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Interleukin 2 activates STAT5 transcription factor (mammary gland factor) and specific gene expression in T lymphocytes

(prolactin/tyrosine phosphorylation)

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ABSTRACT **Although prolactin and interleukin 2 (IL-2) can elicit distinct physiological responses, we have found that their signal pathways share a common signal transducer and activator of transcription, STAT5. STAT5 was originally identified as a mammary gland factor induced by prolactin in lactating breast cells. Here we demonstrate that STAT5 is activated after IL-2 stimulation of two responsive lymphocyte cell lines, Nb2 and YT. Activation of STAT5 is measured both by IL-2-induced tyrosine phosphorylation and by IL-2-induced DNA binding. The STAT5 DNA recognition site is the same as the interferon γ-activated site (GAS) in the interferon regulatory factor 1 gene. We demonstrate that the GAS element is necessary and sufficient for transcriptional induction by both IL-2 and prolactin in T lymphocytes. These results indicate that the role of STAT5 in the regulation of gene expression is not restricted to mammary cells or to prolactin, but is an integral part of the signal pathway of a critical immunomodulatory cytokine, IL-2.**

Recent advances in the field of cytokine research have identified signal transduction pathways that initiate at a cell surface receptor and culminate in the activation of specific gene expression in the nucleus. Such a receptor-to-nucleus signal pathway was originally demonstrated in the interferon system and involves the activation of tyrosine kinases of the Janus kinase (JAK) family that phosphorylate latent cytoplasmic transcription factors called signal transducers and activators of transcription (STATs) (reviewed in refs. 1–3). The tyrosine-phosphorylated STATs translocate to the nucleus and bind to a specific DNA target site containing a palindromic sequence of GAA residues. This response element was first identified in the interferon (IFN)-γ system and is thereby known as a IFN-γ-activated site (GAS). Our studies and those of others have identified distinct STAT-like factors that are induced by heterologous cytokines, including prolactin (PRL), growth hormone, interleukin (IL)-2, IL-3, IL-4, and IL-6 (4–12). In this report we provide evidence that the specific STAT factor activated by IL-2 in lymphocytes is STAT5, also known as the mammary gland factor (MGF), which was first shown to be activated in response to PRL in lactating breast cells (5, 13).

IL-2 and PRL are cytokines responsible for inducing distinct physiological responses, yet they both stimulate T-lymphocyte proliferation and differentiation (14–21). IL-2, produced by T lymphocytes, binds to a transmembrane receptor composed of three subunits (α, β, and γ chains) that oligomerize after ligand binding (20–22). The receptor does not possess intrinsic kinase activity; however, IL-2 binding stimulates the activity of two families of tyrosine kinases. Members of the Src family of tyrosine kinases, Lck, Fyn, and Lyn, were first shown to be activated by IL-2, and, more recently, members of the JAK family, JAK1 and JAK3, have been found to be activated (20, 21, 23, 24). PRL, produced by the anterior pituitary gland as well as by T lymphocytes, stimulates the homodimerization of two transmembrane receptor proteins and the subsequent activation of a distinct JAK kinase, JAK2 (4, 16, 17, 25–31).

IL-2 is best studied by its ability to activate proliferation and/or differentiation of immune cells, whereas PRL is best studied by its ability to activate proliferation of mammary cells and milk production by breast cells (17, 20, 32, 33). However, in T lymphocytes both PRL and IL-2 have been shown to induce the transcription of a common target gene encoding IFN regulatory factor 1 (IRF-1) (4, 15). The IRF-1 gene contains a GAS-like element in its promoter that is recognized by the IFN-γ-induced STAT1 factor (34, 35). We have investigated the ability of PRL and IL-2 to activate latent STAT factors that recognize the IRF-1 GAS site in Nb2 T lymphoma cells. We found that PRL induced the activation of two IRF-1 GAS-binding factors as measured by a gel mobility shift assay, a faster-migrating factor–DNA complex and a unique slower-migrating complex, which we referred to as a PRL-induced factor (PRLIF) (4). In addition, IL-2 stimulation of these T cells activated a STAT-like factor that we referred to as an IL-2 nuclear activated factor (IL-2 NAF). In this study we have analyzed the molecular composition of the STAT factors induced by PRL and IL-2 in lymphocytes. In addition, we have examined the DNA element in the IRF-1 promoter that is transcriptionally responsive to PRL and IL-2.

