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The Roles of Prolactin, Growth Hormone, Insulin-Like Growth Factor-I, and Thyroid Hormones in Lymphocyte Development and Function: Insights from Genetic Models of Hormone and Hormone Receptor Deficiency*

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ABSTRACT

An extensive literature suggesting that PRL, GH, IGF-I, and thyroid hormones play an important role in immunity has evolved. Because the use of one or more of these hormones as immunostimulants in humans is being considered, it is of critical importance to resolve their precise role in immunity. This review addresses new experimental evidence from analysis of lymphocyte development and function in mice with genetic defects in expression of these hormones or their receptors that calls into question the presumed role played by some of these hormones and reveals unexpected effects of others. These recent findings from the mutant mouse models are integrated and placed in context of the wider literature on endocrine-immune system interactions. The hypothesis that will be developed is that, with the exception of a role for thyroid hormones in B cell development, PRL, GH, and IGF-I are not obligate immunoregulators. Instead, they apparently act as anabolic and stress-modulating hormones in most cells, including those of the immune system. (*Endocrine Reviews* **21:** 292–312, 2000)

I. Introduction

STUDIES performed almost 70 yr ago revealed that surgical ablation of the rat pituitary gland resulted in thymic atrophy (1). Although the role of the thymus as the source of T lymphocytes was not appreciated at the time, these results were, in retrospect, among the first to suggest an association between the endocrine and immune systems. Further evidence for functional links between the endocrine and immune systems was subsequently obtained from studies showing that lymphocyte development and function were deficient in Snell dwarf mice (2–4). As discussed in the following section, this strain has a defect in the production of PRL, GH, IGF-I, and thyroid hormones (5–7). Experiments demonstrating that administration of PRL, GH, and/or thyroid hormones to hypophysectomized rats or dw/dw mice corrected their immune system defects (8–11) provided additional confirmation that endocrine-derived signals could regulate immune system development and function.

In the years after the initial description of these rodent models, an extensive literature suggesting that PRL, GH, IGF-I, and thyroid hormones play an important role in im-

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munity has evolved. However, this conclusion has not gained universal acceptance, and a role for PRL, GH, IGF-I, and thyroid hormones in the regulation of the immune system is often not acknowledged in major immunology texts (12–14). Nevertheless, the use of one or more of these hormones as immunostimulants in humans is being considered (15–17), making it of critical importance to resolve the precise role of PRL, GH, IGF-I, and thyroid hormones in immunity.

This review addresses new experimental evidence from mice with genetic defects in expression of these hormones or their receptors (Table 1) that calls into question the presumed role played by some of these hormones and reveals unexpected effects of others in the regulation of lymphocyte development and function. The thesis that will be developed is that, with the exception of a role for thyroid hormones in B cell development, PRL, GH, and IGF-I are not obligate immunoregulators. Instead, they apparently act as anabolic and stress-modulating hormones in most cells, including those of the immune system.

Previous reviews have summarized the vast literature on the effects of PRL, GH, IGF-I, and thyroid hormones on the immune system (15–27), so no attempt will be made to reiterate the conclusions in every paper published on each of these hormones. In addition, other hormones that have been implicated in positive and negative regulation of the immune system (28–31) are considered to be beyond the scope of this review and will not be discussed. Instead, recent findings from the mutant mouse models will be integrated and placed in context of the wider literature on endocrine-immune system interactions to develop a working model of how PRL, GH, IGF-I, and thyroid hormones affect lymphopoiesis.

II. Insights from dw/dw Mice

The dwarf (dw/dw) strain of mouse, first described in 1929 (32), has a primary deficiency in PRL, GH, and TSH due to a mutation in the gene encoding pit-1, a transcription factor that regulates pituitary development and the biosynthesis of PRL, GH, and TSH in anterior pituitary cells (33). The absence of TSH results in secondary thyroid hormone deficiency. Because many effects of GH are mediated through induction of IGF-I (18), dw/dw mice also lack normal IGF-I levels. Another mutant, the Ames Dwarf (df/df) mouse, also lacks production of PRL, GH, and TSH. However, the genetic basis for the defect in that strain is a mutation in a tissue-specific, paired-like homeodomain transcription factor, Prophet of Pit-1 (PROP1), which is required for pit-1 expression and determination of the pit-1 lineage (34).

The definition of immune system abnormalities in dw/dw mice has supported a role for PRL, GH, IGF-I, and/or thyroid hormones in immune system development and function (Table 2). These analyses, combined with results from the hypophysectomy studies, have generally been interpreted to indicate that the endocrine system is a critical source of immunostimulatory signals. However, as reviewed below, numerous contradictions in the dw/dw mouse literature make it difficult to reach definitive conclusions about the role of these hormones in immunity.

A. Primary lymphocyte development

The antigen-independent phase of B and T cell development in the bone marrow and thymus is known as primary lymphocyte development. Primary B lymphopoiesis occurs in the bone marrow of adult mammals, in parallel with the production of erythroid and myeloid cells such as neutrophils and macrophages (35–38), whereas T cell development occurs in the thymus (39, 40). As shown in Figs. 1 and 2, precise stages of lymphopoiesis in each of these primary lymphoid tissues can be identified based on the expression of various cell surface determinants on developing cells. In both the bone marrow and thymus, cell production occurs in association with a fixed population of stromal cells that are the source of various lymphopoietic growth and differentiation factors (41–44).

There is agreement that primary B cell development is abnormal in dw/dw mice, as indicated by the reduced frequency of $CD45R^+$ cells in the bone marrow. In the initial description of this defect, Murphy and colleagues (45) reported that the frequency of B lineage cells was decreased by more than 50%. This deficiency was confirmed by Montecino-Rodriguez *et al.* (46, 47). These data are consistent with findings that the bone marrow of hypophysectomized rats is leukopenic (48).

There is considerable controversy regarding the status of thymopoiesis in dw/dw mice. Baroni and associates (2–4) reported that the thymus of dw/dw mice is characterized by progressive early involution with a marked loss of thymocytes (2–4). In addition, cell proliferation, assayed by tritiated thymidine incorporation, was significantly depressed (2, 3, 49). More recent work in dw/dw mice reported thymic hypoplasia, total disappearance of the $CD4^+CD8^+$ subpopulation by 3 months of age, and premature involution (50). These observations are consistent with the reduction in thymus size and cellularity in hypopyhysectomized rats (1). However, other laboratories, including our own, have reported that the frequency of $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+$, and $CD8^+$ thymocytes in dw/dw mice is similar to that in their normal

