Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6460–6464, August 1990
Physiology/Pharmacology

Regulation of interleukin 2-driven T-lymphocyte proliferation by prolactin

(cell cycle/prolactin receptor/flow cytometry/nucleus)

CHARLES V. CLEVENGER*†, DIANE H. RUSSELL‡§, PIERETTE M. APPASAMY*, AND MICHAEL B. PRYSTOWSKY*

*Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104; and †Department of Pharmacology and Therapeutics, University of Southern Florida, Tampa, FL 33612

Communicated by Robert E. Forster II, May 29, 1990

ABSTRACT **The requirement for prolactin in interleukin 2-driven T-cell proliferation was evaluated. Addition of an anti-prolactin antiserum resulted in the specific inhibition of T-cell proliferation in a time- and dose-dependent manner. Synthesis of prolactin and its mRNA, however, did not occur during interleukin 2 stimulation. Instead, previously internalized prolactin, presumably from fetal bovine serum, appears to serve as the source of prolactin under serum-free conditions. A 7-fold increase in a prolactin receptor occurred as a function of cell cycle progression; accumulation of a 1.6-kilobase prolactin receptor mRNA increased ≈2-fold. Interleukin 2 stimulation induced the translocation of prolactin into the nucleus and prolactin receptor to the nuclear periphery. These data indicate that extracellular prolactin is requisite for T-cell proliferation and suggest that the effects of prolactin are exerted in the nucleus.**

The mechanisms by which interleukin 2 (IL-2) induces cell cycle progression are poorly understood (1). It has been suggested that additional hormonal/autocrine peptides, such as transferrin (2, 3), may act as cell cycle competency factors in concert with the interleukins. Therefore, it is reasonable to assume that other growth-related hormones may participate in IL-2-mediated cell cycle progression. One putative candidate is the neurohormone prolactin (PRL). A growing body of evidence suggests an immunoregulatory role for this hormone. *In vivo* animal studies have induced lymphoid hyperplasia through the injection of PRL (4). Conversely, a reduction of blood PRL levels by bromocryptine or hypophysectomy markedly diminished mouse T-cell responsiveness and function (5, 6). Increased blood PRL levels have been observed in human cardiac allograft recipients undergoing acute transplant rejection (7). At the cellular level, the presence of PRL receptors (PRLr) on the surface of human lymphocytes has been demonstrated (8). Data also suggest that a PRL-like molecule may be secreted by lymphocytes, as such an activity has been identified in the media of a lymphoblastoid cell line (9) and concanavalin A (Con A)-stimulated splenocytes (10). The requirement for PRL in lymphokine- and lectin-driven lymphocyte growth has been revealed (11). Taken together, these data indicate that PRL may act as an important lymphocyte growth factor.

METHODS

Cell Culture. The murine T-helper lymphocyte clone L2 was maintained as described (12, 13). For expansion, 10^6 L2 cells were cocultured with 40×10^6 irradiated allogenic murine CBA splenocytes and 400 Cetus units of highly purified recombinant human IL-2 (Cetus) in 10 ml of culture medium (13) with 10% fetal bovine serum. The L2 cells were harvested (14) and placed in culture medium without fetal bovine serum supplemented with 1% CR-ITS+ (Collaborative Research), modified after the method of Mendelsohn *et al.* (15). After overnight culture the resting L2 cells were then utilized; cell proliferation was reinitiated in specific subcultures by the addition of 100 Cetus units of IL-2 per ml of chemically defined medium containing 10^6 L2 cells per ml.

Immunofluorescence and DNA Content Analysis. Sources of anti-PRL antiserum were Frank Talamontes (University of California, Santa Cruz), Arnel Products (New York), and the National Institute of Diabetes, Digestive, and Kidney Diseases. Rabbit anti-rat PRL was also obtained by a standard rabbit immunization protocol initially utilizing purified rat PRL with subsequent hyperimmunization with purified bovine PRL to obtain anti-bovine PRL. For the growth-inhibition studies, all anti-PRL antisera gave comparable results. The anti-mouse antiserum used for immunoblot analysis has been extensively characterized and does not cross-react appreciably by radioimmunoassay with growth hormone and placental lactogens I and II (Frank Talamontes, personal communication). Mouse anti-human PRLr monoclonal antibody (16) was a gift of John Porter (University of Texas). Anti-rat PRL antiserum was further purified using a rat PRL affinity column. Cells were stained with indirect immunofluorescence (IF) and/or propidium iodide as described (17, 18).

Immunoprecipitation and Immunoblot Analysis. [^{35}S]Methionine incorporation and immunoprecipitation were performed and analyzed as described (19). Immunoblot analysis of cellular lysates and media was performed as previously (18), using an avidin–biotin complex immunoperoxidase kit (Vector Laboratories).