MATERIALS AND METHODS

Cells and Reagents. Nb2 rat T lymphocytes, kindly provided by P. W. Gout (British Columbia Cancer Agency, Vancouver), were maintained as described (36). Prior to cytokine treatment, cells were placed in 8% equine serum for 24 hr. Human YT lymphocytes (0273952), a gift from H. A. Young (National Cancer Institute, Frederick, MD), were maintained in RPMI 1640 medium with 10% fetal bovine serum. Twenty-four hours before cytokine treatment, the cells were resuspended in medium containing 5% equine serum. Rat PRL was a gift from the National Hormone and Pituitary Program (National Institute of Diabetes and Digestive and Kidney Diseases, Rockville, MD) and was used at 250 ng/ml. Human recombinant IL-2 was a gift from the Biological Response Modifiers Program (National Cancer Institute, Frederick, MD) and was used at 500 units (U)/ml. Recombinant murine IFN-α was purchased from GIBCO/BRL. Recombinant murine IL-4 was a gift from Schering–Plough. The rabbit polyclonal anti-STAT1 antibody has been described (4). The anti-STAT3 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and the anti-STAT5 monoclonal antibody was purchased from Transduction Laboratories (Lexington, KY). Anti-phosphoty-

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Abbreviations: IL, interleukin; IFN, interferon; PRL, prolactin; GAS, IFN-γ-activated site; IRF-1, IFN regulatory factor 1; JAK, Janus kinase; STAT, signal transducer and activator of transcription; U, unit.

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rosine antibody (4G10) conjugated to agarose beads was purchased from Upstate Biotechnology (Lake Placid, NY).

Gel Mobility-Shift Electrophoresis. Preparation of nuclear extracts, DNA-binding reactions, and gel electrophoresis were performed as described (4). The double-stranded oligonucleotide used as a probe contained the GAS site from the IRF-1 promoter (nucleotides −129 to −107: 5′-GCCTGATTTCCCCGAAATGACGG-3′) (35). Complexes were formed with radiolabeled DNA and 5 μg of protein.

Phosphotyrosine Analysis of STAT5. Cell lysates were incubated with antiphosphotyrosine antibody conjugated to agarose beads for 6 hr at 4°C (4). Immunocomplexes were collected, separated by SDS/PAGE, and transferred to Immobilon P (Millipore). The membrane was immunoblotted with anti-STAT5 antibody, and STAT5 was detected by using an anti-mouse secondary antibody conjugated to horseradish peroxidase (Amersham) and an enhanced chemiluminescence system (DuPont/NEN).

Gel-Shift/Immunoblot Analysis. DNA-binding reactions were performed as described above except that complexes were formed with 40 μg of protein and an unlabeled oligonucleotide. After electrophoresis, proteins in the gel were transferred to Immobilon P and detected with specific antibodies as described above.

Gene Expression. Nb2 T cells were electroporated with either the wild-type GAS-luciferase construct or the mutant GAS-luciferase construct along with a plasmid expressing β-galactosidase (35). Twenty-four hours after transfection the cells were resuspended in medium containing 10% equine serum. Forty-eight hours after transfection the cells were treated with PRL or IL-2 for 8 hr. Luciferase assays were performed by using Promega reagents and protocol. β-Galactosidase assays were performed as described (37).

Proliferation Assays. Nb2 cells were collected and resuspended in medium with 10% equine serum either unsupplemented or supplemented with PRL at 10 ng/ml, IL-2 at 150 U/ml, both PRL at 10 ng/ml and IL-2 at 150 U/ml, IFN-α at 100 U/ml, or IL-4 at 100 ng/ml.