TABLE 1. Characteristics of hormone-deficient mice

Strain	Defect	Hormone deficiency
Snell dwarf (dw/dw)	Mutation in pit-1 transcription factor	PRL, GH, IGF-I, T3/T4
Ames dwarf (df/df)	Mutation in prop-1 transcription factor	PRL, GH, IGF-I, T3/T4
$PRL^{-/-}$	Targeted disruption of PRL gene	PRL
$PRLR^{-/-}$	Targeted disruption of PRL receptor	Inability to respond to PRL
Little (lit/lit)	Mutation of GH-releasing factor receptor	GH, IGF-I
$IGF\text{-}I^{-/-}$	Targeted disruption of IGF-I gene	IGF-I
Hypothyroid (hyt/hyt)	Mutation of TSH receptor	T_3/T_4
$TR\alpha^{-/-}$	Targeted disruption of TRα gene	Reduced ability to respond to T_3/T_4

TABLE 2. Reported immune system defects in Snell dwarf mice

Reported effect on			
1° Lymphoid Development		2° Lymphoid Development	
B	T	Humoral	Cell-mediated
Reduced frequency of $CD45R^+$ cells in the bone marrow	Hypoplastic thymus; reduced frequency of $CD4^+CD8^+$ thymocytes; premature thymic involution	Depressed humoral immune response to T dependent antigens	Suppressed; delayed skin graft rejection; deficient delayed-type hypersensitivity reaction

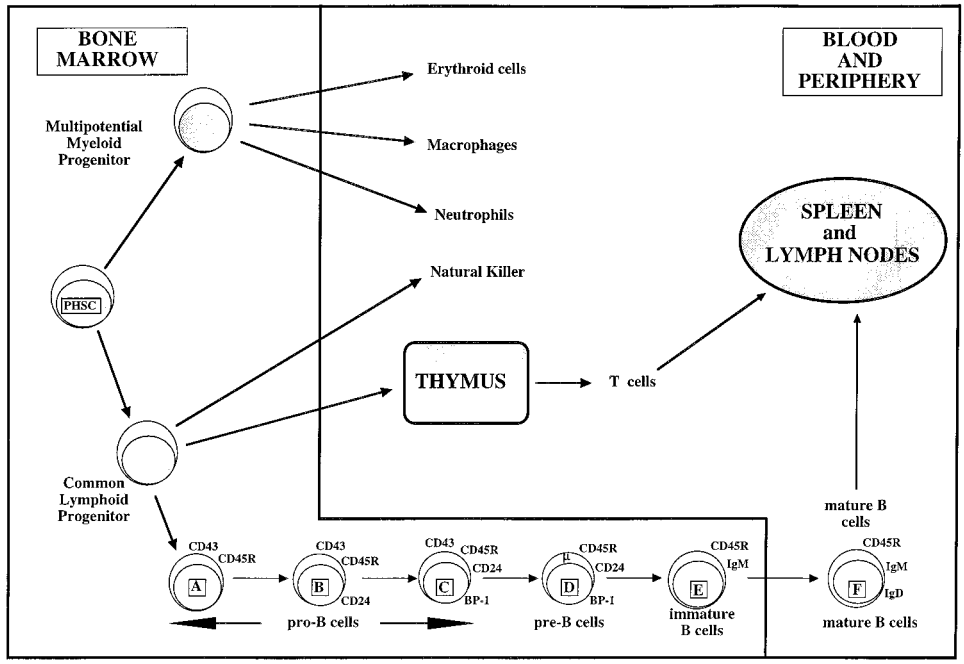

FIG. 1. The hematopoietic hierarchy with an emphasis on lymphopoiesis. Progeny of the pluripotent hematopoietic stem cell (PHSC) include myeloid- and lymphoid-restricted progenitors. The former generate erythroid and myeloid cells that include mediators of innate immunity, such as granulocytes and macrophages. Progeny of the common lymphoid progenitor include B cells, T cells, and, possibly, NK cells. The process of B cell development occurs in the bone marrow. Stages (A–F) of B cell development defined by the expression of CD43 (leukosialin), CD24 (heat-stable antigen), CD45R (a phosphatase), and surface immunoglobulin (sIg) as defined by Hardy *et al.* (38) are indicated. A bone marrow-derived precursor, which may include the common lymphoid progenitor, migrates to the thymus and sustains cell production in that organ (Fig. 2).

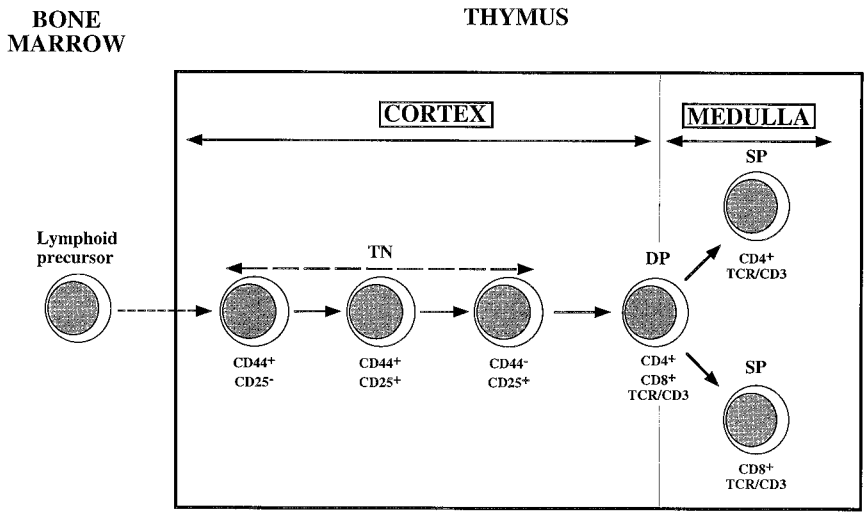

FIG. 2. T cell development in the thymus. Upon entering the thymus, multipotential cells commit to the T cell developmental pathway. The initial triple negative populations can be subdivided into four distinct stages based on the expression of CD44 (the hyaluronate receptor) and/or CD25 (the IL-2Rα receptor). As these cells initiate T cell receptor gene rearrangements, they mature to the double positive stage where they coexpress CD4 and CD8. Subsequent development results in the production of CD4 helper and CD8 cytotoxic T cells.

littermates (Refs. 47, 51, and 52 and Fig. 3). Furthermore, when corrected for the reduced size and weight of the mice, there were no significant defects in thymus cellularity in dw/dw mice (47).