Northern Blot Analysis. RNA from L2 cells was prepared for Northern blot analysis (12). Mouse and rat PRL cDNAs were gifts of Daniel Linzer (Northwestern University) and Richard Maurer (University of Iowa), respectively. PRLr cDNA was a gift of Paul Kelly (McGill University). Granulocyte/macrophage-colony-stimulating factor (GM-CSF) cDNA was a gift of Verner Paetkau (University of Alberta). Probe inserts were labeled with [α-^{32}P]dCTP by the random primer method (20). Radiolabeled mouse PRL cDNA readily identified the 0.9-kilobase PRL RNA present in total RNA isolated from mouse pituitary (data not shown).

RESULTS

Anti-PRL Antiserum Inhibits L2 Cell Proliferation in a Specific and Dose-Dependent Manner. The role of PRL in T-cell proliferation was examined by the addition of various

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL-2, interleukin 2; IF, indirect immunofluorescence; PRL, prolactin; PRLr, prolactin receptor; GM-CSF, granulocyte/macrophage-colony-stimulating factor.

†To whom reprint requests should be addressed.
§Deceased, September 16, 1989.

concentrations of anti-PRL or control (anti-vimentin) antiserum into the chemically defined medium of an IL-2-stimulated cloned murine T-lymphocyte line, L2. L2 cells were stimulated with IL-2 in the absence of exogenous PRL and cell cycle status was assessed at 30 hr by flow cytometric analysis of DNA content. Affinity-purified anti-PRL antiserum inhibited IL-2-induced T-cell proliferation in a dose-dependent fashion (Fig. 1, open circles). The addition of exogenous rat PRL reversed the antiproliferative effect of the affinity-purified anti-PRL antiserum (Fig. 1, filled circle). Neither preimmune serum (data not shown) nor irrelevant, control anti-vimentin antiserum demonstrated the inhibitory effect.

Inhibition of T-Lymphocyte Proliferation by Anti-PRL Antiserum Is Temporally Dependent. The temporal requirement for a lymphocyte-secreted PRL was examined by adding anti-PRL antiserum at various times after stimulation with IL-2 and measuring DNA content at 30 hr. Anti-PRL antiserum inhibited IL-2-driven growth when added 12 hr after culture initiation (Fig. 2, open circles) but did not inhibit proliferation when added 18 hr or more after culture initiation. Addition of exogenous PRL into resting L2 cell culture in the absence of IL-2 (Fig. 2, filled circle) did not stimulate proliferation, indicating that a lymphocyte-secreted PRL is temporally necessary, but not sufficient, for proliferation.

Lymphocyte-Secreted PRL Is Not Biosynthesized by Resting or IL-2-Stimulated L2 Cells. Since it appeared that immunoreactive PRL was secreted by L2 cells, immunoblot analysis was performed on both cell lysates and concentrated cell culture media. From resting and IL-2-stimulated cultures, equal quantities of total protein from cell lysates and equal volumes of media concentrates were analyzed. A single band at ≈24 kDa was identified in both lysates and media concentrates (Fig. 3*A*), which comigrated with purified rat PRL and was not seen in the blot labeled with preimmune serum. Densitometric analysis of this blot revealed comparable levels of accumulated PRL in resting- and stimulated-cell lysates and a 2.5-fold increase in the extracellular PRL concentration as a function of IL-2 stimulation.

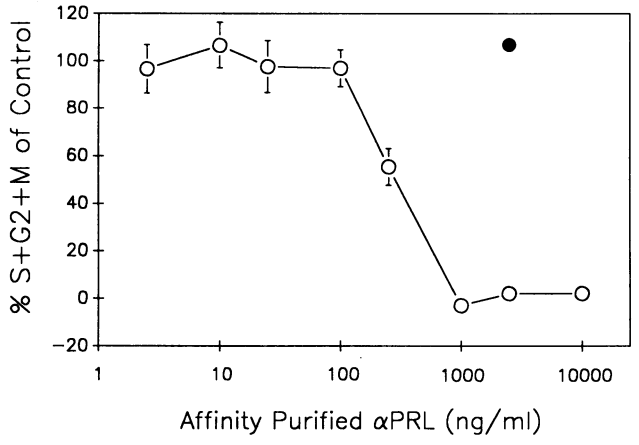

FIG. 1. Growth-inhibitory effect of anti-PRL antiserum (αPRL) on L2 cells is specific and dose-dependent. Various dilutions of affinity-purified anti-PRL or control (anti-vimentin) antiserum were added at culture initiation with IL-2, and at 30 hr DNA content was analyzed. Each data point (open circles) represents the following percentage obtained from the means of three separate cell cultures: 100 × [(fraction of cycling cells in anti-PRL antiserum-treated, IL-2-stimulated L2 cell culture) − (fraction of cycling cells in resting L2 cell culture)]/[(fraction of cycling cells in control antiserum-treated, IL-2-stimulated L2 cell culture) − (fraction of cycling cells in resting L2 cell culture)]. The filled circle represents cultures to which both anti-PRL antiserum and 10 μg of exogenous PRL per ml of medium were added. Where not shown, the 95% confidence limits fall within the symbols.

