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The Effects of Novel Growth Factors on T-Lymphocyte Proliferation

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Introduction

The major growth factor for T lymphocytes is interleukin 2 (IL2); however, a number of studies have implicated prolactin (PRL) and other lactogens (e.g., human growth hormone, hGH) as important immune regulators. Receptors for lactogens are present on lymphocytes (17,18,20) and lactogens have been shown to promote lymphocyte proliferation (20). PRL can restore immunocompetence in hormone-deficient animals (12,13), it induces the secretory immune system in the mammary gland (24) and decreased PRL binding/action may play a role in mediating the immunosuppressive actions of cyclosporin (8). Recently it has become possible to examine the immunostimulatory actions of PRL using a rat T-cell line, the Nb2 lymphoma (20,22). These cells proliferate in response to low concentrations of lactogens (i.e., PRL. hGH) and in response to the normal T-cell mitogen (IL2 (14).

Recent studies suggest that a number of novel growth factors may regulate T-cell function. Transforming growth factor-β (TGF-β), which is produced by a number of tissues including monocytes (1) and T lymphocytes (9), inhibits the activity of immune cells. In tonsillar T-lymphocytes, TGF-β inhibits IL2-stimulated proliferation and it inhibits induction of the IL2 receptor by concanavalin A (9). TGF-β also inhibits concanavalin A-stimulated growth of mouse thymocytes (15). Activation of T lymphocytes increases the synthesis and secretion of TGF-β and up-regulates the receptor for TGF-β (9). TGF-β has been shown to decrease the cytolytic activity of human natural killer cells and to decrease the sensitivity of these cells to α-interferon (16). TGF-β also stimulates the expression of the protooncogene c-*sis* (11), a molecule which codes for one subunit of platelet derived growth factor (PDGF), and it has been proposed that PDGF may mediate some actions of TGF-β (11). In this regard, PDGF has been shown to decrease natural killer cell activity (6). Another novel factor that regulates cells of the immune system is tumor necrosis factor (TNF). It has been shown recently that TNF inhibits proliferation of HL-60 cells (10), a human lymphocyte cell line. Like TGF-β, TNF is produced by mononuclear phagocytes (4).

In this study, we have used Nb2 cells to examine the effects of these novel growth factors on lactogen-stimulated T-cell growth. The data show that TGF-β, TNF and PDGF inhibit proliferation of Nb2 cells in response to lactogens. We also examined the actions of fibroblast growth factor FGF), but this factor had no effect on lactogen-stimulated Nb2 cell growth. Overall, these data further support immunosuppresive roles for TGF-β, TNF and PDGF.

Methods and Materials

Materials—hGH (79-7-23H) and TGF-β were gifts of Dr. H.G. Friesen and Dr. M.B. Sporn, respectively. Rat IL2 and PDGF were obtained from Collaborative Research, Inc. (Lexington, MA), FGF (basic) was purchased from R&D Systems, Inc. (Minneapolis, MN) and TNF was donated by Genentech, Inc. (South San Francisco, CA). Ovine PRL (oPRL) was obtained from the Hormone Distribution Program, NIH.

Cell culture and quantitation—The Nb2 cells were grown in Fischer's medium supplemented with 10% fetal bovine serum, 10% horse serum, 1 mM 2-mercaptoethanol, 100 U/mL penicillin and 100 μg/mL streptomycin, and were maintained in a humidified atmosphere of 5% CO_2/95% air at 37 °C. Cell proliferation was quantified according to the bioassay of Tanaka *et al.* (22). Approximately 24 h prior to the addition of test substances, the Nb2 cells were growth-arrested by removing fetal bovine serum from the medium (i.e., resuspended in "assay medium"). Cells were exposed to test substances for 3 days except where indicated. Cell number was determined using a Coulter model ZM counter (Coulter Electronics, Inc., Hialeah, FL).