B. Secondary lymphocyte development

Humoral immunity in dw/dw mice has generally been assessed by measuring antibody production after immunization with sheep red blood cells. The response to this T-dependent antigen by B cells is dependent on signals from $CD4^+$ T helper cells. T-independent B cell responses, elicited by multivalent antigens that are able to cross-link surface immunoglobulin (sIg), have also been described in rodents (53). Initial studies of dw/dw mice concluded that humoral immunity to sheep red blood cell immunization was deficient and that hemagglutinating antibodies were almost un-

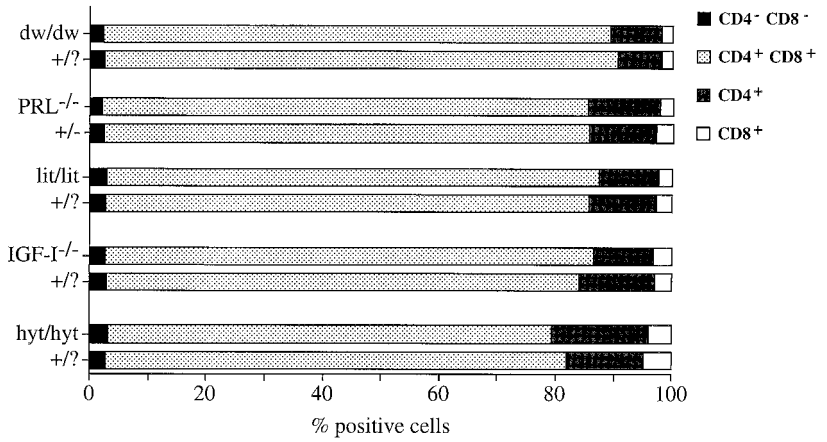

FIG. 3. Frequency of thymocyte subpopulations in hormone-deficient mice and their normal (+/? = +/+ or +/−) littermates. The frequency of $CD4^-CD8^-$, $CD4^+CD8^+$, and single positive populations defined by CD4 and CD8 expression is indicated. Data determined by two-color flow cytometric analysis. [Some of these data are taken from Refs. 46, 47, and 90.] There are no statisticaly significant differences between the frequency of thymocyte subsets between the hormone-deficient mice and their normal littermates.

detectable (3, 4, 49, 54–58). Similar defects in immune cell function in Ames df/df mice have been observed (59). These results are consistent with similar findings in hypophysectomized rats (10, 11, 57). However, studies from other laboratories have concluded that dw/dw mice respond normally to antigenic challenge (51, 52, 59a).

Contradictions also exist regarding cell-mediated immune responses. Delayed type hypersensitivity responses (56) and skin graft rejection (55, 58) in dw/dw mice were reportedly deficient. Similar findings have been observed in hypophysectomized rats (10, 11, 57). Delayed type hypersensitivity responses primarily involve $CD4^+$ T cells while rejection of skin grafts is dependent on $CD4^+$ and $CD8^+$ T cells (60, 61). However, defective cell-mediated immune responses are not uniformly observed in other studies. For example, Schneider (52) reported the response of dw/dw mice to contact-sensitizing agents to be normal. Infection of dw/dw mice with lymphocytic choriomeningitis virus (LCMV) and *Listeria monocytogenes* (LM) has been performed in our laboratory. The ultimate control of LCMV is completely dependent upon $CD8^+$ T cells. LM clearance is dependent on both $CD4^+$ and $CD8^+$ T lymphocytes, although the initial (day 2 post-infection) response is mediated by cells of the innate immune system (62). Neither pathogen was fatal to dw/dw mice, a finding consistent with a normal cell-mediated response of dw/dw mice on day 6 post-LM infection (59a). However, the frequency of LCMV-responsive $CD8^+$ T cells may be lower in dw/dw mice than in their +/? littermates (our unpublished observations).

C. Innate immunity

The principal cellular effectors of innate immunity are phagocytic neutrophils, macrophages, and natural killer (NK) cells (Fig. 1), and these populations provide defenses that are in place before challenge by antigens (63, 64). Detailed studies of innate immunity in dw/dw mice have not been made, but studies of hypophysectomized rats suggest a role for pituitary hormones in innate immunity. Hypophysectomized rats are more susceptible to the lethal effects of bacteria such as *Salmonella typhimurium* than are sham-operated animals due to an inability of macrophages to become activated normally (65, 66).

Preliminary data showing that bacterial loads in dw/dw mice 2 days after LM infection were significantly higher than in their littermates (59a) corroborate this finding and suggest that one or more hormones may be important for innate immunity.

D. Hormones and stress

The dw/dw mouse studies suggest a role for PRL, GH, IGF-I, and/or thyroid hormones in various aspects of immunity, but the above inconsistencies, which have not been emphasized in previous reviews, must be reconciled to define the instances in which this is the case. The unifying concept that emerges from reconsidering these studies is that immune system defects in dw/dw mice, and the positive effects of hormones on restoring thymopoiesis or antigen-responsiveness to normal (49, 54, 55, 67), have been observed primarily when animals were stressed because of suboptimal environmental conditions. Hypophysectomized rats are also hypersensitive to environmental stress (57), so this premise may apply to that model as well. Thus, a hypothesis that will be developed in this review is that positive hormonal effects on immunity occur primarily as an adaptation to stress.

The conditions under which dw/dw mice have been bred and maintained appear to be a major stress determinant. For example, the age at which dw/dw mice are weaned has been shown to affect thymopoiesis (68). Pups weaned at 21 days of age have a reduced number of thymocytes, followed 1 week later by a reduced number of $CD4^+CD8^+$ cells. However, if mice are weaned at 30 days of age, their thymus phenotype does not differ from that in their normal littermates. A prolonged period of nursing might provide either general metabolic support or transfer of maternal cytokines to the pups (69), either of which could influence thymopoiesis.

Close examination of the data showing that the immune response is impaired in dw/dw mice also provides support for the position that deficient husbandry may result in severe stress, which the mice cannot manage. Initial studies showing that humoral and cell-mediated immunity were defective were performed at least 20 yr ago when conditions in animal colonies were likely to be more crowded and unsanitary than they are today. This, in turn, may explain why the dw/dw mice used in those studies had a life span of only 45–60 days (58, 59). In view of this observation, the possibility that the animals used in the earlier studies were not healthy must be

considered. Mice of either the Snell or Ames dwarf genotype housed under high-quality contemporary animal care conditions thrive and actually have a longer than normal life span. In the Ames strain, mice live at least 50% longer than their normal controls (70). However, even under modern conditions, dw/dw mice may remain very sensitive to stress. For example, the use of LCMV necessitated that dw/dw mice be removed to quarters that were not as rigorously maintained as the room in which they had been initially housed, and this change in conditions may have been stressful to the animals.

This point suggests that even in carefully controlled, contemporary colonies, how dw/dw mice are housed can affect the status of their immune system, and there is evidence that this is, in fact, the case. In the studies reported from our laboratory, dw/dw mice were caged separately from their normal littermates. Under these conditions, thymopoietic effects were not evident. Schneider (52) corroborated this finding and speculated that dw/dw mice housed apart from their littermates were under less stress and that this, in turn, resulted in a normal thymus. Recent experiments by Murphy (personal communication) indicate that housing dw/dw mice separately appears to reduce stress by removing larger, potentially dominant normal littermates that compete for food and water. Those studies confirmed our results that if dw/dw mice are separated from the normal mice, no thymus defects were observed.

