and 90° light scatter was used to gate on cells to be included in analysis; data were collected

in list mode format. For dual-colour experiments, the four quadrants for analysis were defined using positive control samples with both FITC and PE.

Data was analysed using the Epics Elite software package. Log integrated fluorescence distributions were generated and analysed to determine percentages of cells that were positively fluorescent. In dual-colour experiments, dot plots with log intensity yellow fluorescence as the vertical axes and log intensity green fluorescence as horizontal axes were created. Duplicate samples were averaged. The percentages of positive cells from proper control samples, exhibiting non-immune fluorescence, were substracted from the percentages of positive cells. The Macintosh StatView SE+ program was used to determine mean values $\pm$SEM. StatView, Fisher PLSD test, was used and significant differences at 95% (P=0.05) and 99% (P=0.008) confidence intervals were determined.

RESULTS

PRL-R expression by splenocytes

Cell surface expression of PRL-R was seen on rat splenocytes with antibodies to extracellular region of PRL-R along with flow cytometry. Approximately 20.6$\pm$2% (mean$\pm$SEM; n=16) of splenocytes expressed PRL-R regardless of the hormonal states of the animals from which the spleens were taken. The log intensity fluorescence distribution indicated that most of the PRL-R positive lymphocytes stained relatively dimly compared to the highly fluorescent peaks obtained from the fluorescence distribution of CD4 positive and CD8 positive lymphocytes (Figure 1a–c).

Because splenocyte preparations contain heterogeneous subpopulations of lymphocytes, we identified some of these cells. Splenocytes were stained with anti-CD4 for helper T-cells (T_H), anti-CD8 for cytotoxic T-cells (T_C) and anti-kappa light chain for B-cells. Flow cytometric analysis showed that the percentage of each lymphocyte subpopulation in total splenocytes was 24.3$\pm$0.8% CD4+; 19.5$\pm$0.7% CD8+; and 55.5$\pm$0.9% B-cells (mean$\pm$SEM; n=16, Table 1).

Percentages of lymphocyte subpopulations expressing PRL-R

Dual-colour flow cytometric analysis revealed that a greater portion of B lymphocytes expressed surface PRL-R (mean, 31.5$\pm$3%; n=16) compared to either CD4+ cells (mean, 9.8$\pm$1.4%; n=16 of CD4+ cells) or CD8+ cells (5.4$\pm$1.2% of CD8+ cells). In other words, the average percentage distributions of B-cells, CD4+ cells and CD8+ cells among PRL-R positive splenocytes were approximately 85%, 11% and 4.3%, respectively (Table 1). This distribution of PRL-R expression among splenocyte subpopulations may be due to the observation that the numbers of B lymphocytes in the spleen outnumber both T lymphocyte subpopulations.

When fluorescence distributions of PRL-R expression in each subpopulations were compared, B lymphocytes stained more brightly for PRL-R than the T lymphocytes (Figure 2a–c). The brightly stained PRL-R positive B lymphocytes were more fluorescent than PRL-R positive T-cells. The results indicate that more B-cells than T lymphocytes expressed PRL-R, and that B lymphocytes also expressed the PRL-R more densely.

PRL-R expression by splenic lymphocytes from rats at various hormonal stages

In order to determine if responsiveness to PRL as a mitogen *in vitro* was related to the percentages of splenocytes expressing PRL-R, splenocytes from rats at different hormonal states were subjected to a single-colour and dual-colour flow cytometric analysis. OVX rats,

oestradiol-injected OVX rats, intact females at oestrus and males were compared. The average percentage of splenocytes expressing PRL-R from oestrus females was lower than that of splenocytes from other groups of rats (Table 2). However, there were no significant differences in the percentages of cells expressing PRL-R from animals in the various physiological stages examined.