RESULTS

STAT5 Is Activated by Both PRL and IL-2 in Rat T Lymphocytes. STAT5 has been shown to be activated by PRL in mammary epithelial cells, and both PRL and IL-2 induce transcription of the IRF-1 gene in T lymphocytes (4, 5, 15). For these reasons we determined whether STAT5 is activated in Nb2 T cells in response to stimulation by PRL or IL-2. The results of a gel mobility-shift assay performed with 5 μg of protein from nuclear extracts isolated from control, PRL-treated, or IL-2-treated Nb2 T cells and a radiolabeled IRF-1 GAS oligonucleotide are shown in Fig. 1*A*. Two major DNA-binding complexes are induced by PRL (lane 2) and one complex is induced by IL-2 (lane 3). The identity of the STAT factors in these complexes was revealed by an immunoblot analysis of a gel-shift assay performed with an unlabeled DNA probe. This experimental method was chosen because the anti-STAT5 antibody recognizes the STAT5 protein more efficiently after its denaturation on a solid support. A DNA-binding assay was performed with nuclear protein and an unlabeled probe at the same time as the DNA-binding assay with radiolabeled probe, and the reaction products were electrophoresed side-by-side on the same gel. Following electrophoresis, radioactive STAT–DNA complexes were identified by autoradiography and their relative migration was measured (circle and diamond symbols in Fig. 1*A*). Proteins in the unlabeled gel complexes were transferred to a membrane and immunoblotted with either anti-STAT5 antibody (Fig. 1*B*) or anti-STAT1 antibody (Fig. 1*C*), and the mobility of the STAT was compared with that of the radiolabeled complexes. Untreated cells did not contain activated STAT-like factors, but the slow-migrating complexes induced by PRL and IL-2 both contained STAT5 (migration noted by circle). These results indicate that both PRL and IL-2 activate STAT5 and that the previously described DNA-binding factors PRL-induced factor and IL-2 nuclear activated factor are composed of STAT5 (4). The fast-migrating complex induced by PRL (noted by a diamond) was found to contain both STAT5 and STAT1. The presence of STAT1 in this complex is in accordance with our previous results demonstrating the ability of

