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Effects of Prolactin on Cloned Human T-Lymphocytes

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To evaluate the possible role of prolactin (PRL) in T-lymphocytes, we monitored gene induction in one cytotoxic T-lymphocyte (CTL) clone derived from a patient with hemochromatosis and in several T-helper clones generated from a normal donor and a patient with multiple sclerosis. The CTL clone expressed conventional PRL receptor (PRLR), and PRL induced the expression of suppressor of cytokine signaling-3 (SOCS-3) and increased the expression of SOCS-2 and cytokine-inducible src homology-2–containing protein (CIS, another member of the SOCS family). As is the case in granulocytes, expression of a conventional receptor for PRL could not be shown by polymerase chain reaction analysis on three helper clones. In addition, as in granulocytes, PRL modulated the expression of genes such as the interferon-regulatory factor-1, inducible nitric oxide synthase, CIS, and SOCS-2. These effects were also elicited with ovine PRL and could be prevented by anti-PRL antibodies. Thus, the use of clones allowed the detection of direct effects of PRL on T-cells, even when these have few or no detectable PRLR, confirming that human T-lymphocytes are targets for PRL.

Key Words: Prolactin; T-cell clone; signal transduction; suppressor of cytokine signaling; multiple sclerosis.

Introduction

Biochemical and biologic effects of the hormone prolactin (PRL) have been described in the immune system of rodents and humans *(1–7)*. Yet, PRL is clearly dispensable for the murine developing immune system *(6–9)*. Receptors for PRL are found on a large proportion of immune and hemopoietic cell types. B-lymphocytes and monocytes express more receptors per cell than resting T-cells *(1,10)*. The PRL receptor (PRLR) is a member of the cytokine-hemopoietin receptor family and signals mainly through JAK2-Stat5 and mitogen-activated protein kinases *(1,2,10–14)*.

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We have recently reported that PRL stimulates the expression of genes important for leukocyte function in mononuclear and polymorphonuclear cells from normal donors *(2)*. To better identify the primary target cell of PRL, the use of very pure populations, preferably cloned cell lines, is highly desirable. We have now extended this analysis to non-transformed human T-cell clones.

Results

PRLR was detected by polymerase chain reaction (PCR) on the cytotoxic T-lymphocyte (CTL) clone but on none of the helper clones (not shown). Gene expression was documented by PCR. In the CTL clone CTL 435/A9 MAGE1-A3, PRL induced the expression of suppressor of cytokine signaling-3 (SOCS-3) and increased the expression of cytokine-inducible src homology-2–containing protein (CIS) and (SOCS-2 and, to a minor extent, of interferon regulatory factor-1 (IRF-1) and inducible nitric oxide synthase (iNOS) (Fig. 1). In the helper clones, PRL stimulated to various extents the expression of IRF-1, CIS-1, SOCS-2, and iNOS in most clones studied. Figure 1 shows one such clone, 10D9. Another helper clone, 4B11, however, did not respond in this way to PRL; actually several genes were down-regulated on addition of PRL. Expression of SOCS-3 was never found in the helper clones. Similar results were obtained with rec hPRL from two sources (own preparation and from R&D) and with ovine PRL (oPRL), although quantitative and even qualitative differences were observed. Furthermore, the time course varied. Smaller or no effects were seen when PRL was preincubated with rabbit anti-PRL serum (not shown).

Discussion

The use of clones allowed the detection of direct effects of PRL on T-cells, even when these had few or no detectable PRLR.