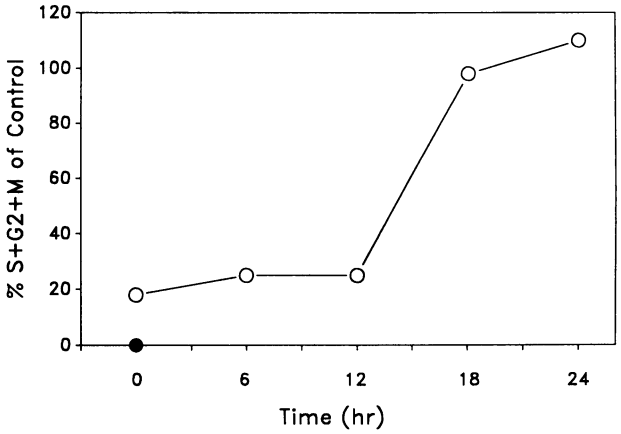

FIG. 2. Temporal dependence of growth inhibition by anti-PRL antiserum. Open circles, IL-2-stimulated cells to which a 1:7000 dilution of either anti-PRL or control antiserum was added at various intervals after culture initiation with IL-2; filled circle, resting L2 cells that received 10 μg of rat PRL per ml of medium at culture initiation without IL-2. Each point represents the mean of three separate cultures; percentages were calculated as in Fig. 1. The 95% confidence limits fall within the symbols. Mean absolute % (S + G2 + M) values; resting L2 cultures, 4.0 ± 0.0%; anti-vimentin antiserum-treated, IL-2-stimulated cultures, 35.0 ± 0.3% (mean ± SEM).

To determine whether L2 cells synthesized the immunoreactive PRL species, biosynthetic labeling with [^{35}S]methionine followed by immunoprecipitation using anti-PRL (or control) antiserum was performed. Attempts to demonstrate an ^{35}S-labeled lymphocyte PRL were not successful. Immunoreactive PRL was revealed by immunoblot analysis of the immunoprecipitates with anti-PRL antiserum (Fig. 3*B*, panels a and b). Subsequent autoradiography of the electrophoretically transferred proteins (Fig. 3*B*, panels c and d) showed that the antigenically detectable PRL was not radiolabeled.

Uptake of exogenous bovine PRL from the fetal bovine serum-containing culture medium used during maintenance culture could account for the intracellular pool of PRL within L2 cells. To examine this possibility, L2 cell lysates and purified rat, mouse, and bovine PRL were blotted onto nylon membranes. These blots were then labeled either with crude anti-PRL antiserum or with anti-PRL antiserum that had been affinity-purified using Sephadex-conjugated bovine PRL. Crude anti-PRL antiserum labeled all species of PRL, whereas the affinity-purified antiserum recognized only bovine PRL and the PRL within L2 cells (Fig. 3*C*).

PRLr mRNA, But Not PRL mRNA, Is Expressed in Resting, IL-2-, and Con A-Stimulated L2 Cells. Steady-state levels of PRL and PRLr mRNA were determined in resting, IL-2-, and Con A-stimulated L2 cells (Fig. 4). Despite repeated attempts no PRL RNA was found in resting or stimulated L2 cells raised in chemically defined or serum-containing medium. The lack of detectable PRL mRNA further confirms the above biosynthetic-labeling data indicating that PRL is not synthesized by L2 cells during IL-2 stimulation in chemically defined medium. Resting and stimulated cells, however, did express a 1.6-kilobase PRLr mRNA. The level of PRLr mRNA increased ≈2-fold after stimulation with Con A or IL-2 for 30 hr. In comparison, GM-CSF mRNA was induced ≈30-fold in cells stimulated with Con A for 8 or 30 hr.

IL-2 Induces the Accumulation of PRLr, But Not PRL, as a Function of Cell Cycle Progression. Only a modest increase (25% on a per-cell basis) in the intracellular PRL content of cycling IL-2-stimulated cells was observed as compared to resting cells, as measured by flow cytometry (Fig. 5*A*). These relative levels of intracellular PRL in L2 cells measured by flow cytometry are in keeping with the above immunoblot