Moreover, the percentage of each lymphocyte subpopulation expressing PRL-R was also investigated in rats at various hormonal states, CD4+, CD8+ and B lymphocytes of splenocytes from each group were analysed. Although there were no statistically significant differences in the percentage of each lymphocyte subpopulation expressing PRL-R during different hormonal states, the percentage of B lymphocytes expressing surface PRL-R from

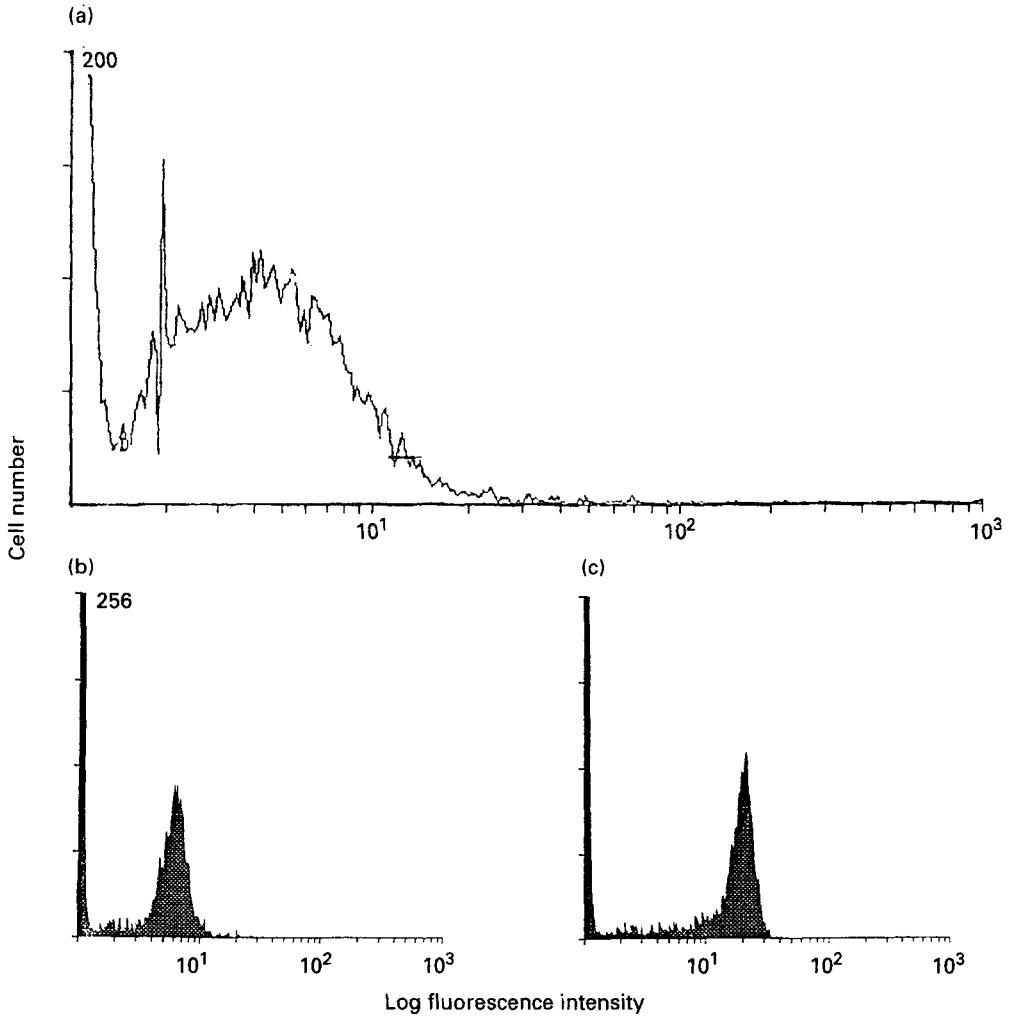

Figure 1. Fluorescent distribution of splenocytes stained with antibodies against PRL-R, CD4, or CD8 and FITC-conjugated secondary antibody. Histograms show the log integrated green fluorescence intensity *vs.* cell size. The average percentages of PRL-R+ (a), CD4+ (b) or CD8+ (c) cells are represented in Table 1.