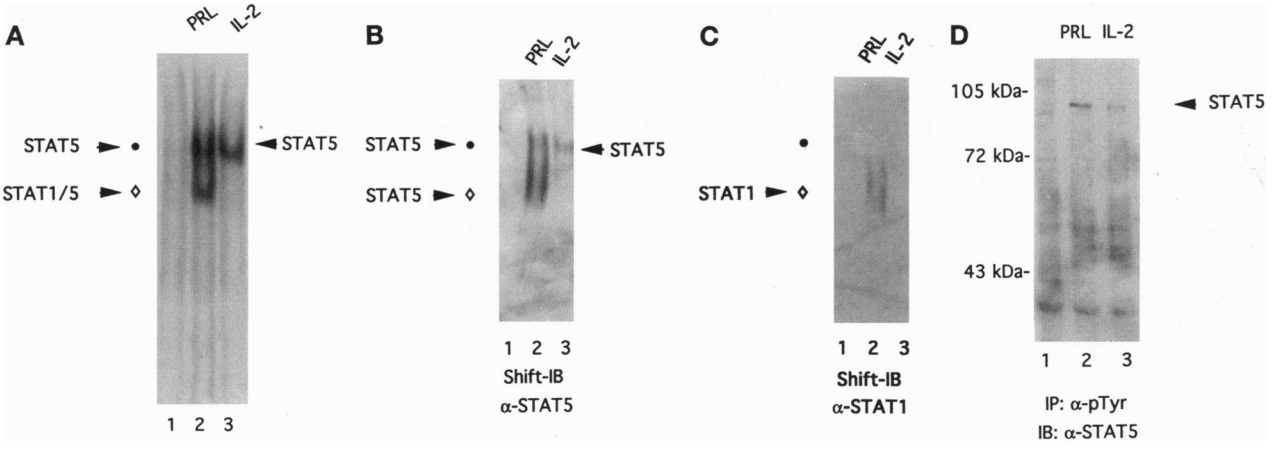

FIG. 1. PRL and IL-2 induce the tyrosine phosphorylation and DNA-binding activity of STAT5 in Nb2 T lymphocytes. (*A*) Nuclear extracts from control cells (lane 1) or cells treated with PRL (250 ng/ml; lane 2), or IL-2 (500 U/ml; lane 3) for 20 min were incubated with a radiolabeled IRF-1 GAS probe for gel shift analysis. Arrows indicate the mobility of the DNA-binding complexes. Characters ● and ◇ are positioned as an electrophoretic mobility reference for the immunoblots in *B* and *C*. (*B*) Nuclear extracts were prepared from Nb2 T cells and used in a gel mobility-shift assay as described for *A* but with unlabeled oligonucleotide GAS probe. The protein–DNA complexes in the shift gel were electrophoretically transferred to Immobilon and immunoblotted (IB) with anti-STAT5 antibody (α-STAT5). The complexes that reacted with anti-STAT5 antibody are indicated with the arrows. Characters ● and ◇ mark the position of the slow- and fast-migrating complexes shown in *A*. (*C*) Gel mobility-shift/immunoblot (IB) assay as described for *B* performed with anti-STAT1 antibody (α-STAT1). The complexes that reacted with anti-STAT1 antibody are indicated with the arrows. Characters ● and ◇ mark the position of the slow- and fast-migrating complexes shown in *A*. (*D*) Nb2 T cells were untreated (lane 1), treated with PRL at 250 ng/ml (lane 2), or treated with IL-2 at 500 U/ml (lane 3). Tyrosine-phosphorylated proteins from 5×10^7 cells were immunoprecipitated (IP) with anti-phosphotyrosine antibody (α-pTyr) and immunoblotted (IB) with anti-STAT5 antibody (α-STAT5). Molecular mass standards are indicated on the left and the mobility of tyrosine-phosphorylated STAT5 is indicated by the arrow.

anti-STAT1 antibody in the DNA-binding reaction to eliminate the appearance of the PRL-induced fast-migrating complex (4). In addition, the migration of this faster complex is similar to the IFN-induced STAT1 (4). For these reasons STAT1/STAT5 heterodimers may be present in the faster-migrating complex and may be involved in the response to PRL.

To determine if STAT5 is activated by tyrosine phosphorylation in T lymphocytes by stimulation with PRL or IL-2, we analyzed the status of STAT5 tyrosine phosphorylation after cytokine stimulation. After ligand binding we measured STAT5 specific tyrosine phosphorylation by an immunoprecipitation/immunoblot assay (Fig. 1*D*). Nb2 T cells were untreated or treated with PRL or IL-2 for 7 min. Cells were lysed, and the tyrosine-phosphorylated proteins were immunoprecipitated with anti-phosphotyrosine antibodies. The immunoprecipitated proteins were separated by SDS/PAGE, transferred to a membrane, and immunoblotted with specific anti-STAT5 monoclonal antibodies. A rapid induction of STAT5 tyrosine phosphorylation can be detected readily in response to both PRL and IL-2 in the T cells. These results suggest that PRL signal transduction in mammary cells and T cells leads to the activation of STAT5. Furthermore, although PRL and IL-2 bind to distinct receptors and stimulate distinct tyrosine kinases, they share the ability to activate STAT5.

Activation of STAT5 in Human YT Lymphocytes by IL-2. To be sure that STAT5 activation is a general response to IL-2, we examined another lymphocyte cell line, YT (38). YT cells appear to be human natural killer lymphocytes that constitutively synthesize and secrete IFN-γ (Howard A. Young, personal communication). Since IFN-γ has been demonstrated to activate both STAT1 and STAT3, the autocrine effect of IFN-γ would be predicted to produce a constitutive GAS-binding activity (12, 39). A gel mobility-shift assay of nuclear extracts from control and IL-2-treated YT cells with a radiolabeled IRF-1 GAS probe revealed a slow-mobility constitutive DNA-binding complex (noted by circle) and a fast-mobility complex specifically induced by IL-2 (noted by diamond) (Fig. 2*A*).