Results and Discussion

As shown in earlier studies (14,20,22) the Nb2 cells proliferated in response to the lactogen hGH and in response to IL2 (Fig. 1). Proliferation in response to both mitogens

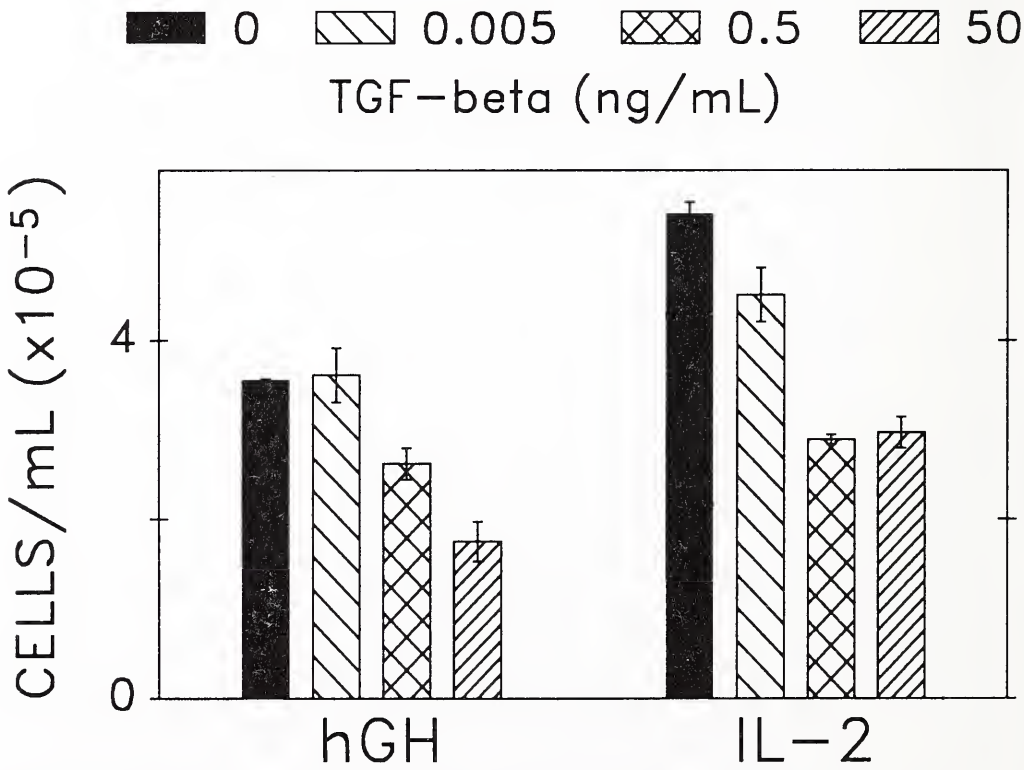

FIGURE 1. Inhibitory effects of TGF-β on mitogen-stimulated proliferation of Nb2 cells. Cells were plated in assay medium at a concentration of 1 x 10^5 cells/mL in the wells of 24-well culture dishes. The cells were exposed to hGH (1 ng/mL) or rat IL2 (20 half-maximal units/mL) ± TGF-β (concentrations indicated) for three days.

was inhibited by low concentrations of TGF-β, but proliferation could not be totally blocked even by high concentrations of TGF-β. Preliminary data suggest that this incomplete inhibition of Nb2 cell growth stems from the fact that the inhibitory actions of TGF-β are not expressed in the first round of cell division but only in the second or subsequent rounds (data not shown). This inconsistent timing in the expression of the actions of TGF-β led to considerable variability in the level of inhibition detected in the 3-day assays; however, the results of 15 studies with TGF-β indicated that this factor is a potent immunosuppressive agent.

PDGF also inhibited the proliferation of Nb2 cells in response to the lactogen PRL (Fig. 2). As observed with TGF-β, only partial suppression was obtained using a high