A prediction, based on the hypothesis that PRL, GH, IGF-I, and/or thyroid hormones are stress-modulating hormones, is that they are not required for the generation of an immune response in normal, healthy animals. With the exception of a role for thyroid hormones in B cell development, the analysis of the hormone/hormone receptor-deficient mice generally supports this prediction. In view of this point, the organization of this review is based on the following two questions:

1. Is expression of endogenous PRL, GH, IGF-I, thyroid hormones, or their receptors required for development or function of the immune system in healthy animals?

2. Are there conditions in which endogenous or exogenously administered PRL, GH, IGF-I, and thyroid hormones hormone mediate positive immunoregulatory effects?

III. PRL

Numerous reviews, including a recent 1998 *Endocrine Reviews* article, have summarized the literature indicating that PRL can have effects on the immune system (20, 21, 24, 27, 71). However, data showing that immune system development and function are normal in PRL receptor (PRLR)$^{-/-}$ and PRL$^{-/-}$ mice indicate that it is not an obligate lymphopoietic hormone. After summarizing these results, a case will be made that when potentiating effects of PRL on the immune system are observed, it is under conditions when the organism is subjected to stress.

A. PRLR$^{-/-}$ and PRL$^{-/-}$ mice

In addition to dw/dw mice and hypophysectomized rats, anti-PRL neutralizing antibody-treated (72) or bromocriptine-treated (73–77) rodents have been used as models of PRL deficiency. Each has inherent limitations. For example, both dw/dw mice and hypophysectomized rats have multiple hormone deficiencies, making it difficult to ascribe particular effects solely to PRL. An additional complication arises from the fact that extrapituitary cells, such as lymphocytes, may secrete PRL by pit-1-independent mechanisms (78). If antibodies are used to neutralize PRL *in vitro* or *in vivo*, it is critical to confirm their specificity by parallel use of nonneutralizing antibodies of the same isotype. This essential control has not always been performed (79, 80). Even if specificity of the antibody for PRL is ensured, the resulting immune complexes are important complicating factors to be considered.

Numerous studies of immunity in bromocriptine-treated rodents also have been conducted. Bromocriptine is a type-2 dopamine receptor agonist that results in suppression of circulating PRL (73). An inherent assumption in using this drug is that its effects are attributable to PRL deficiency. Studies that have used bromocriptine treatment *in vitro* or *in vivo* must be interpreted cautiously in view of data showing that direct effects on B and T cells (81, 82), possibly mediated through binding of bromocriptine to D2-like receptors on lymphocytes (83), can occur. Thus, specific immune defects in bromocriptine-treated animals may correlate with a decline in circulating PRL concentrations, but these effects may not be causally attributable to PRL deficiency. For example, the reduced proliferative response of lymphocytes harvested from bromocriptine-treated mice in a mixed lymphocyte reaction (75) may be due to direct effects of bromocriptine on responding T cells (81). Direct effects of bromocriptine on immune cells may also explain why its use impaired the response to LM in mice (84). Nevertheless, the precise means by which bromocriptine inhibits lymphocyte function is controversial (85).

These concerns precipitated the development of additional models to assess the effects of PRL and PRL-signaling deficiency. Mice with deficiencies in PRL signal-transducing molecules, such as signal transducer and activator of transcription 5 (Stat5) and Janus kinase 2 (Jak2), have been described (86–88), but these proteins transduce signals from a variety of cytokines in addition to PRL. In 1997 Kelly and colleagues (89) and Horseman *et al.* (90) described mice with targeted disruptions of the PRL receptor (PRLR$^{-/-}$) and PRL (PRL$^{-/-}$) genes, respectively. Females of both of these knockout strains exhibited profound defects in reproduction and mammary gland development, as would be expected from the classically described actions of PRL. However, they have also been of great value in determining whether or not PRL is required for normal development and function of immune cells. Transgenic mice overexpressing PRL have also been described (91), but no data on their immune system have been reported.

B. Primary lymphocyte development in PRLR$^{-/-}$ and PRL$^{-/-}$ mice

Hematopoietic cells in murine bone marrow and thymus express the PRLR (92), but the precise frequency is unclear. One study reported that more than 90% of cells in these primary lymphoid tissues were PRLR^{+} (93), but a subsequent report from that same group has called into question the specificity of the anti-PRLR antibody employed (94). The

expression of the PRLR on bone marrow (95) and thymic (96) stromal cells has also been reported, raising the possibility that PRL may have both direct and indirect effects on lymphopoiesis. The functional importance of stromal cell expression of the PRLR has not been resolved. The highest level of PRLR expression on marrow stroma is on adipocytes, which are normally associated with inactive hematopoiesis. In the thymus, PRL stimulated secretion of extracellular matrix components and thymic hormones by stroma (97). However, the precise role of thymic hormones in T lymphopoiesis is controversial (98).

1. Thymopoiesis. Data showing that thymopoiesis was normal in healthy dwarf mice (Refs. 47 and 52 and Fig. 3) provided strong evidence that PRL is not an obligate thymopoietic hormone, and this view has been recently corroborated in studies of the $PRLR^{-/-}$ and $PRL^{-/-}$ mice. The number of thymocytes present in each of these knockout strains is comparable to that in their normal littermates, and there is a normal distribution of CD4- and CD8-expressing populations (Refs. 90 and 99 and Fig. 3). Taken together, these data indicate that PRL deficiency has no adverse effect on normal thymopoiesis in healthy animals.

2. B lymphopoiesis. Analysis of $PRLR^{-/-}$ and $PRL^{-/-}$ mice indicate no requirement for PRL in B cell development. The frequency and absolute numbers of B lineage cells are normal in the knockout mice (90, 99), a finding consistent with results demonstrating that B cell developmental defects in dw/dw mice are not corrected by PRL administration (90).

C. Secondary lymphocyte development in $PRLR^{-/-}$ and $PRL^{-/-}$ mice

Expression of the PRLR by peripheral blood lymphocytes and hematopoietic cells in secondary lymphoid tissues has been detected at the mRNA and protein levels (94, 100–106). Approximately 80% of the cells in the spleen and 50–70% of cells in lymph nodes are $PRLR^{+}$. Within these tissues, virtually all B cells and macrophages are $PRLR^{+}$, while approximately 70% of T cells express the receptor (93). Again, because of concerns regarding the specificity of the anti-PRL antibody used (94), the precise frequency of $PRLR^{+}$ cells may be lower.

Scatchard analysis demonstrated that B and T cells express approximately 360 receptors per cell (104). This compares with 12,000 receptors per cell on the PRL-dependent Nb2 lymphoma cell line (105, 107). Based on phenotypic analysis, it has been suggested that Nb2 arose from a thymocyte at some intermediate stage of differentiation (108), but the relationship of this cell line to normal thymocyte development remains open to interpretation. Although the relatively low number of receptors on normal lymphocytes might imply a relatively low sensitivity to the hormone, PRL target tissues such as mammary epithelium express the receptors at a comparably modest level (109). There are multiple receptor isoforms as well (71). Thus, it is difficult to correlate PRLR number with sensitivity to PRL.