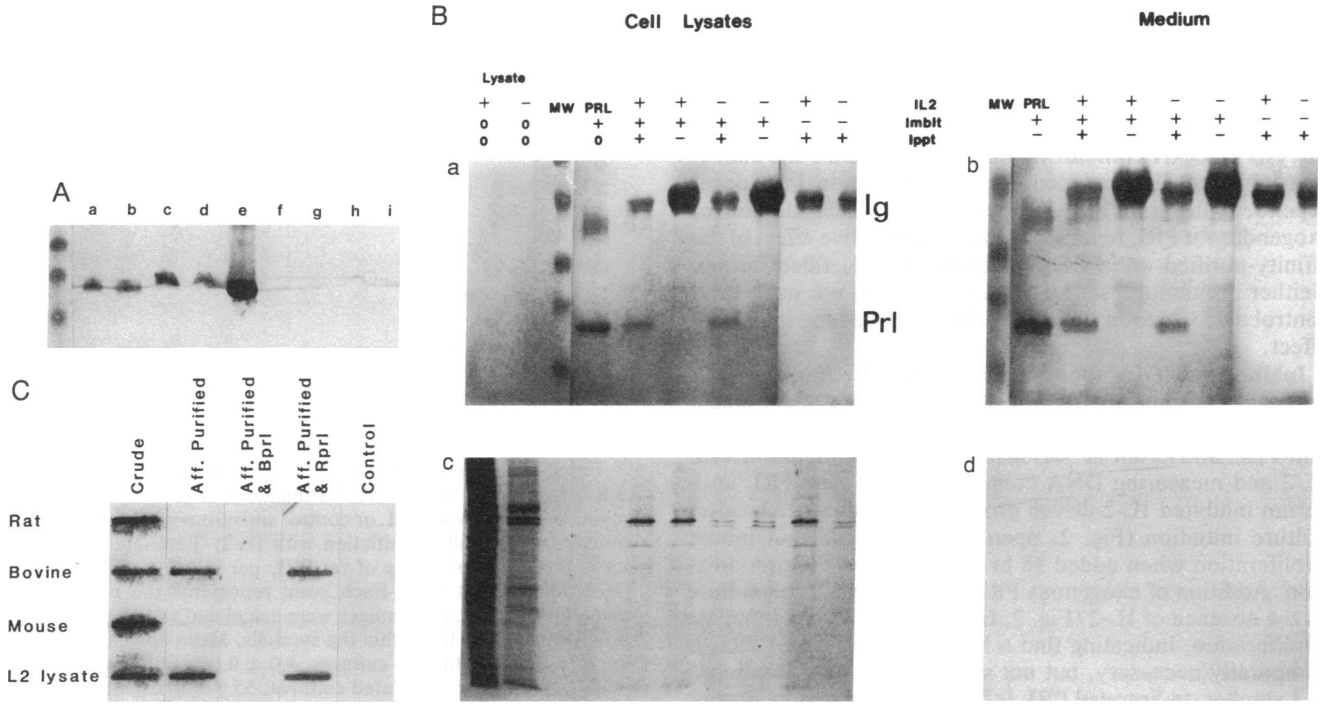

FIG. 3. (*A*) Immunoreactive PRL in resting and IL-2-stimulated cells. Values represent the densitometric quantitation of each band (seen on the anti-PRL antiserum-stained blot) reported in arbitrary units (au). Lanes A–F, anti-PRL antiserum; lanes G–I, preimmune serum. Lanes A and G, IL-2-stimulated cell lysate (8.7 au); lane B, resting cell lysate (8.2 au); lanes C and H, IL-2-stimulated culture medium concentrate (14.3 au); lane D, resting culture medium concentrate (5.7 au); lanes E and I, 1 μg of purified rat PRL (45.6 au); lane F, blank well. Both cell lysates were loaded with equal quantities of total cell protein, 10 μg per well. Equal volumes of cell media were loaded (20 μl per well). Bands at extreme left are molecular size markers of (top to bottom) 39, 27, and 17 kDa. (*B*) Immunoreactive PRL in resting and IL-2-stimulated cells is not biosynthetically labeled. Resting and IL-2-stimulated L2 cells were biosynthetically labeled with [^{35}S]methionine. Cell lysates and nonconcentrated medium were immunoprecipitated with anti-PRL antiserum or normal rabbit serum; the immunoprecipitates were subjected to SDS/PAGE and electrophoretically transferred to nitrocellulose. The blots were stained with anti-PRL or control antiserum (panels a and b) prior to autoradiography (panels c and d). Lysate + and − represent total cellular lysates from IL-2-stimulated and unstimulated cultures, respectively, stained with amido black. Molecular size standards (lane M, top to bottom), 66, 39, 27, and 17 kDa. Labels: IL-2, IL-2-stimulated (+) or unstimulated (−) cells; PRL, purified rat PRL positive control; Ippt, immunoprecipitation with either anti-PRL antiserum (+) or control antiserum (−); Imblt, immunoblotting with either anti-PRL antiserum (+) or control serum (−). The band at 24 kDa represents PRL, while the band migrating at 55–60 kDa represents immunoglobulin heavy chain from the immunoprecipitation. (*C*) Immunoreactive PRL in L2 cells shares immunologic identity with bovine PRL. For immunoblot analysis, protein suspensions were blotted directly on a nylon membrane with a slot blot apparatus under vacuum. One microgram of purified rat, mouse, and bovine PRL and 50 μl of an L2 cell lysate (10^7 cells per ml) were used in this study. Crude and affinity-purified anti-rat PRL was obtained as described in *Methods*. To demonstrate specificity, affinity-purified antiserum was incubated with purified bovine (Bprl) or rat (Rprl) PRL prior to use. Preimmune serum was used as a control. After affinity purification with Sephadex-conjugated bovine PRL, the anti-rat PRL antiserum recognized only bovine PRL and L2 cell lysate.

data, and demonstrated that a cell cycle-associated accumulation of PRL did not occur.