Table 1. Surface PRL-R expression on subpopulations of splenic lymphocytes

Subpopulations of splenocytes	Percentage of total lymphocytes	Percentage of each subpopulation expressing PRL-R	Relative brightness of PRL-R expression
CD4+ (T_H)	24.3±0.80	9.8±1.4	Low
CD8+ (T_C)	19.5±0.7	5.4±1.2	Low
sIg+ (B)	55.5±0.9	31.5±3.1	High

The percentages of splenocyte subpopulations expressing surface PRL-R was determined by dual-colour flow cytometric analysis. Splenocytes were isolated from rats at different hormonal states and stained with PRL-R antisera and either anti-CD4, anti-CD8, or anti-kappa light chain (specific for T_H, T_C and B lymphocytes, respectively). The PRL-R was detected using PE conjugated anti-rabbit IgG antibody, and the splenocyte subpopulations with FITC conjugated anti-mouse IgG antibody. The relative brightness of PRL-R staining was gauged by the positions of cells in the second quadrant along the yellow fluorescence intensity axis of dual-colour dot plots. Data represent the mean±SEM (n=16).

OVX rats was significantly greater (mean 40.3±5%; n=6) than that of B lymphocytes from rats in oestrus (mean 20.1±3.8%; n=4). In addition, a number of differences among rats from different hormonal backgrounds were observed in the percentages of three lymphocyte subpopulations in the spleen (Table 2). Male rats had a greater percentage of CD4+ lymphocytes (mean 27±2%; n=3) than OVX (mean 23.1±0.3%; n=6) or OVX+E2 rats (mean 21.4±2.3%; n=3); fewer CD8+ cells (mean 16.8±2%; n=3) than oestrus females (mean 22.0±1.5%; n=4) more B lymphocytes (mean 59.6±2.9%; n=3) than either OVX or oestrus females (mean 54.3±0.9%; n=6; and mean 52.5±0.9%; n=4, respectively). Females in oestrus were observed to have a greater percentage of CD4+ lymphocytes (mean 26.5±1.2%; n=4) than OVX rats (mean 23.1±0.3%; n=6) or OVX+E2 rats (mean 21.6±3.9; n=3); fewer B lymphocytes (mean 52.5±0.9%; n=4) than OVX+E2 (57.9±1.4%; n=3) or male rats (mean 59.6±2.9%; n=3).

DISCUSSION

In this study, we investigated how percentages of splenocyte subpopulations and the percentage of each subpopulation expressing PRL-R alter during various hormonal stages in order to determine if a change in these parameters may be responsible for the PRL responsiveness of splenocytes from OVX rats. We found that approximately 24%, 20% and 56% of splenocytes were CD4+, CD8+ and B lymphocytes, respectively. When the percentage of each splenocyte subpopulation from rats at different hormonal states was compared, a few significant alterations have been observed. First, the percentage of CD4+ cells from OVX rats or from OVX+E2 rats were significantly less than that of CD4+ cells from oestrus rats or from male rats (Table 2). Second, the percentage of CD8+ cells from rats at oestrus was statistically greater than that of CD8+ cells from male rats. Finally, the percentage of B-cells from male rats was higher than that of B-cells from rats at oestrus or from OVX rats. These results indicate that there was no significant alteration in the percentage of CD8+ cells and B-cells between rats at oestrus and OVX rats. Based on this finding, we concluded that the PRL responsiveness of splenocytes from OVX rats but not from rats at oestrus is not due to the alteration of the percentage of lymphocyte subsets, suggesting that a more subtle difference must exist between PRL-responsive and PRL-unresponsive splenocytes. Furthermore, it seems that a significant decrease in the percentage of CD4+ cells in OVX rats compared to

rats at oestrus may not be one of the possibilities for PRL-responsiveness of lymphocytes because the percentage of CD4+ cells from OVX+E2 was similar to that of CD4+ cells in OVX rats.