Although correlative studies suggest a link between humoral immunity and PRL levels (110), studies of $PRLR^{-/-}$ and $PRL^{-/-}$ mice indicate that PRL is not required for the generation of an immune response in healthy mice. Antibody production in response to immunization with T-dependent and T-independent antigens is normal in both strains (59a, 99). Cell-mediated immunity has also been evaluated in LCMV- and LM-infected PRLR or PRL knockout mice. Both strains exhibited a normal response to LM, and $PRL^{-/-}$ mice were also able to respond to LCMV comparable to their normal littermates (Refs. 59a and 99 and our unpublished observations). These data are consistent with studies showing a normal proliferative response of $PRLR^{-/-}$ splenocytes (99) and observations that the number and frequency of B and T cells in the spleen and lymph nodes of the $PRL^{-/-}$ mice are comparable to values in their normal littermates (90).

D. Innate immunity in $PRLR^{-/-}$ and $PRL^{-/-}$ mice

The PRLR has been reported to be expressed on cells that mediate natural immunity, including NK cells (111, 112) and macrophages (93, 100). Effects on NK cells may occur, although the data in this regard have been inconsistent and dependent upon the PRL concentration used (113–116). Innate immunity appears to be unaltered in the absence of PRL signaling, since both $PRLR^{-/-}$ and $PRL^{-/-}$ mice elicited a normal response to LM during the initial stages of the infection (59a, 99). As noted above, these results directly contradict a report that the response to LM is deficient in bromocriptine-treated mice (84).

E. PRL as a stress-adaptation hormone

Although the $PRLR^{-/-}$ and $PRL^{-/-}$ mouse studies indicate that PRL does not play an obligate role in the immune system, effects of PRL on hematopoietic cells can occur. For example, under selected conditions PRL can stimulate lymphocyte proliferation (117, 118), and in some cases this may be mediated through effects on induction of the interleukin-2 (IL-2) receptor (119, 120). It has also been shown that PRL can enhance production of granulocytes and macrophages (121).

A common thread among those studies that have documented PRL effects on immunity is once again stress. For example, the most marked *in vivo* effects of PRL commonly employed rodents that were either chemically (bromocriptine or azidothymidine) treated (75, 84, 121), surgically manipulated (1, 10, 11), or handled repeatedly to administer injections. The thesis in this review is that it is under these conditions that PRL effects on the immune system are manifest. This premise is consistent with the well documented, stress-induced release of PRL in a variety of species. Whether or not the actions of stress-induced PRL are specific for the immune system is not clear, and given the pleiotropic effects of this hormone (71), this seems unlikely. Nevertheless, it has been suggested previously that the stress-induced release of PRL may have positive consequences for the maintenance of the immune system (122–124), particularly, as discussed below, in countering the negative effects of glucocorticoids.

If PRL effects on the immune system are manifest primarily during stressful challenge to the organism, one prediction is that mice subjected to systemic stress would fare better if PRL levels were elevated. This prediction is consistent with experiments demonstrating a protective effect of PRL ad-

ministration after hemorrhagic shock (125, 126). A second prediction is that PRL should in general have minimal effects on the immune system in animals not subjected to such challenges. This also appears to be the case.

No effects of exogenous PRL on primary B cell development in healthy dw/dw mice were observed (90). Although Fig. 4A demonstrates an increased number of thymocytes in PRL-treated dw/dw mice, this is not a consistent effect (our unpublished observations), and some studies have even reported a decline in thymus cellularity after PRL treatment (127). However, a consistent observation in our studies is that no effect of PRL administration on the frequency of CD4- and CD8-expressing thymocyte subpopulations in dw/dw mice was ever observed (Ref. 47 and Fig. 4B).

There is also little evidence to support a role for exogenous PRL on immune cell function in healthy animals. Murphy *et al.* examined the effects of PRL on the immune response to keyhole limpet hemocyanin (KLH) in dwarf mice and their normal littermates. These mice were divided into saline- and PRL-treated groups. Seven days after KLH immunization, T cells were harvested from the draining lymph nodes and rechallenged with KLH *in vitro*, and the extent of antigen-specific proliferation was measured. There was only a modest increase in antigen-specific proliferation by T cells from the normal, PRL primed animals and, as noted by the authors, this was not a consistent finding. In this experiment, the PRL-treated dwarf mice did exhibit increases in lymph node cellularity and antigen-specific proliferative responses to KLH. However, these dwarf mice were housed with their normal littermates (127), which, as pointed out, may have subjected the animals to stress. Finally, the effects of PRL on granulocyte and macrophage production in the bone marrow of normal mice are also modest (121).

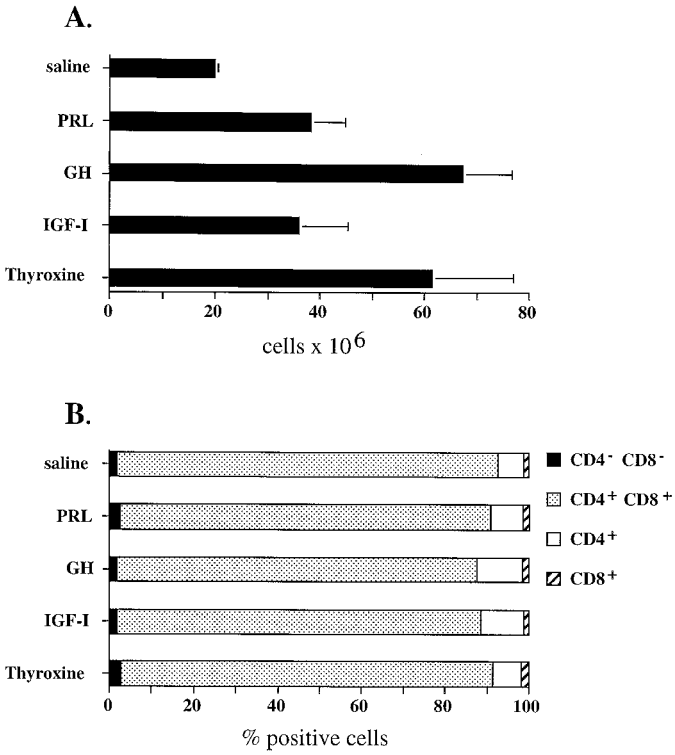

FIG. 4. Effects of PRL, GH, IGF-I, and T_4 on (A) total number of thymocytes and (B) the distribution of thymocyte subpopulations in the thymus of dw/dw mice. Mice received the indicated hormone treatment for approximately 2 weeks by sc injection. [Data based on results amended from Refs. 46, 47, and 90, and our unpublished observations.] There are no statistically significant differences in the frequency of thymocyte subpopulations (panel B) between the saline- and hormone-treated mice.