Analysis of anti-PRLr IF in resting and IL-2-stimulated L2 cells revealed two populations of cells within the G_0/G_1 phase of the cell cycle, with levels of anti-PRLr IF differing by ≈7-fold (Fig. 5*B*). Only increased levels of PRLr IF were noted in the S- and G_2/M-phase cells. The ratio of the number of cells containing high levels of PRLr to the number of cells containing low levels of PRLr within the G_0/G_1 phase was increased 4-fold in IL-2-stimulated cultures as compared to resting cultures. This suggests that increased levels of PRLr correlate with cellular activation. In addition, the relative level of anti-PRLr IF of L2 cells containing low levels of PRLr was quantitatively similar to that seen in resting mouse splenocytes (data not shown).

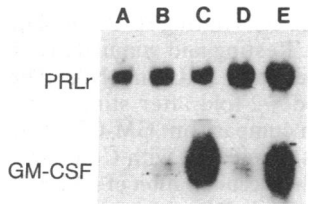

FIG. 4. Northern blot analysis with PRLr and GM-CSF cDNA probes. Lane A, resting cells; lane B, cells stimulated with IL-2 for 8 hr; lane C, cells stimulated with Con A for 8 hr; lane D, cells stimulated with IL-2 for 30 hr; lane E, cells stimulated with Con A for 30 hr.

PRL and PRLr Are Translocated Intracellularly as a Function of IL-2 Stimulation. IF microscopy was performed to identify changes in the subcellular localization of the PRL and PRLr in resting and IL-2-stimulated L2 lymphocytes. In resting L2 cells (Fig. 6*A*), a perinuclear cytoplasmic pool of PRL was seen with little to no apparent anti-PRL IF noted within the nucleus. In contrast, in IL-2-stimulated L2 cells significant levels of anti-PRL IF were noted within the nucleus (Fig. 6*B*). As an antibody specificity control, exogenous PRL was added with anti-PRL antiserum; this reduced IF to background levels (Fig. 6 *C* and *D*), demonstrating the specificity of the anti-PRL IF. Similarly cells stained with preimmune serum demonstrated low levels of nonspecific staining (data not shown).

Anti-PRLr IF revealed the presence of PRLr on the cell surface and within the cytoplasm of L2 cells (Fig. 6 *E* and *F*). Stimulation with IL-2 led to a partial translocation of the PRLr to the nuclear periphery of L2 cells. It is uncertain, given the limited resolving powers of IF microscopy, whether