In a previous study, we detected the expression of PRLR mRNA in peripheral blood mononuclear cells (PBMCs) but not in granulocytes *(2)*. Fu et al. *(15)* however, reported the presence of functional PRLR on human neutrophils, and PRL has clearcut effects in rat neutrophils *(16)*. So far, only one gene coding for a PRLR has been identified. We have used several sets of primers that would allow the detection

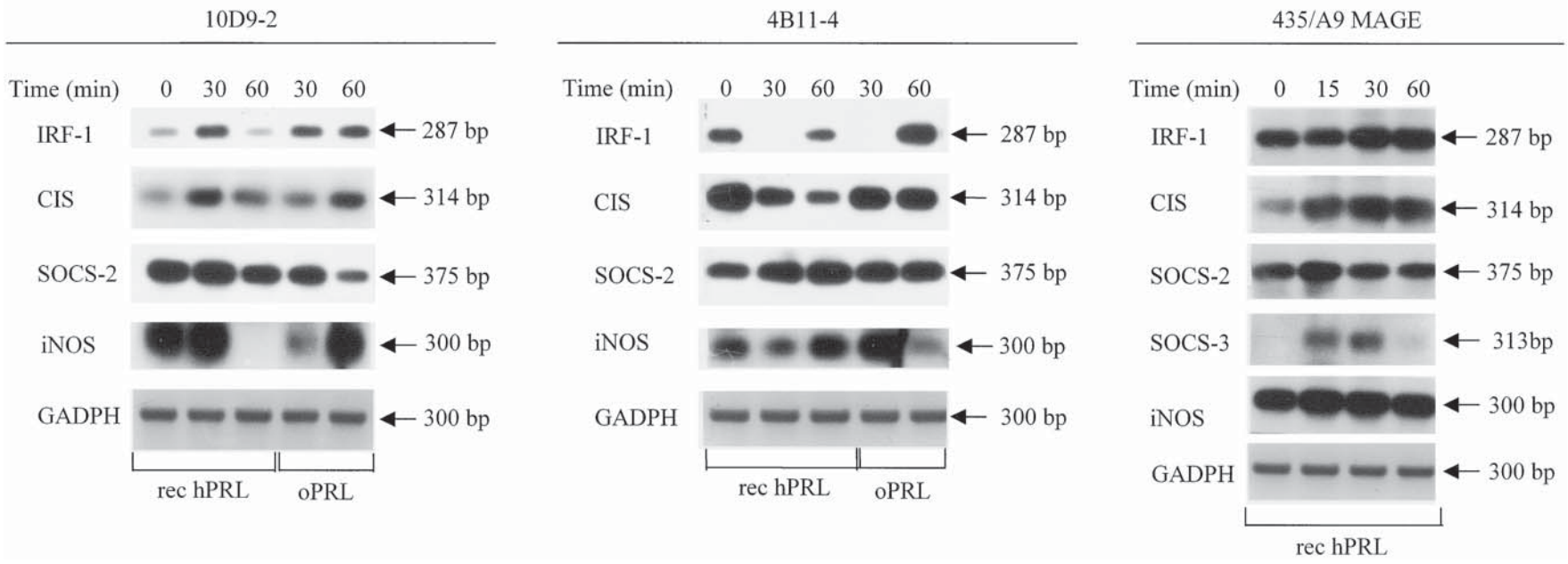

Fig. 1. Expression of IRF-1, iNOS, and SOCS mRNAs in T-cell clones. Cells were treated with rec hPRL for 15, 30, or 60 min. RNA was prepared as described. After reverse transcriptase PCR amplification, agarose gel electrophoresis, and transfer, IRF-1, iNOS, and SOCS PCR products were analyzed by Southern blotting. The position of the expected PCR products is marked. Data are representative of three independent experiments. Clone10D9 and clone 4B11 were treated with 100 ng of PRL/mL, and cells from CTL 435/A9 MAGE1-A3 were treated with 10 ng of PRL/mL from R&D.

of most but not all of the recently described variants of the PRLR. In particular, the ΔS variant of the PRLR would have escaped detection and could thus be expressed in the helper clones *(12–14)*. The existence of a new PRLR, encoded by a different gene, cannot be excluded. Evidence has been provided in favor of a receptor (which, however, has not been cloned) for a 16-kDa proteolytic fragment of PRL *(17)*, and we have shown that 23-kDa hPRL, incubated for 30 min in the presence of normal human leukocytes, is degraded, yielding, among other products, molecules of about 16 kDa (unpublished results).

The response to PRL of the CTL clone studied here shares characteristics with that of normal PBMCs: expression of a bona fide PRLR mRNA and stimulation of SOCS-3 expression by PRL *(2)*. In addition, PRL upregulates the expression of CIS in the CTL clone.