In fact, elevated PRL concentrations may be detrimental. For example, high doses of PRL may depress anti-tumor cell responses, possibly by inhibiting NK cell function (114, 115, 128). Reports that elevated levels of PRL are found in autoimmune diseases, and that lowering them is beneficial, raises the possibility that elevated levels of PRL could interfere with key immunoregulatory functions (129–131).

The PRL and PRL receptor knockout mice provide excellent models to examine the hypothesis that PRL is a stress-adaptation hormone. Subjecting these mice to one or more stressful stimuli will provide a context for studying the adaptive role of PRL. Under such conditions, it may be possible to examine the capacity of PRL treatment to restore immune function to normal. Documentation that PRL is effective under such conditions could have highly significant clinical consequences.

F. Molecular mechanisms of stress modulation by PRL

Glucocorticoids are critical for survival during stress, but a negative side effect is their potentiation of apoptosis in lymphoid cells (132–134). Thus, positive regulatory signals that counteract this effect may be critical to lymphocyte survival, and recent studies have provided evidence of a PRL signaling pathway that may do so and thus mediate many of the stress-modulating actions of PRL (Ref. 135 and Fig. 5).

After glucocorticoids bind to their cytoplasmic glucocorticoid receptors (GRs), the ligand-receptor complex then recognizes a specific DNA response motif, the glucocorticoid response element (GRE; Fig. 5). Groner and colleagues demonstrated that activated Stat5, a signaling molecule required to confer the response to PRL, associates with the glucocorticoid-GR complex. The binding of activated Stat5 to GR inhibits signaling through the GRE by about 75%. Simultaneously, signaling through a Stat5-responsive element is enhanced (135).

The potent inhibition of glucocorticoid signaling by activated Stat5 provides a mechanism by which PRL may act as a positive immunoregulator in a limited way. Under conditions in which glucocorticoid levels are elevated, PRL may reduce the immunosuppressive effects of glucocorticoids, thus providing a mechanism that leads to a homeostatic normalization of cellular functions. Conversely, if glucocorticoid levels are not elevated, PRL would have no immunoprotective or immunostimulatory effect through this mechanism (135).

The amplification of PRL signaling by glucocorticoids depends on physical interactions of Stat5, but not the GR, with DNA (136). The mechanism for the suppression of glucocorticoid signaling is unclear. Although both Stat proteins and the nuclear hormone receptors interact with the common coactivators CBP (CREB-binding protein) and p300, competition for these coactivators did not account for negative

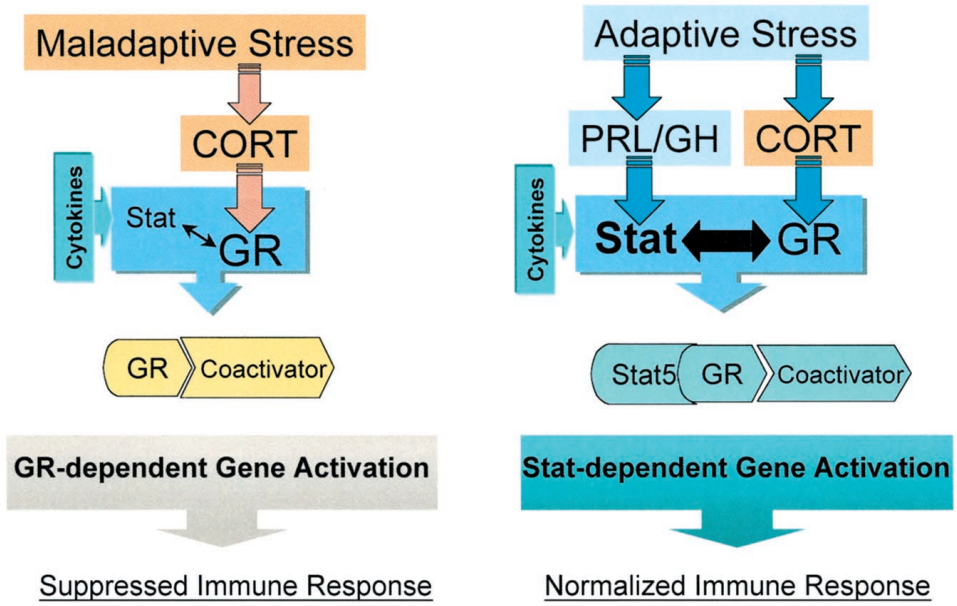

FIG. 5. Hypothetical mechanisms for interactions between endocrine pathways in stress. Glucocorticoids (CORT) cause immunosuppression through GR-mediated mechanisms. Chronic, uncompensated elevation of GR signaling would be considered maladaptive with regard to the immune system. Immune cells express active PRL and/or GH signaling systems. In these cells activated Stat5 interacts with GR, thereby preventing GR-mediated signaling, and facilitating Stat-driven gene expression. This adaptive stress response is proposed to be a means of normalizing the functions of immune cells (and other cells) in the presence of chronically elevated glucocorticoids.

regulation of GR signaling (137). One of the responses to glucocorticoids that is inhibited by PRL is apoptosis of lymphocytes in culture through signaling pathways that include induction of pim-1 and members of the bcl-2 family (138–141). It is unknown whether the antiapoptotic action of PRL on lymphocytes is mediated by the interaction between Stat5 and GR. There may be additional mechanisms by which PRLR activation modulates stress responses in the immune system, including direct induction of antiapoptotic or lymphoproliferative genes (138). Nevertheless, the interaction of PRL-activated Stat5 with GR provides a specific mechanism to test in the context of stress-modulating actions of PRL.

The distribution of receptors for PRL and glucocorticoids supports the concept that these hormones interact in specific tissues to optimize adaptations to stress. Liver and skeletal muscle are critical tissues for mediating the metabolic (*i.e.*, gluconeogenic) actions of glucocorticoids. Skeletal muscle has the lowest level of PRLR expression of any tissue, and the predominant PRLR expressed in liver is a short form that does not mediate Stat5 activation (142). Therefore, in these tissues there is no provision for PRL to inhibit glucocorticoid signaling through activation of Stat5. However, immune tissues, as well as other cell types that coexpress the long form of the PRLR and GR (92), could be protected from the chronic effects of GR activation by the negative interaction of Stat5 with GR.

It is important to recognize that the primary physiological context for PRL action is during pregnancy and lactation. These states can be considered to be conditions in which the physiology is under a chronic stress, requiring mobilization of a wide range of metabolic adaptations. During these times, both glucocorticoids and PRL are elevated, leading to an adaptive stress response. Glucocorticoids provide for glucose mobilization, insulin resistance, and protein flux. Suppression of GR signaling by PRL-activated Stat5 during pregnancy and lactation in immune cells and other tissues provides one potentially important way to minimize the negative effects of high glucocorticoid levels in pregnant or lactating females (Fig. 5). It is less clear how these systems might interact in other types of stress. Further research in this area is warranted.