A few studies have examined the distribution of surface PRL-R on lymphocyte subpopulations from rats at different hormonal stages. Therefore, we firstly determined the percentage of each splenocyte subset expressing PRL-R, regardless of *in vivo* hormonal states of the rats.

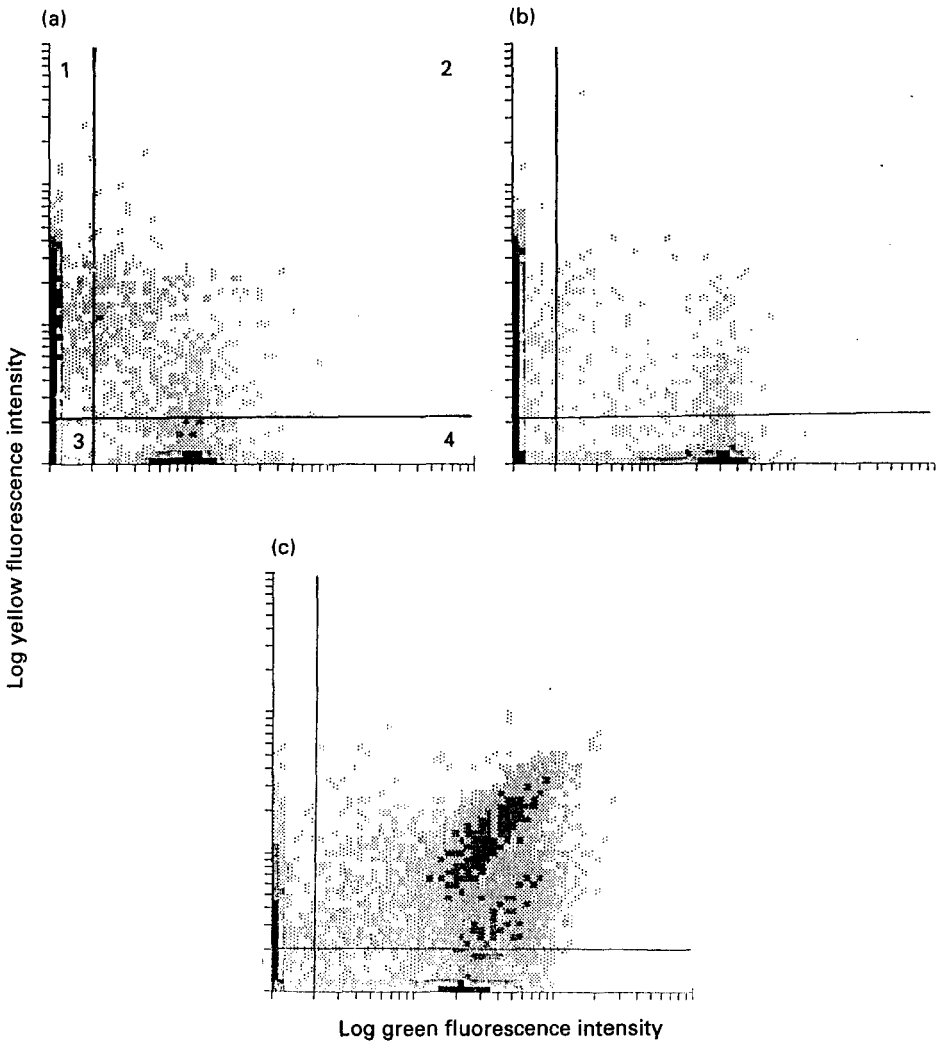

Figure 2. Dual-colour fluorescence dot plot demonstrating the surface membrane expression of PRL-R on CD4+ (a), CD8+ (b) or B- (c) cells. Splenocytes were stained with PRL-R antisera and either anti-CD4, anti-CD8, or anti-kappa light chain. PRL-R positive cells are represented by a log yellow fluorescence intensity distribution. CD4+, CD8+ or kappa light chain+cells are represented by a log green fluorescence intensity distribution. Quadrants 1 and 2 contain cells expressing surface PRL-R; quadrants 2 and 4 contain cells expressing leukocyte surface antigen being considered; quadrant 2 contains a particular phenotype (e.g. CD4+ T-cells) expressing surface PRL-R. Average percentages ($n=16$, mean$\pm$SEM) of cells in the dot plot quadrants are represented in the Table 1.