To determine if STAT5 was contained in the specific IL-2-induced DNA-binding complex, a gel-shift/immunoblot assay was performed as described for Fig. 1. The assay was performed with nuclear protein and an unlabeled probe at the same time as the DNA-binding assay with radiolabeled probe, and the reaction products were electrophoresed side-by-side on the same gel. After electrophoresis, radioactive STAT–DNA complexes were identified by autoradiography and their relative migration was measured (circle and diamond symbols in Fig. 2*A*). Complexes formed with unlabeled probe were transferred to a membrane and immunoblotted with anti-STAT5 antibody (Fig. 2*B*). No reactivity was detected in the lane containing protein extracts from control cells; however, both the slow- and fast-migrating complexes formed with IL-2-treated extracts reacted with the STAT5 antibody (Fig. 2 *A* and *B*).

A gel-shift/immunoblot assay performed with anti-STAT1 antibody demonstrated that STAT1 was contained in the slow-migrating DNA–protein complex (Fig. 2*C*). It was present constitutively, as was expected from the autocrine activity of IFN-γ, and it was also slightly induced by IL-2 treatment. A similar result was obtained by analyses with anti-STAT3 antibody (Fig. 2*D*). Therefore these cells are in a continuous state of activation of STAT1 and STAT3 apparently due to the presence of IFN-γ. But more importantly, the YT lymphocytes respond to stimulation with IL-2 with the specific activation of the STAT5 transcription factor.

The ability of IL-2 to stimulate the tyrosine phosphorylation of the STAT5 factor also was examined in YT lymphocytes. Cell lysates were prepared from untreated cells or cells treated with IL-2, and tyrosine phosphorylated proteins were detected by immunoprecipitation with anti-phosphotyrosine antibodies followed by immunoblotting with anti-STAT5 antibodies (Fig. 2*E*). Several immunoreactive proteins were present in the untreated cells, and one new tyrosine-phosphorylated STAT5