IV. GH and IGF-I

Although the functions of GH and IGF-I do not overlap entirely, many GH effects are mediated through IGF-I induction (18, 143). Also, in rodents and primates, human GH binds to and activates the PRLR, so some GH effects may be mediated through the PRLR (144). This may be of consequence in pharmacological cases of hGH administration. The effects of GH and IGF-I on the immune system have been surveyed in several recent reviews (18, 20, 27), but whether or not they play an obligate role in immune system development and function has not been addressed.

The analysis of IGF-I$^{-/-}$ and little (lit/lit) mice strongly suggests that neither GH nor IGF-I is required for primary lymphopoiesis, and additional studies with the latter strain further indicate that these hormones are not essential for lymphocyte effector function. Nevertheless, there are clearly instances in which GH and IGF-I are immunostimulatory. After reviewing the status of the immune system in GH- and IGF-I-deficient mice, a case will be made in *Section IV.D* that, as with PRL, these stimulatory effects are most beneficial to the animal in times of growth or stressful challenge to the organism. In this instance, however, the immune potentiating effects of GH and PRL are not immune system specific and are most appropriately appreciated in terms of their actions as general anabolic/somatogenic hormones.

A. lit/lit and IGF-I$^{-/-}$ mice

The lit/lit strain of mice was described by Eicher and Beamer in 1976 (145) and is characterized by an approximately 90% deficiency in serum GH levels. The lack of GH, in turn, results in 90–95% suppression of circulating IGF-I (146, 147). The autosomal recessive defect in lit/lit mice is

due to the mutation of a single nucleotide in the gene encoding the GHRF receptor. As a result, somatotrophs in the anterior pituitary gland fail to secrete GH because they do not bind GHRF (148). This defect produces a condition that mimics human isolated GH deficiency type 1 (149). lit/lit Mice have normal levels of TSH, and thus thyroid hormones, and slightly reduced-to-normal levels of PRL (7). Thus, the overall hormone defect in little mice is less severe than observed in dwarf mice.

A caveat that must be raised in use of lit/lit mice is whether or not all GH production is deficient in that strain, since local production of GH by extrapituitary cells, including lymphocytes, has been described (27, 150–153). In view of the small size of these mice, it is clear that low levels of hormone are not sufficient to mediate normal function. It is reasonable to expect that some demonstrable immune system defect would be observed in lit/lit mice if GH and/or IGF-I were required for normal immunity. Nevertheless, it is important when using little mice to correlate the data acquired with results obtained by analysis of other GH-deficient strains. It is for this reason that analysis of the immune system in mice with a targeted defect in GH or its receptor would be important. The latter strain of mice has recently been described, but its immune system status has not been reported (154).

There is less ambiguity in the analysis of IGF-I's role in the immune system. Two laboratories have produced mice in which the gene encoding IGF-I (155, 156) or its receptor (157) has been disrupted. As described below, these mice have been useful for evaluating the effects of IGF-I on the immune system. However, generating sufficient numbers of IGF-I$^{-/-}$ animals with which to work is a challenge, because the lack of IGF-I causes developmental abnormalities that result in a high death rate of homozygotes soon after birth. Mortality is due to respiratory problems that result from poor development of muscles such as the diaphragm. Thus, an extensive breeding program must be maintained to generate enough viable mice for analysis, and the animals that survive are only a subpopulation of the IGF-I$^{-/-}$ progeny in any particular litter.

B. Primary lymphocyte development in lit/lit and IGF-I$^{-/-}$ mice

Compared with the wide distribution of the PRL receptor on thymus and bone marrow cells, GH receptor (GHR) expression is more restricted. Flow cytometric analysis indicates that 1–4% of thymocytes and 20–40% of bone marrow cells, depending on the strain of mouse analyzed, are GHR$^+$ (158, 159). Among the CD45R$^+$ bone marrow cells, 29% are GHR$^+$ (159). The IGF-I receptor (IGF-IR) has been identified on rodent and human thymocytes (160–163) and, in view of effects on B lineage cells, its expression on that lineage can be inferred.

Although the pituitary gland is the major source of GH, cells in the thymus can produce both GH (158, 164) and IGF-I (165, 166). GH mRNA (167) and IGF-I (168, 169) production in the bone marrow have also been detected. In particular, stromal cells have been shown to be a source of IGF-I (168, 169). The distribution of GH/IGF-IRs in primary lymphoid tissues (18, 170) and the potential for local production of GH and IGF-I raise the possibility that complex autocrine- and paracrine-regulatory interactions occur. For example, anti-IGF-I antibodies can abrogate GH effects in the thymus, suggesting that GH effects are mediated through local induction of IGF-I (171, 172).

Does the widespread distribution of GH/IGF-IRs and their ligands and reported lymphopoietic effects of these hormones imply that GH and IGF-I play an obligate role in lymphopoiesis? As reviewed below, the analyses of the genetically mutant mice suggest that they do not.

1. Thymopoiesis. The possibility that GH or IGF-I is required for thymopoieis has been assessed in lit/lit and IGF-I$^{-/-}$ mice. The results of these studies suggest that neither is an obligate thymopoietic hormone. In both strains the absolute number of cells in the thymus, corrected for the smaller size of the mice, and the proportions of CD4$^-$CD8$^-$, CD4$^+$CD8$^+$, CD4$^+$, and CD8$^+$ thymocytes were normal when mutant mice were compared with normal littermates (Ref. 47 and Fig. 3).

2. B lymphopoiesis. B cell development in lit/lit and IGF-I$^{-/-}$ mice is normal. In both strains, the frequency of B lineage cells and their absolute number, when corrected for the size of the mice, were not different from controls (47). Taken together with observations that neither GH nor IGF-I restored defective B lymphopoiesis to normal in dw/dw mice (46, 47), these data indicate that neither GH nor IGF-I is an obligate B lineage immunoregulatory hormone.

C. Secondary lymphocyte development in lit/lit mice

Lymphocytes in peripheral blood and in secondary lymphoid tissues express the GH and IGF-IRs (159, 173). Approximately 50% of B cells and macrophages and 20% of T cells in spleen, lymph nodes, and peripheral blood are GHR$^+$. The IGF-IR is also expressed on helper and cytotoxic T cells and B cells (Refs. 174–176; reviewed in Refs. 18, 20, and 27). Immunoreactive IGF-I has been detected in a small population of rat splenocytes (177). Consequently, autocrine and paracrine effects of GH and IGF-I in secondary lymphoid tissues are possible.

Humoral immunity has been assessed in lit/lit mice immunized with T-independent and T-dependent antigens, and the responses observed were comparable to those in normal littermates (59a). Similarly, no defects in cellular immunity were detected in lit/lit mice infected with LCMV or LM. While it will be important to substantiate these data further by studies of humoral immunity in GHR$^{-/-}$ mice, the lit/lit studies are consistent with reports that GH-deficient children and GHR-deficient Laron dwarf children are not clinically immunodeficient (reviewed in Refs. 18 and 20).