Table 2. Percentages of splenocytes expressing PRL-R and their distribution among splenic lymphocyte subpopulations in rat at various hormonal stages

Splenocyte phenotype	Hormonal stages			
	OVX	OVX+E2	Oestrus	Male
PRL-R+ (total splenocytes)	25.0±4.7	21.6±3.9	13.6±4.1	20.3±3.0
T_H cells (CD4+)	23.1±0.3	21.4±2.3	26.5±1.2*	27.1±2.0*
T_H cells expressing PRL-R (%)	11.2±3.1	12.7±1.2	6.1±2.0	8.8±1.8
T_C cells (CD8+)	19.9±0.4	18.7±1.4	22.0±1.5†	16.8±2.0
T_C cells expressing PRL-R (%)	5.3±1.1	2.1±1.2	4.5±2.2	4.4±0.9
B-cells (kappa+)	54.3±0.9	57.9±1.4	52.5±0.9‡	59.6±2.9§
B-cells expressing PRL-R (%)	40.3±5.5¶	29.2±6.6	20.1±3.8	30.2±0.9

Splenocytes were isolated from rats in different hormonal stages, OVX rats (n=6), OVX rats administered oestradiol (OVX+E2; n=3), females in oestrus (n=4), and males (n=3). The splenocytes were stained and analysed with dual-colour flow cytometry as in the legend to Table 1. Statistical analysis was done using the Fisher PLSD test, and differences at a confidence level of 95% were considered significant. Data represent the mean±SEM. The significant differences among groups were shown by different letters. *Different from OVX and OVX+E2; †different from male; ‡different from OVX+E2 and male; §different from OVX and oestrus; and ¶different from oestrus.

Our results show that 10% of CD4+, 5% of CD8+ and 30% of B splenocytes express surface PRL-R, and that the majority (85%) of total splenocytes expressing PRL-R are B lymphocytes. Similarly, Gagnerault *et al.* (1993) reported that nearly all B lymphocytes express PRL-R and approximately 95% of B-cells stain brightly for the receptor by using biotinylated anti-PRL-R monoclonal antibody U5. They also found that approximately 50% of CD4+ and CD8+ lymphocytes express PRL-R and most of these cells stain dimly for the receptor. Gala & Shevach (1993) indicated that 5% of CD4+, 20% of CD8+ and 20% to 80% of B lymphocytes express surface PRL-R by using the five monoclonal antibodies to PRL-R, directly conjugated to fluorescein (Okamura *et al.* 1989). Viselli & Mastro (1993) reported a much more even distribution of PRL-R among splenocyte subpopulations using a polyclonal antibody, anti-PRL (Bajpai *et al.* 1991). They found that approximately 25% of CD4+ cells, 25% of CD8+ cells and 20% of B-cells displayed PRL-R. The variability in the percentage of B or T lymphocytes staining positive for PRL-R among these reports is most probably due to different monoclonal or polyclonal antibodies to PRL-R, different species and sensitivity of the techniques used in these studies.

In the present study, the percentage of CD4+ and CD8+ lymphocytes expressing PRL-R did not exhibit a significant change during different hormonal states. Based on the result of Viselli (1992), T-cells but not B lymphocytes proliferated after stimulation with PRL. Therefore, our finding indicates that there is no correlation between responsiveness to PRL as a mitogen for lymphocytes and levels of surface PRL-R expression. This result is in agreement with that of Gala & Shevach (1993) who examined the percentage of lymphocyte subsets from intact and OVX BALB/c mice and found that the percentage of lymphocyte subsets (CD4+, CD8+, B-cells) expressing PRL-R did not markedly change in OVX mice. Unlike their findings, we observed that the percentage of B lymphocytes expressing PRL-R was the greatest in the OVX rats compared to intact females in oestrus (Table 2).