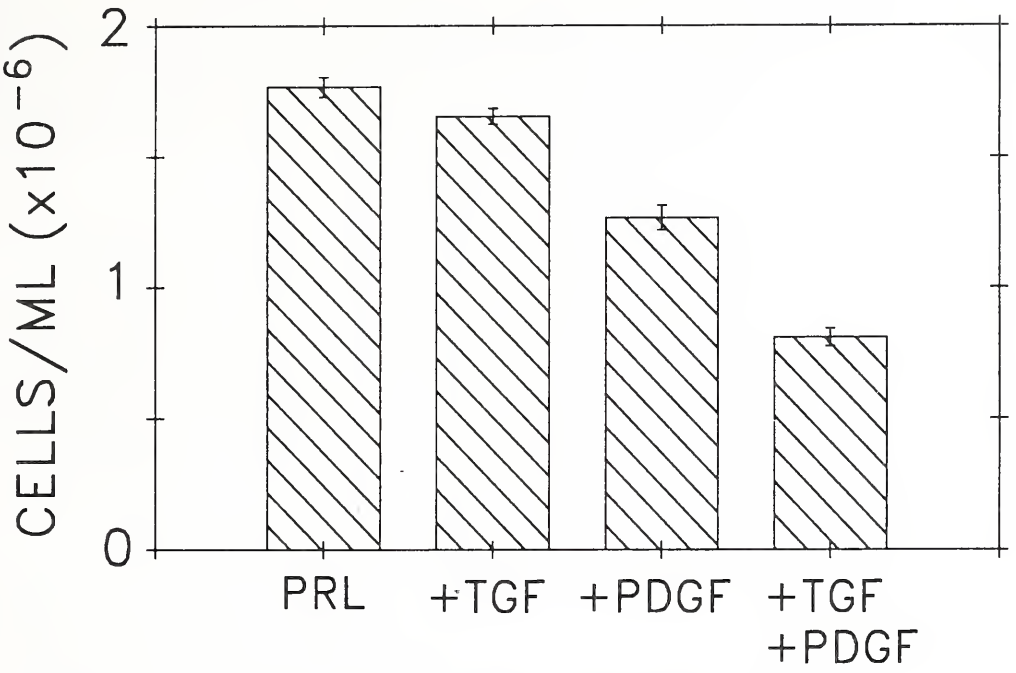

Figure 2. Inhibitory effect of PDGF on PRL-stimulated Nb2 cell growth. Cells were plated in assay medium at a concentration of 1 x 10^5 cells/mL in the wells of 96-well culture dishes. Cells were exposed to ovine PRL (5 ng/mL) ± PDGF (50 half-maximal units/mL) and/or TGF-β (5 ng/mL) for three days.

concentration of PDGF. When PDGF was combined with TGF-β, the inhibition of Nb2 cell proliferation appeared to be more than additive. However, the inhibition attained using TGF-β in these experiments was minimal; therefore, it is not possible to state at this time whether there is synergism between the two factors. Inasmuch as TGF-β stimulates c-*sis* expression, it is possible that a PDGF-like molecule contributes to the actions of TGF-β.

A preparation of TNF prepared by recombinant technologies exerted strong immunosuppressive actions in the Nb2 cell assay. At a concentration of 1 μg/mL, TNF totally blocked cell proliferation in response to PRL (Fig. 3). It is unknown, however, whether the high concentrations of TNF required for inhibition of Nb2 cell growth would be encountered under physiological conditions. If these concentrations can be generated locally, then TNF, or perhaps the closely related protein lymphotoxin (7), may be an important immunoregulatory factor.

FGF, an agent which has not been reported to affect immune function, did not alter Nb2 cell proliferation when used at concentrations comparable to those used in other proliferation studies (Fig. 4). Thus, our data suggest that FGF does not play a role in regulation of T-lymphocyte proliferation.

The mechanisms through which TGF-β, PDGF and TNF inhibit the actions of lactogens and IL2 are unknown at this time, however, all three of the immunosuppressive factors have been shown to stimulate prostaglandin synthesis (5,19,23). It is possible, therefore, that these factors inhibit lymphocyte proliferation by increasing prostaglandin levels. In this regard, it has been shown that prostaglandins inhibit the proliferation