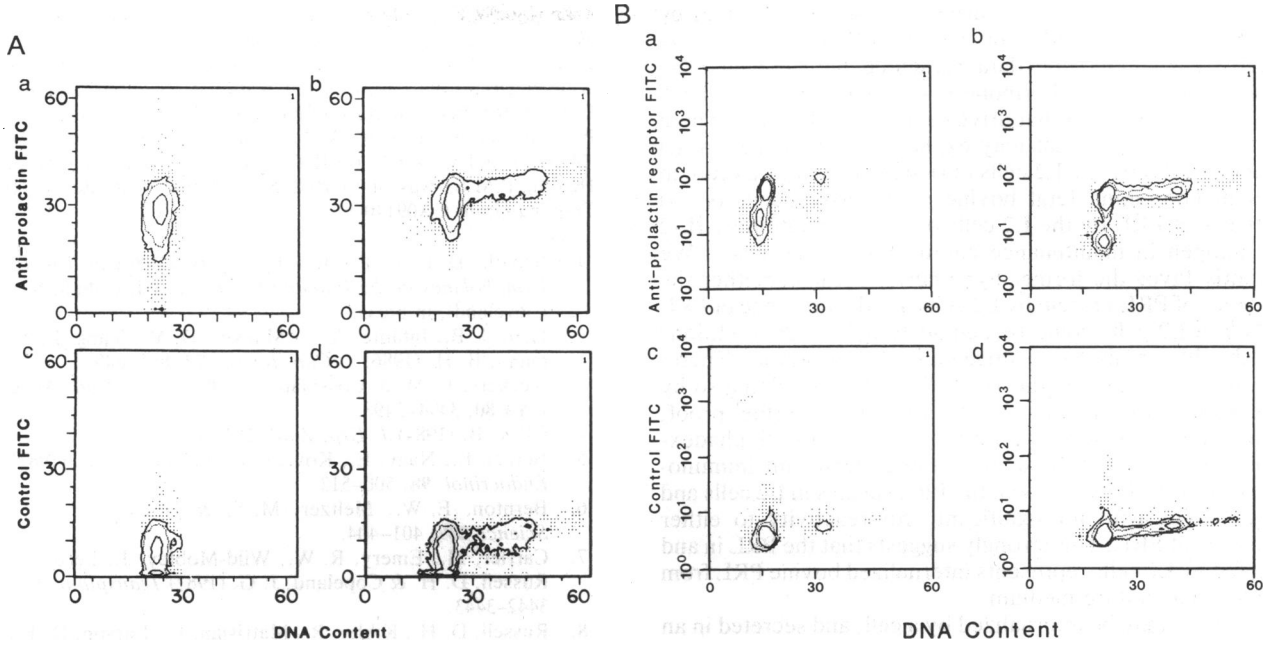

FIG. 5. (*A*) Flow cytofluorometric analysis of resting and IL-2-stimulated cells simultaneously stained with anti-PRL IF and propidium iodide. PRL and DNA content were quantified on the basis of anti-PRL IF [green; fluorescein isothiocyanate (FITC)-conjugated second antibody] and propidium iodide (red) fluorescence. Cells were stained with either a 1:600 dilution of anti-PRL antiserum (histograms a and b) or preimmune control serum (histograms c and d). Resting L2 cell cultures are represented in histograms a and c; IL-2-stimulated cultures in histograms b and d. Controls demonstrated an overall level of IF 5-fold less than the above histograms, with no population overlap with the anti-PRL IF-stained cells. (*B*) Flow cytofluorometric analysis of resting and IL-2-stimulated cells simultaneously stained with anti-PRLr IF and propidium iodide. As in *A*, PRLr and DNA content were quantified with anti-PRLr IF and propidium iodide fluorescence. The *y* axis is logarithmically scaled. Cells were stained with anti-PRLr monoclonal antibody (histograms a and b) or isotype-control monoclonal antibody (histograms c and d). Resting L2 cell cultures are represented in histograms a and c; IL-2-stimulated cultures in histograms b and d.

the redistributed PRLr was associated with the nuclear membrane or peripheral heterochromatin. Cells stained with an isotype control gave no IF (data not shown).

DISCUSSION

Although the interaction of IL-2 and the IL-2 receptor has been well characterized, the mechanisms surrounding the signal transduction of this event, and the subsequent induced expansion of T cells, remain poorly understood. Many of the factors associated with T-cell activation, such as protein phosphorylation/protein kinase activation (21, 22) and Ca^+, K^+, and H^+ concentrations (23, 24), have been extensively studied in IL-2-dependent T-cell lines. To date, however, no single intracellular transduction molecule has been conclusively demonstrated to mediate directly cell cycle progression in T cells binding IL-2 (1). Alternatively, the coordinate secretion and/or uptake of lymphokines and hormonal factors, such as PRL, might trigger the cascade of intra- and extracellular events that mediate cell cycle progression (2).

We demonstrate here that the extracellular secretion of PRL by L2 cells is required for their proliferation in chemically defined medium. This was shown by the specific and