Surprisingly, the results obtained with several T-helper clones were in most cases similar to those recently reported with granulocytes: no detectable PRLR mRNA but nevertheless modulation of IRF-1, CIS-1, SOCS-2, and iNOS expression by PRL, with undetectable levels of SOCS-3 mRNA at any time point *(2)*.

There is good evidence that PRL has effects on murine T-cells but the evidence is less compelling in human T-cells *(1,6,7)*. Our results suggest that human T-lymphocytes are indeed targets for PRL, even when the expression of PRLR is low or undetectable. The different results obtained with ovine and human PRL are somewhat puzzling. Several variants of the human PRLR have recently been described, and it is possible that PRL of different sources act preferentially on one of these *(12,13)*.

Gene expression in response to PRL has been studied in great detail in the rat Nb2 T lymphoma cells *(18)*. In normal rat leukocytes, PRL stimulates the expression of IRF-1 and iNOS *(16,19)*. In human PBMCs, an increased expression of SOCS3 and iNOS was induced by PRL. In granulocytes, CIS and iNOS were induced by PRL and the expression of SOCS-2 and IRF-1 was increased. In the present study, PRL again modulated the expression of SOCS genes. Several SOCS factors limit cytokine signal transduction *(20)*. SOCS factors induced by one cytokine (e.g., PRL) can also limit signal transduction by other cytokines, and this points to a possible role of PRL in the fine tuning of lymphocyte activity *(21,22)*.

It has been proposed, on the basis of animal and clinical data, that PRL favors the breakdown of tolerance and plays an aggravating role in some autoimmune diseases such as systemic lupus erythematosus *(1,23)*. Hyperprolactinemia is present in a subgroup of multiple sclerosis (MS) patients with optic nerve involvement, and small cohorts of MS patients have been treated with the PRL-lowering drug bromocriptine, without clear benefit *(24,25)*. Our data support the claim that PRL can have immunomodulatory activity but do not indicate whether these effects would be beneficial or harmful in the case of autoimmune diseases such as MS. Most effects of PRL on the immune system reported so far were stimulatory *(1–7)*. In that case, effects of PRL on the development or progression of MS would rather be detrimental. It will be interesting to compare the properties of clones obtained from MS vs control donors. So far, the limited number of clones investigated does not allow meaningful comparisons.

Table 1
T-Cell Clones

Donor[a]	Clone	Antigen reactivity	Phenotype	Cytokine profile
P129	10D9-2	MBP	$TCR\alpha\beta^+CD4^+$	Th1
P129	4B11-4	TT	$TCR\alpha\beta^+CD4^+$	Th1
P129	5F11-2	MBP	$TCR\alpha\beta^+CD4^+$	Th1
MED	1E8-25	MBP	$TCR\alpha\beta^+CD4^+$	Th1
HEM	435/A9	MAGE1	CTL[b]	TNF[c]

[a]P129 is a normal donor, MED is an MS patient, and HEM is a patient with hemochromatosis.
[b]Cytotoxic in a ^{51}Cr release assay against autologous EBV-B cells infected with vaccinia-MAGE-A1 *(16)*.
[c]Produced tumor necrosis factor (TNF) on stimulation by HeLa cells transfected with MAGE-A1 and HLA-A3 *(16)*.

Stimulatory effects of PRL on natural killer (NK) activity, on the generation of lymphokine-activated killer cells, and on the susceptibility of target cells have been documented *(3,26)*. The administration of PRL in vivo must be avoided when PRL is a growth factor for the tumor cells, as is the case for some mammary carcinomas, but the use of PRL for the activation of cytotoxic cells in vitro is being considered *(27,28)*.