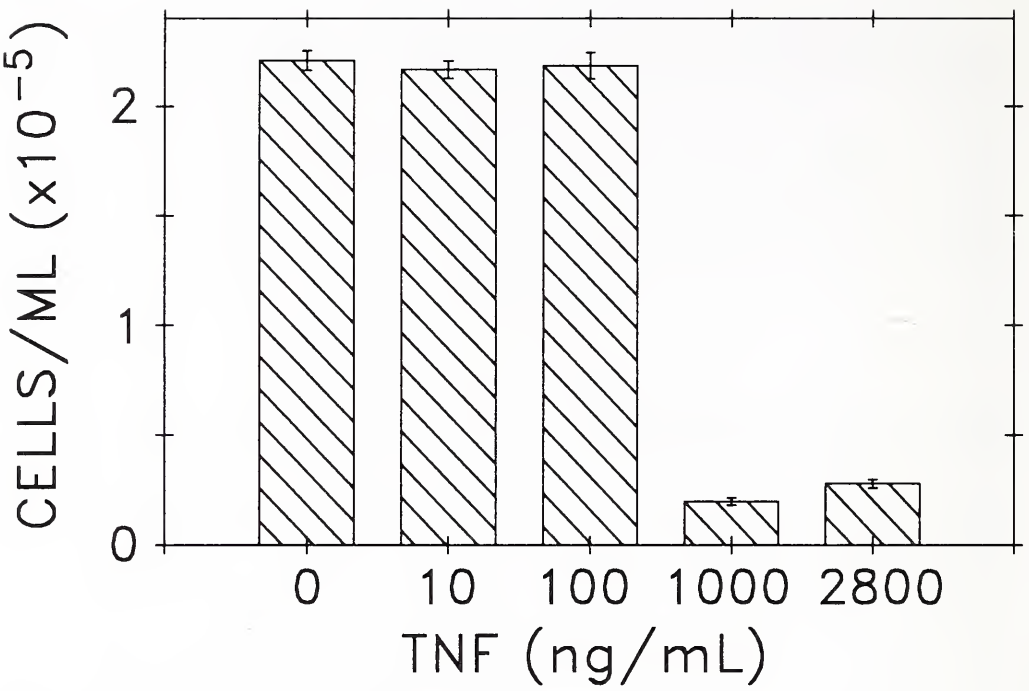

FIGURE 3. Inhibitory effect of TNF on PRL-stimulated Nb2 cell growth. Cells were plated in assay medium at a concentration of 2 x 10^4 cells/mL in the wells of 96-well culture dishes. Cells were exposed to ovine PRL (5 ng/mL) ± TNF (concentrations indicated) for five days.

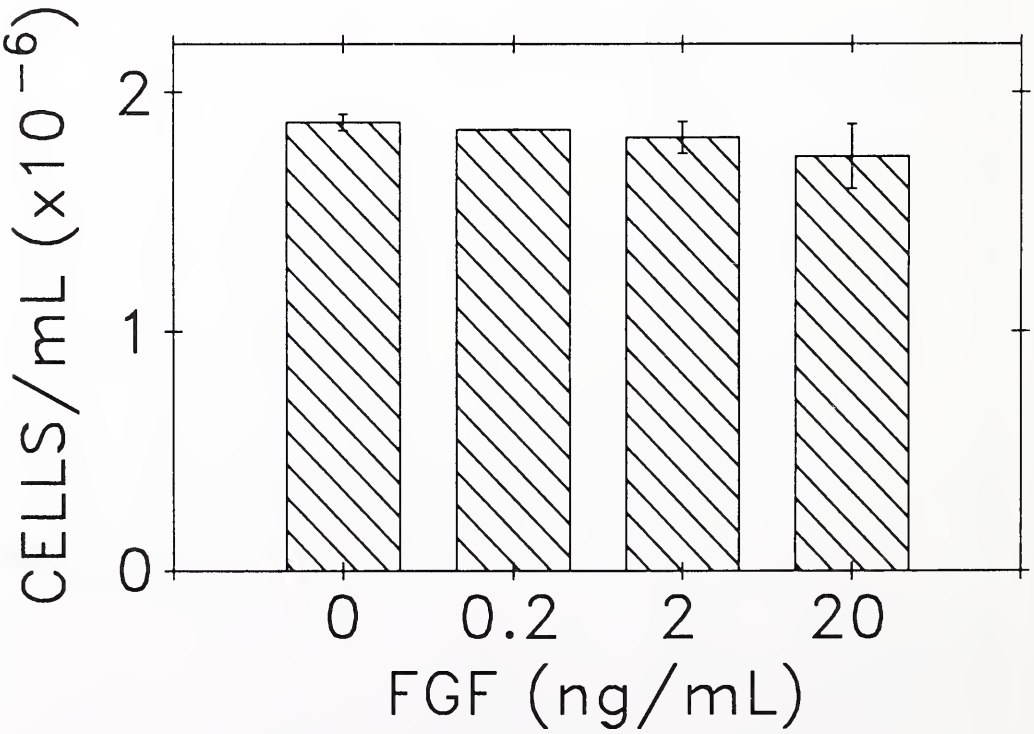

FIGURE 4. Effect of FGF on PRL-stimulated Nb2 cell growth. Cells were plated in assay medium at a concentration of 1 x 10^5 cells/mL in the wells of 96-well culture dishes. Cells were exposed to ovine PRL (5 ng/mL) ± FGF (concentrations indicated) for three days.

of T lymphocytes (2), perhaps through an increase in cAMP (3). Thus, our working hypothesis at this time is that TGF-β, PDGF and TNF regulate lymphocyte proliferation by increasing the production of prostaglandins.

Acknowledgments

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