Both the present study and the studies of others (Viselli & Mastro 1993, Gagnerault *et al.* 1993, Gala & Shevach 1993) indicate that B lymphocytes display cell surface PRL-R. Among the lymphocyte subsets expressing surface PRL-R, the percentage of B lymphocytes is higher

than that of CD4+ and CD8+ lymphocytes. However, the role of PRL in regulating the activity of each splenocyte subset is not known yet. When splenocytes are stimulated with PRL and stained for DNA content with propodium iodide, the CD4+ and CD8+ lymphocytes but not B lymphocytes move beyond G_0/G_1 and increase in DNA content (Viselli 1992). After 96 h of stimulation with PRL, the percentages of CD4+ and CD8+ lymphocytes in culture increased significantly (Viselli & Mastro 1991). Gala & Shevach (1993) observed that in ConA stimulated cultures, the T lymphocytes increase their surface membrane expression of PRL-R but B lymphocytes do not. In addition, in ConA stimulated lymphocyte cultures, a molecule with prolactin-like bioactivity is secreted (Montgomery *et al.* 1987). Because ConA is a T-cell mitogen, it was proposed that T-cells secreted the PRL. In a further study, T-cells separated from ConA stimulated cultures were shown to express PRL mRNA. These data give some clue about the prolactin's role in regulation of the T lymphocyte activity but they do not explain why B lymphocytes express PRL-R. Hartmann *et al.* (1989) demonstrated that the antisera to prolactin can abrogate the mitogenic response of lymphocytes to both ConA and the B-cell mitogen, lipopolysaccharide. Therefore, prolactin appears to have a role in regulating B-cell, as well as T-cell, proliferation. However, more experiments need to be carried out to elucidate the role of prolactin in the B lymphocytes.

How responsiveness to PRL is linked to the hormonal status of the rat is still unknown but there are possibilities that need to be examined in future studies. For example, Guillaumot & Cohen (1994) reported that depending on the various physiological stages of the rats, different molecular forms of PRL-R were observed by ^{125}I-oPRL binding studies. Therefore, it could be possible that during different hormonal states, a certain molecular form of PRL-R may appear in the spleen and transduce the mitogenic signal of PRL to splenocytes. In addition, antibody to the long form of PRL-R recognized two different molecular forms (42 kDa and 84 kDa) of PRL-R in the spleen (Güneş & Mastro 1996), and ovariectomy may cause differential expression of these two molecular forms of the receptor. In further studies, ^{125}I-oPRL binding experiments as well as Western blot analysis might distinguish among these possibilities.

Both long and short forms of the PRL-R are expressed in the rat. The long form of PRL-R is capable of transducing signal for induction of milk gene expression when transfected into Chinese hamster ovary cells whereas the short form does not (Ali *et al.* 1992). Therefore, it is likely that these receptors function differently. If this is the case, the circulating steroid hormones could control the expression of both forms of the PRL-R, and responsiveness to PRL as a mitogen would correlate to predominantly expressed form of the receptor. Indeed, ovariectomy resulted in a decrease in mRNA levels of both short and long forms of the PRL-R in rat brain (Sugiyama *et al.* 1994). Because effect of hormones on the expression of forms of PRL-R may be tissue specific (Güneş 1995), ovariectomy could possibly exhibit more different effects on the expression level of long and short forms of PRL-R in spleen than it does in the brain.