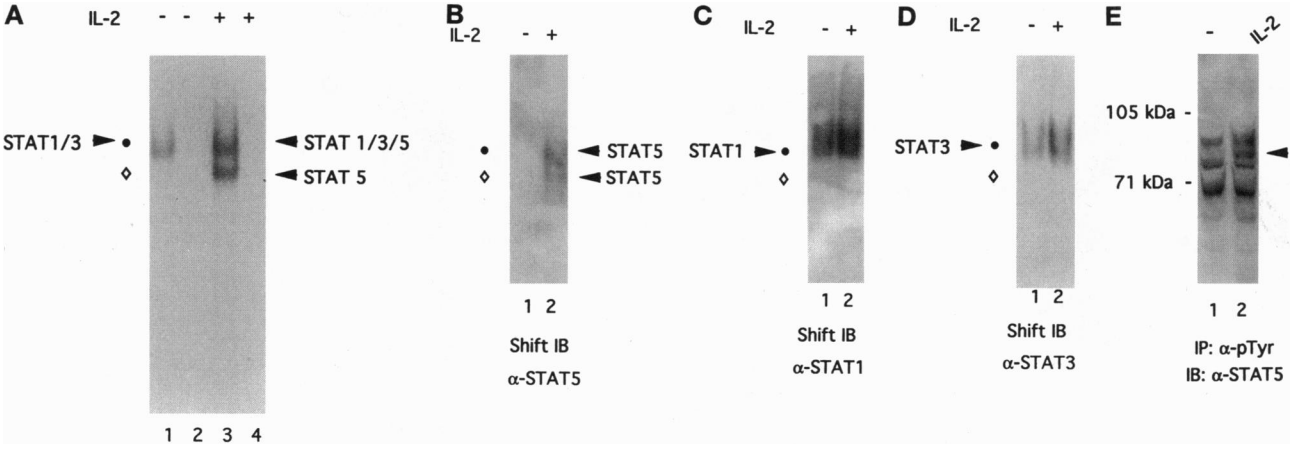

FIG. 2. IL-2 stimulates the activation of STAT5 in YT lymphocytes. (*A*) Nuclear extracts were prepared from untreated cells (lanes 1 and 2) or cells treated with IL-2 for 20 min (500 U/ml) (lanes 3 and 4), and a gel mobility-shift analysis was performed. Lanes 2 and 4 represent a DNA-binding reaction in the presence of 100-fold molar excess of an unlabeled GAS oligonucleotide to demonstrate binding specificity. The DNA-binding complexes and the STATs present in the complexes are indicated with the arrows. Characters ● and ◇ are positioned as an electrophoretic mobility reference for the immunoblots in *B, C,* and *D.* (*B*) Gel mobility-shift assay was performed with nuclear extracts from untreated cells (lane 1) or cells treated with IL-2 for 20 min (500 U/ml) (lane 2) and unlabeled oligonucleotide probe. The complexes were transferred to Immobilon and immunoblotted (IB) with anti-STAT5 antibody (α-STAT5). The complexes that reacted with α-STAT5 are indicated with the arrows. Characters ● and ◇ mark the position of the slow- and fast-migrating complexes shown in *A.* (*C*) Gel mobility shift and immunoblot (IB) with anti-STAT1 antibody (α-STAT1) as described for *B.* The complexes detected by α-STAT1 are indicated with the arrow. Characters ● and ◇ mark the position of the slow- and fast-migrating complexes shown in *A.* (*D*) Gel mobility-shift and immunoblot (IB) with anti-STAT3 antibody (α-STAT3) as described for *B.* The complexes detected by α-STAT3 are indicated with the arrow. Characters ● and ◇ mark the position of the slow- and fast-migrating complexes shown in *A.* (*E*) Lysates were prepared from 2.5×10^7 untreated YT cells (lane 1) or cells treated with IL-2 (500 U/ml) for 7 min (lane 2) and immunoprecipitated with anti-phosphotyrosine antibody. The immunoprecipitates were separated by SDS/PAGE, transferred to Immobilon, and incubated with anti-STAT5 antibody. Molecular weight markers are indicated on the left. The IL-2-induced tyrosine-phosphorylated protein detected with the anti-STAT5 antibody is indicated with the arrow on the right.

protein appeared after IL-2 stimulation. The appearance of this phosphorylated protein correlates with the induced appearance of STAT5 DNA-binding activity in the gel mobility-shift analyses (Fig. 2*B*). The presence of multiple immunoreactive STAT5 proteins that are tyrosine phosphorylated in untreated cells may represent splice variants that are not capable of binding DNA (since untreated cells did not contain STAT5 DNA-binding activity; Fig. 2*A*). Protein degradation does not seem to account for the proteins, since protease inhibitors were included in the lysis, and this result was very reproducible. The tyrosine phosphorylation of the defective STAT5 variants may be due to the activated state of the cell by autocrine IFN-γ. The molecular mass of the tyrosine-phosphorylated STAT5 in YT cells appears to be approximately 10 kDa less than STAT5 in Nb2 cells as estimated by SDS/PAGE. This correlates with the position of the STAT5 DNA-binding complex relative to the STAT1 complex in the gel mobility-shift assays of the two cell lines when electrophoresed on the same gel (data not shown). This result may reflect differential splicing or species variation.

The GAS Element Is Necessary and Sufficient for Gene Expression Induced by Prolactin or IL-2 in T Cells. To examine if the GAS element is sufficient for transcriptional induction by PRL and IL-2 in T lymphocytes, Nb2 cells were transfected with luciferase reporter constructs containing either a wild-type GAS element from the IRF-1 gene or a mutated GAS element (Fig. 3) (35). Transfected cells were untreated or treated with PRL or IL-2 for 8 hr, cell lysates were prepared, and luciferase activity was measured. As a control for transfection efficiency, a construct of a β-galactosidase gene with a Rous sarcoma virus (RSV) promoter was included in the transfection, and luciferase values were normalized to β-galactosidase levels. Both PRL and IL-2 induced expression of the gene containing the wild-type GAS by 4- to 5-fold. However, neither PRL nor IL-2 induced expression of the gene containing the mutated GAS. In addition, the parent vector,