Materials and Methods

Reagents

The following cDNA probes were used: hCIS from S. Chrétien (Paris, France), hSOCS-2 and -3 from A. Yoshimura (Kurume, Japan). A plasmid containing the coding sequence of hPRL (pT7L) was obtained from J. Martial (Liège, Belgium) and expressed in *Escherichia coli* BL21. Recombinant (rec) hPRL was purified as described *(29)*. Some experiments were also performed with rec hPRL obtained from R&D (Minneapolis, MN), the latter with low endotoxin content, and with oPRL obtained from Dr. A. F. Parlow, NHPP, Harbor-UCLA Medical Centre (Torrance, CA). All PRL preparations used contained endotoxin, but the observed effects did not correlate with endotoxin levels. The bioactivity of PRL preparations was checked in the Nb2 bioassay *(30)*. Antisera to oPRL and hPRL were provided by A. F. Parlow.

Cells and Treatments

The T-cell clones used are listed in Table 1. Myelin basic protein (MBP)–and tetanus toxoid (TT)–reactive T-cell lines

and clones were isolated as described *(31)*. Briefly, PBMCs were plated at 10^5 or 2×10^5 cells/well in the presence of 40 μg/mL of human MBP or at 4×10^4 cells/well in the presence of 20 Lf/mL of TT. Seven days later, cultures were restimulated with autologous irradiated (60 Gy) PBMCs (10^5 cells/well), used as antigen-presenting cells (APCs), in the presence of MBP or TT, followed by the addition of rat interleukin-2 (5 U/mL) after 48 h. On d 15, cultures were tested for specific responses in a proliferation assay (^{3}H-thymidine incorporation). A T-cell line was considered to be antigen reactive when the counts per minute in the presence of antigen exceeded the control counts per minute (without antigen) at least threefold. MBP- and TT-specific T-cell lines were cloned at single-cell densities in the presence of phytohemagglutinin (PHA) and allogeneic accessory cells *(31)*. MBP- and TT-specific T-cell clones were expanded by alternate restimulation with either irradiated allogeneic PBMCs and PHA or with irradiated autologous PBMCs and MBP or TT. Cells were used 1 wk after final restimulation with irradiated antigen-pulsed APCs. At this point, the T-cells are still in an activated state but other cell types (e.g., monocytes) are no longer present in the cultures (these cells were irradiated). The cells used for the PRL experiment are therefore pure T-cell clones (as tested by flow cytometry).

Clone CTL 435/A9 has been described previously *(32)*. Briefly, monocyte-derived dendritic cells infected with a recombinant canarypoxvirus containing the entire MAGE-A1 gene were used to stimulate $CD8^+$ peripheral blood T-lymphocytes from an HLA-A3 patient with hemochromatosis. After 3 wk of culture, responder cell microcultures that specifically lysed autologous cells expressing MAGE-A1 were cloned using autologous stimulator cells transduced with a retrovirus coding for MAGE-A1. CTL clones were tested for lysis on autologous EBV-B cells infected with vaccinia-MAGE-A1. Once established, one of the positive clones, CTL 435/A9, was cultured (6×10^5 cells/well in 24-well plates in the presence of 1.2×10^6 irradiated allogeneic EBV-B cells [LG2-EBV] as feeder cells and 1×10^5 HLA-A3 tumor cells previously incubated for 1 h at 37°C with 1 μg/mL of peptide and washed) *(32)*.

T-cells were transferred to fresh serum-free RPMI medium (since fetal calf serum has lactogenic activity) the day before the experiment (i.e., 7–10 d after the last stimulation). Stimulator and feeder cells were dead by the time of the experiment and their debris was removed through a washing step. At time 0, PRL (or phosphate-buffered saline vehicle only) was added to the cultures to a final concentration of 100 ng/mL, without change of medium. All experiments were done on three separate occasions.

Polymerase Chain Reaction

The methods used for RNA preparation, cDNA synthesis, and PCR and the sequence of the PCR primers used have been reported previously *(2)*. PCR products were resolved by agarose gel electrophoresis, stained with ethidium bromide, and transferred overnight onto Hybond-N nylon membranes (Amersham-Pharmacia) for Southern analysis, as reported previously *(2)*. Criteria for specificity of the PCR reaction included in all cases length of the product, Southern hybridization, and, in the case of SOCS-2, sequencing of the PCR product.

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