Alternatively the level of PRL-R expression may not be correlated to PRL responsiveness of splenocytes at all. Recent studies have indicated an association between each PRL-R isoform and a *src* family protein thyrosine kinase ($p59^{fyn}$) in NB2 cells (Clevenger & Medaglia 1994). Prolactin stimulation resulted in activation of *fyn* and proliferation of NB2 cells. In addition, JAK family tyrosine kinase JAK2 have been shown to be associated with PRL-R (Rui *et al.* 1994). However, the function of these protein thyrosine kinases in signal transduction pathway through PRL-R in order to stimulate lymphocytes to divide is not yet well known. Therefore, it is possible that the key to whether lymphocytes are responsive or unresponsive to prolactin as a mitogen lies within the signal transducing network in the cell.

Perhaps circulating steroid hormones could preside over whether or not the signal to divide is transduced to the nucleus. Elucidation of signal transduction pathway through PRL-R may help us understand how hormones affect signal transduction for lymphocyte proliferation upon stimulation with PRL.

In conclusion, the hormonal status of the animal can influence PRL-R expression. Specifically a greater percentage of B lymphocytes express PRL-R in OVX rats than rats at oestrus. However, no correlation was found between the responsiveness to PRL as a mitogen and the levels of cell surface PRL-R expression by lymphocytes during different hormonal stages. The underlying mechanism for the initial observation that PRL serves as a mitogen for lymphocytes *in vitro* only in rats of certain hormonal backgrounds has yet to be determined. Research to elucidate the signal transduction pathway by PRL-R and how sex steroid hormones modulate the signal transduction by PRL-R may reveal the mechanism. Nevertheless, the finding that PRL-R expression can be modulated by hormonal status gives us clues for future investigations.

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REFERENCES

Ali S, Pelligrini I, Kelly PA. (1991) A prolactin dependent immune cell line (Nb2) expresses a mutant form of prolactin receptor. *J. Biol. Chem.* **266**, 20110.

Ali S, Edery M, Pellegrini I *et al.* The Nb2 form of prolactin receptor is able to activate a milk protein gene promoter. *Mol. Endocrinol.* **1242**, 1248.

Bajpai A, Hooper KP, Ebner KE. (1991) Interactions of antisense peptides with ivine prolactin. *Biochem. Biophys. Res. Commun.* **180**, 1312.

Berczi I, Nagy E, Toledo SM, Matusik RJ, Friesen HG. (1991) Pituitary hormones regulate c-myc and DNA synthesis in lymphoid tissue. *J. Immunol.* **146**, 2201.

Bernton EW, Meltzer MS, Holaday JW. (1988) Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. *Science* **239**, 404.

Boutin Jm, Jolicoer C, Okamura H *et al.* (1988) Cloning and expression of the rat prolactin receptor, a member of the growth hormone/prolactin receptor gene family. *Cell* **53**, 69.

Clevenger CV, Medaglia MV. (1994) The protein tyrosine kinase p59 fyn is associated with prolactin receptor and is activated by prolactin stimulation of T-lymphocytes. *Mol. Endocrinol.* **8**, 674.

Edery M, Jolicoer C, Levi Meyrueis C *et al.* (1989) Identification and sequence analysis of a second form of prolactin receptor by molecular cloning of cDNA from rabbit mammary gland. *Proc. Natl. Acad. Sci. USA* **86**, 2112.

Gagnerault M, Touraine P, Savino W, Kelly PA, Dardenne M. (1993) Expression of prolactin receptors in murine lymphoid cells in normal and autoimmune situations. *J. Immunol.* **150**, 5673.

Gala RR, Shevach EM. (1993) Identification by analytical flow cytometry of prolactin receptors on immunocompetent cell populations in the mouse. *Endocrinol.* **133**, 1617.

Guillaumot P, Cohen H. (1994) Heterogeneity of the prolactin receptor in the rat mammary gland and liver during various physiological states. *J. Endocrinol.* **141**, 271.

Güneş H. (1995) The expression of forms of prolactin receptor in splenic and thymic tissues of rats at different developmental and physiological stages. Submitted in partial fulfillment of the requirements for the degree of PhD at The Pennsylvania State University.

Güneş H, Mastro AM. (1996) Prolactin receptor gene expression in rat splenocytes and thymocytes from birth to adulthood. *Mol. Cell. Endocrinol.* **117**, 41.