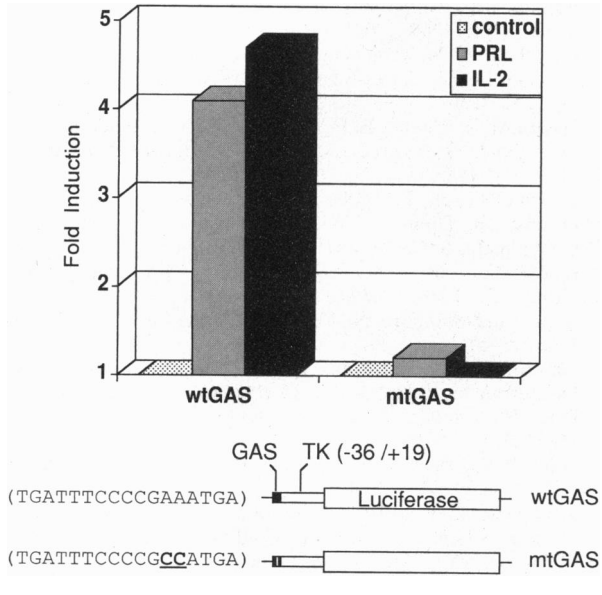

FIG. 3. PRL and IL-2 induce transcription of a GAS-luciferase construct in T cells. Nb2 T cells were cotransfected with wild-type (wt) GAS-luciferase and RSV-β-galactosidase or mutant (mt) GAS-luciferase and RSV-β-galactosidase genes. The luciferase constructs are presented below the graph. TK, thymidine kinase. Cells were untreated or treated with PRL at 250 ng/ml or IL-2 at 500 U/ml for 8 hr. Cell lysates were prepared, and luciferase and β-galactosidase activities were measured. Luciferase activity was normalized to β-galactosidase activity, and the fold increase was calculated with the untreated cells as baseline (1-fold). The graph presents the mean fold increase from three experiments.

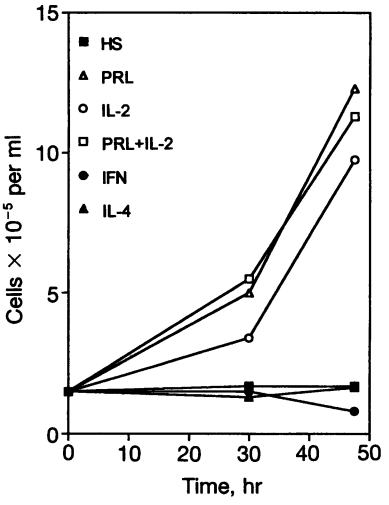

FIG. 4. IL-2 stimulates proliferation of PRL-dependent T lymphocytes. Nb2 T cells were collected and resuspended at 10^5 cells per ml in Fisher's medium containing 10% horse serum. Cultures were supplemented with one of the following: 0.1% horse serum (HS), PRL at 10 ng/ml, IL-2 at 150 U/ml, PRL at 10 ng/ml + IL-2 at 150 U/ml, IFN-α at 100 U/ml, or IL-4 at 100 ng/ml. The numbers of cells per ml are indicated at the time points noted. The data graphed in this experiment are representative of three different experiments.

containing a luciferase gene and herpes simplex virus thymidine control elements, failed to be induced by PRL or IL-2 (K.C.G. and N.C.R., unpublished data). These results demonstrate that the wild-type GAS element is necessary and sufficient for transcriptional induction by PRL and IL-2 in T cells. Since the mutated GAS fails to bind the STATs (K.C.G. and N.C.R., unpublished data), STAT binding to the GAS site appears to be necessary for gene activation.