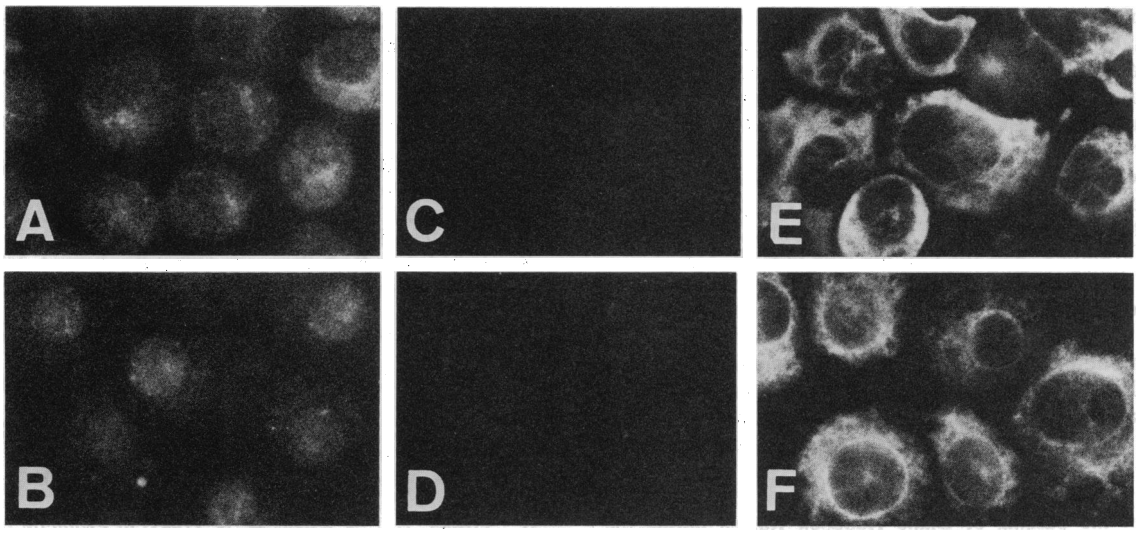

FIG. 6. IF photomicrographs of anti-PRL- and anti-PRLr-stained L2 cells. Resting (*A*, *C*, and *E*) and IL-2-stimulated (*B*, *D*, and *F*) L2 cells were stained with anti-PRL (*A* and *B*), anti-PRL that had been incubated with PRL (*C* and *D*), or anti-PRLr (*E* and *F*). IL-2-stimulated cells demonstrated a redistribution of PRL from the cytoplasm into the nucleus. Controls demonstrated only low levels of nonspecific IF (preimmune serum and anti-PRLr isotype control are not shown and were comparable to the levels of IF seen in *C* and *D*). (×1350.)

reversible inhibition of IL-2-driven L2 cell proliferation by the addition of anti-PRL antiserum. Although PRL is secreted by L2 cells, the data presented here conclusively demonstrate that this hormone is not synthesized by the T cells under conditions that drive cellular proliferation. There are two hypotheses that may explain this phenomenon: (*i*) uptake of PRL by the L2 cells during maintenance culture in medium containing fetal bovine serum may occur or (*ii*) synthesis of PRL by the L2 cells during stimulation by IL-2 plus antigen in maintenance culture could take place. We currently favor the former hypothesis, because neither the synthesis of PRL protein by L2 cells nor the presence of PRL mRNA in L2 cells could be demonstrated under conditions that should stimulate the antigen and IL-2 receptors. Therefore, if L2 cells are synthesizing PRL, they are doing so by a mechanism we cannot currently identify. As further proof, an anti-PRL antiserum affinity-purified with Sephadex-conjugated bovine PRL demonstrated significant immunologic crossreactivity between the PRL species in L2 cells and bovine PRL, with no significant crossreactivity to either mouse or rat PRL. This strongly suggests that the PRL in and secreted by L2 cells represents internalized bovine PRL from maintenance culture medium.

That PRL may be internalized by a cell, and secreted in an unchanged form, is not without precedent. Giss and Walker (25) have demonstrated that primary-culture pituitary cells take up radiolabeled PRL and subsequently secrete this hormone in an unchanged form over a period of hours. The significance of re-use of a secretory product such as PRL by either pituitary cells or T cells is unknown. It is possible, however, that the enhanced secretion of PRL or binding of PRL by increased numbers of PRLr during IL-2 stimulation may serve as a transducer in L2 cells of IL-2/IL-2 receptor binding. Indeed, work with the PRL-dependent rat lymphoma cell line NB2 suggests that only the secreted form of PRL, and not internalized PRL, may serve as a factor capable of stimulating cell growth (26).

PRL appears to be necessary, but not sufficient, for T-cell proliferation during the transit of L2 cells across the G_1 phase of the cell cycle. How the PRL–PRLr interaction mediates this effect is not clear. The primary sequence of PRLr does not demonstrate homology to other receptors with known enzymatic activities (27). One potential mechanism is that PRLr associates with another protein that transduces the signal of PRL–PRLr interaction. Alternatively, the translocation of PRL into the nucleus and PRLr into a perinuclear localization is perhaps of functional significance. This would suggest that PRLr may exert its immunomodulatory activity by direct action as a transport protein, translocating PRL to the nucleus. The IF data presented here indicate the likelihood of this latter possibility. Analogously, PRL stimulation of pituitary cells and PRL-responsive tissues (i.e., breast, lever, adrenal, and ovary) appears to be mediated by a cell surface receptor (28). The PRL–PRLr complex in these tissues is translocated into the Golgi apparatus, endosomes/secretory granules, and the cell nucleus (25, 29–31).

The continued study of PRL-mediated, IL-2-induced T-cell proliferation should provide further insights into the neurohormonal mechanisms of lymphocyte growth regulation. Further characterization of the cellular channeling and recycling of PRL, and the molecular events triggered by these events, may prove central to a complete knowledge of the molecular cascade that ultimately leads to clonal T-cell expansion.