Hartmann DP, Holaday JW, Bernton EW (1989) Inhibition of lymphocyte proliferation by antibodies to prolactin. *FASEB* **3**, 2194.

Kelly PA, Ali S, Rozakis M *et al.* (1993) The growth hormone/prolactin receptor family. *Rec. Progress Hor. Res.* **48**, 123.

MONTGOMERY DW, ZULOSKI CF, SHAH GN, BUCKLEY AR, PACHOLCZYK T, RUSSELL DH. (1987) Concanavalin-A stimulated murine splenocytes produce a factor with prolactin-like bioactivity and immunoreactivity. *Biochem. Biophys. Res. Commun.* **145**, 692.

MOORE RC, OKA T. (1993) Cloning and the sequencing of the cDNA encoding the murine mammary gland long-form prolactin receptor. *Gene* **134**, 263.

MUKHERJEE P, MASTRO AM, HYMER WC. (1990) Prolactin induction of interleukin-2 receptors on rat splenic lymphocytes. *Endocrinol.* **126**, 88.

NAGY E, BERCZI I. (1978) Immunodeficiency in hypophysectomized rats. *Acta. Endocrinol.* **89**, 530.

NIALL HD, HOGAN ML, SAYER R, ROSENBLUM IY, GREENWOOD IC. (1971) Sequences of pituitary and placental lactogenic and growth hormones. Evolution from a promordial peptide by gene duplication. *Proc. Natl. Acad. Sci. USA* **68**, 866.

NICOLL CS, BERN HA. (1972) On the actions of prolactin among vertebrates: is there a common denominator? In: Wolstenholme GEW, Knight J, eds. *Lactogenic Hormones*. London: Churchill Livingstone, 299.

OKAMURA H, ZACHWIEJA J, RAGUET S, KELLY PA. (1989) Characterization and applications of monoclonal antibodies to the prolactin receptor. *Endocrinol.* **124**, 2499.

RUI H, KIRKEN RA, FARRAR WL. (1994) Activation of receptor-associated tyrosine kinase JAK2 by prolactin. *J. Biol. Chem.* **269**, 5364.

SHIROTA M, BANVILLE D, ALI S *et al.* (1990) Expression of two forms of prolactin receptor in rat ovary and liver. *Mol. Endocrinol.* **4**, 1136.

STEVENS AM, YU-LEE LY. (1992) The transcription factor interferon regulatory factor-1 is expressed during both early G1 and G1/S transition in the prolactin-induced lymphocyte cell cycle. *Mol. Endocrinol.* **6**, 2236.

SUGIYAMA T, MINOURA H, KAWABE N, TANAKA M, NAKASHIMA K. (1994) Preferential expression of long form of prolactin receptor mRNA in the rat brain during the estrus cycle, pregnancy and lactation: hormones involved in its gene expression. *J. Endocrinol.* **141**, 325.

TANAKA T, SHIU RPC, GOUT PW, BEER CT, NOBLE RL, FRIESEN HG. (1980) A new sensitive and specific bioassay for lactogenic hormones: measurement of prolactin and growth hormone in human serum. *J. Clin. Endocrinol. Metabl.* **51**, 1058.

VISELLI SM, MASTRO AM. (1991) Prolactin-induced mitogenesis of lymphocytes from ovariectomized rats. *Endocrinol.* **129**, 983.

VISELLI SM. (1992) Characterization of prolactin-induced lymphocyte proliferation. Submitted in partial fulfillment of requirements for the degree of PhD at Pennsylvania State University.

VISELLI SM, MASTRO AM. (1993) Prolactin receptors are found on heterogenous subpopulations of rat splenocytes. *Endocrinol.* **132**, 571.

YU-LEE LY. (1990) Prolactin stimulates transcription of growth related genes in Nb2 T lymphoma cells. *Mol. Cell. Endocrinol.* **68**, 21.