PRL or IL-2 Can Stimulate Proliferation of Nb2 T Lymphocytes. Nb2 T lymphocytes were isolated originally as PRL-dependent cells (36). These cells proliferate in the presence of fetal bovine serum, which serves as a source of PRL, or in the presence of PRL at concentrations as low as 50 pg/ml. However, when Nb2 cells are placed in 10% equine serum, they fail to proliferate and they die. Since PRL and IL-2 both activate STAT5 and induce transcription through the GAS element, we examined if IL-2 could substitute for PRL in inducing proliferation of Nb2 cells. As Fig. 4 demonstrates, PRL at 10 ng/ml induces proliferation of Nb2 cells (10 ng/ml induces maximal proliferation of Nb2 cells; unpublished data of K.C.G. and N.C.R.). In addition, IL-2 can also induce proliferation of Nb2 cells, with a maximal response at 150 U/ml. The combination of IL-2 and PRL at levels that induced maximal proliferation fails to induce more proliferation than PRL alone. This suggests that PRL- and IL-2-induced proliferation of Nb2 cells shares a common limiting factor. Treatment with IL-4, IFN-α, or horse serum was unable to induce proliferation of Nb2 cells, although IL-4 and IFN activate STAT6 and STAT1/STAT2, respectively (refs. 1–3, 8, and 9; K.C.G. and N.C.R., unpublished data). Over an extended period of time no viable cells were observed in the IL-4-, IFN-, or horse serum-treated cultures. Although PRL and IL-2 bind to different receptors and activate different JAKs, they are both able to induce proliferation of Nb2 T lymphocytes.

DISCUSSION

Knowledge of the molecular mechanism by which IL-2 transmits a signal from the receptor to the nucleus is critical to our understanding of the biological effects of IL-2, and knowledge of the signal transduction pathways of PRL that lead to gene activation in T cells should enhance our understanding of its role in the immune system. In this report we demonstrate that

IL-2, an immunomodulatory and T-cell growth cytokine, and PRL, a lactogenic and immunoregulatory hormone, both activate the same specific transcription factor, STAT5. Activation of STAT5 was demonstrated both by cytokine-induced DNA-binding activity and by tyrosine phosphorylation. In addition, both PRL and IL-2 are capable of activating transcription by means of the IRF-1 GAS element in T lymphocytes. This GAS element has homology with the STAT5 binding site in the β-casein promoter (4, 5); however, the STAT complexes induced by PRL in T cells bind preferentially to the IRF-1 GAS element rather than the β-casein GAS element (4).

Although the signal transduction pathways of IL-2 and PRL are distinct, the ability of both cytokines to activate STAT5 and transcription through the GAS element may explain their ability to activate the same gene, IRF-1. Our results demonstrating that IL-2 can substitute for PRL in inducing T-cell proliferation further demonstrate that PRL and IL-2 have shared immunological functions. The ability of PRL to activate both STAT1 and STAT5 in Nb2 T cells while IL-2 is able to activate only STAT5 in these cells may contribute to different physiological effects in T cells in response to PRL or IL-2.

The mechanism of STAT5 activation by PRL and IL-2 remains to be elucidated. IL-2 and PRL bind to distinct receptors and activate different tyrosine kinases, but they share a critical transcription factor, STAT5, that appears to be involved in mediating their effects on specific gene expression through the GAS element. It is possible that there is an unidentified signaling component that may be activated by the different receptors or tyrosine kinases leading to STAT5 activation. Alternatively, since the STAT molecules contain SH2 domains that associate with phosphorylated tyrosine residues, STAT5 may associate directly with regions of the different receptors that are phosphorylated after ligand binding (21, 28). This mechanism would recruit the STAT5 molecule to the proximity of the different activated kinases for subsequent tyrosine phosphorylation. This later mechanism has been proposed for the IL-4 and IFN-γ signal pathways and may constitute a general means of STAT activation (9, 40).

Note. During the preparation of this manuscript Hou *et al.* (41) published the purification of human STAT5 from IL-2 treated YT cells and recombinant cloning of the human STAT5 gene.

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