We regret the passing of Diane Haddock Russell during the preparation of this manuscript and dedicate this work to her. We acknowledge the technical assistance of Ms. Nancy Thornton and Ms. Amy Sillman. We are grateful for the advice of Dr. Daniel Linzer. We thank the National Institute of Diabetes, Digestive, and Kidney Diseases as well as the National Hormone and Pituitary Program for their generous gifts of rat and bovine PRL and anti-rat PRL antiserum. This work was supported by Grants GM36962 (M.B.P.) and CA48673 (D.H.R.) from the National Institutes of Health. P.M.S. was supported by National Institutes of Health Training Grant CA09140-12.

1. Sabath, D. E. & Prystowsky, M. B. (1989) in *The Role of Lymphokines in the Immune Response*, ed. Cohen, S. (CRC, Cleveland), in press.
2. Lum, J. B., Infante, A. J., Makker, D. M., Yang, F. & Bowmann, B. H. (1986) *J. Clin. Invest.* **77**, 841–849.
3. Neckers, L. M. & Cossman, J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3494–3498.
4. Glick, B. (1984) *J. Exp. Zool.* **232**, 671–682.
5. Berczi, L., Nagy, E., Kovacs, K. & Horvath, E. (1981) *Acta Endocrinol.* **98**, 506–513.
6. Bernton, E. W., Meltzer, M. S. & Holaday, J. W. (1988) *Science* **239**, 401–404.
7. Carrier, M., Emery, R. W., Wild-Mobley, J., Perotta, N. J., Russell, D. H. & Copeland, J. G. (1987) *Transplant. Proc.* **29**, 3442–3443.
8. Russell, D. H., Kibler, R., Matrisian, L., Larson, D. F., Poulos, B. & Magun, B. E. (1985) *J. Immunol.* **134**, 3027–3031.
9. DiMattia, G. E., Gellersen, B., Bohnet, H. G. & Friesen, H. G. (1986) *Endocrinology* **122**, 2508–2517.
10. Montogomery, D. W., Zukoski, C. F., Shah, G. N., Buckley, A. R., Pacholczyk, T. & Russell, D. H. (1987) *Biochem. Biophys. Res. Commun.* **145**, 692–698.
11. Hartmann, D. P., Holaday, J. W. & Bernton, E. W. (1989) *FASEB J.* **3**, 2194–2202.
12. Shipman, P. M., Sabath, D. E., Fischer, A. H., Comber, P. G., Sullivan, K., Tan, E. M. & Prystowsky, M. B. (1988) *J. Cell. Biochem.* **38**, 39–48.
13. Prystowsky, M. B. (1989) in *Cell Growth and Division*, ed. Baserga, R. (Oxford Univ. Press, Oxford), pp. 95–103.
14. Lee, S. C., Sabath, D. E., Deutsch, C. & Prystowsky, M. B. (1986) *J. Cell. Biol.* **102**, 1200–1208.
15. Mendelsohn, J., Caviles, A. & Castagnola, J. (1988) *Cold Spring Harbor Symp.* **9**, 677–690.
16. Sissom, J. M., Eigenbrodt, M. L. & Porter, J. C. (1988) *Am. J. Pathol.* **133**, 589–595.
17. Clevenger, C. V., Epstein, A. L. & Bauer, K. D. (1985) *Cytometry* **6**, 208–214.
18. Clevenger, C. V., Epstein, A. L. & Bauer, K. D. (1987) *J. Cell Physiol.* **130**, 336–343.
19. Jaffe, B. D., Sabath, D. F., Johnson, G. D., Moscinski, L. C., Johnson, K. R., Rovera, G., Nauseef, W. M. & Prystowsky, M. B. (1988) *Oncogene* **3**, 167–174.
20. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
21. Farrar, W. L. & Anderson, W. B. (1985) *Nature (London)* **315**, 233–235.
22. Manager, B., Weiss, A., Imboden, J., Laing, T. & Stobo, J. D. (1987) *J. Immunol.* **139**, 2755–2760.
23. Gelfand, E., Mills, G. B., Cheung, R. K., Lee, J. W. W. & Grinstein, S. (1987) *Immunol. Rev.* **95**, 59–88.
24. Imboden, J. B. & Weiss, A. (1987) *Biochem. J.* **247**, 695–700.
25. Giss, B. J. & Walker, A. M. (1985) *Mol. Cell. Endocrinol.* **42**, 259–267.
26. Davis, J. A. & Linzer, D. I. H. (1988) *Mol. Endocrinol.* **2**, 740–746.
27. Davis, J. A. & Linzer, D. I. H. (1989) *Mol. Endocrinol.* **3**, 674–680.
28. Katoh, M., Raguet, S., Zachwieja, J., Dijane, J. & Kelly, P. A. (1987) *Endocrinology* **120**, 739–749.
29. Nolin, J. M. (1978) *Endocrinology* **102**, 402–406.
30. Nolin, J. M. (1980) *Peptides* **1**, 249–255.
31. Posner, B. I. & Khan, M. N. (1983) in *Prolactin and Prolactinomas*, ed. Tolis, G. (Raven, New York), pp. 9–18.