D. Innate immunity in lit/lit mice

Receptors for GH and/or IGF-I are expressed on myeloid and NK cells (15), raising the possibility of GH or IGF-I effects on these populations. lit/lit Mice were able to clear LM infection during the initial phase of the infection as efficiently as their normal littermates (59a), suggesting that innate immunity is not GH or IGF-I dependent.

E. The relationship between immune and somatic actions of GH and IGF-I

The above observations strongly suggest that neither GH nor IGF-I is essential for immune cell development or function. However, the literature documenting their potential to stimulate the immune system at multiple levels is extensive (20, 27). For example, the administration of GH or IGF-I to normal or Snell dwarf mice consistently and reproducibly increases thymus size and thymocyte numbers (Refs. 161, 178, and 179 and Fig. 4A). IGF-I can potentiate B lymphopoiesis by promoting maturation of $CD45R^+/c\mu^+$ pre-B cells from $CD45R^-$ precursors (168) *in vitro*, and *in vivo* treatment of normal mice with IGF-I also results in an increase in the total number of B lineage cells in the bone marrow (180). Additionally, IGF-I can synergize with IL-7 to stimulate proliferation of $CD45R^+$ cells (181).

GH and IGF-I can also stimulate immune cell function. Treatment of 9-month-old mice with recombinant human IGF-I significantly enhanced the initial response to a T-dependent antigen and subsequent rechallenge with the antigen. These augmented responses were paralleled by an increase in the size and cellularity of secondary lymphoid organs such as the spleen (182). Similar stimulatory effects of IGF-I in mice after bone marrow transplantation have been observed (180).

GH and IGF-I could affect lymphocyte function in several ways. One possibility is that they stimulate the growth of antigen-responsive clones of B and T cells, thereby increasing the number of responding cells. *In vivo* studies showing that the frequency and absolute number of splenic $CD45R^+$ cells in the S phase of the cell cycle are significantly increased in IGF-I-treated mice are consistent with this possibility (180). These hormones could also increase the survival of antigen-responsive cells (183).

Additionally, GH and/or IGF-I can enhance innate immunity through effects on myeloid and NK cells. In concert with lineage-specific growth factors, such as the colony stimulating factors, GH and IGF-I can stimulate myelopoiesis (184), possibly through antiapoptotic effects (183, 185). The latter action may increase the number of cells that can respond to lineage-specific cytokines. GH or IGF-I could also act indirectly by enhancing production of myeloid-potentiating cytokines from stromal cells in the bone marrow microenvironment. Exposure of mature myeloid cells to GH and IGF-I may also lead to their activation. Granulocytes and macrophages have been demonstrated to increase production of reactive oxygen metabolites after IGF-I treatment (186, 187). Effects of IGF-I on increasing NK cell cytotoxicity have also been reported (174).

Understanding the role of GH and IGF-I on the immune system is dependent upon reconciling these effects with the normal immunity in GH- and IGF-I deficient mice. In this regard, it is useful to consider GH and IGF-I in terms of their well defined roles as general growth-promoting mediators, with actions on multiple organs. In addition to their expression on cells in lymphoid organs, receptors for GH and IGF-I are widely distributed on nonlymphoid populations as well. As a result, administration of these hormones may have salutary effects on multiple organ systems, including the immune system, through direct or indirect pathways. Thus, after 2 weeks of GH or IGF-I treatments, Snell dwarf mice exhibit a weight gain of up to 4 g, which is caused by increased size of multiple tissues that include those of the immune system (Ref. 47 and Fig. 6). Even under conditions when obvious weight increases in experimental animals are not observed, GH or IGF-I may alter growth and metabolism in various cells. In view of these general anabolic actions, it is important that the effect of GH and IGF-I on immune cells be considered in the context of somatic growth and metabolism rather than as immune specific events. This point will not be appreciated if studies of GH and IGF-I are limited to measuring effecs on isolated lymphoid populations *in vitro*.

In primary lymphoid organs, GH and IGF-I appear to act as "proportionate" regulators. Thus, marrow cellularity increases, but there is no preferential growth of particular lineages. For example, IGF-I treatment increased the absolute number of cells of all lineages, including $CD45R^+$ cells, in the marrow of normal mice, but it had no effect on their frequency (180). Such growth-promoting effects of IGF-I on the marrow have been previously observed (188). One interpretation of these results is that, as a consequence of their somatogenic effects, GH or IGF-I treatment increased overall bone volume with an accompanying increase in bone marrow cellularity. Even in adults where overall growth of long bones is limited, increases in bone density with remodeling of the medullary cavity have been observed (149). The premise that GH and IGF-I are proportionate growth regulators is in agreement with previous studies showing that GH treatment of dwarf mice had no specific effects on the cellular components of the bone marrow (46). Similarly, these hormones increase thymus cellularity (Fig. 4A) but have no effect on the relative proportions of thymocyte subpopulations (Fig. 4B).

Precisely how GH and IGF-I act on cells within a tissue such as the bone marrow and thymus to promote proportionate growth is unclear, and the actions on particular cell populations may be distinct. Thus, GH or IGF-I could promote differentiation of some cells, proliferation of others, and survival in yet other populations. The potential of these hormones to promote survival of hematopoietic populations may be particularly important. In cells with normally short life spans, particularly some myeloid cells, a modest increase in longevity may have significant effects on the overall number of cells responding to a particular challenge.

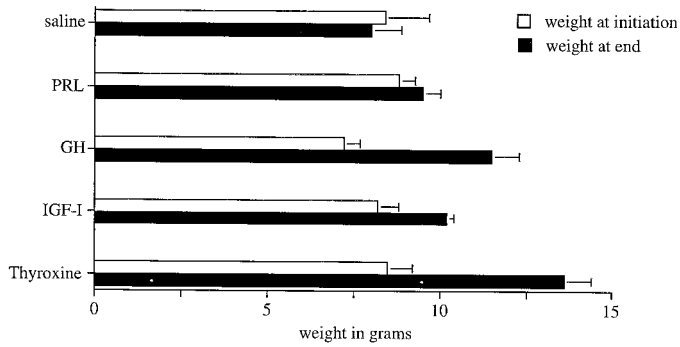

FIG. 6. Effects of PRL, GH, IGF-I, and T_4 on the weight of dw/dw mice. [Data based on Refs. 46, 47, 59a, and 90.]

Despite the fact that their actions may not be immune system specific, GH and IGF-I may have highly beneficial effects on the immune system in times of stress. Such immune potentiating effects of GH/IGF-I may be clinically exploited. For example, chemotherapeutic regimens have deleterious effects on cell production in multiple systems. The growth-enhancing effects of GH and IGF-I on the thymus and spleen suggest that one or both of these hormones could be used to accelerate the recovery of lymphopoiesis after such treatments. Indeed, effects of IGF-I on the recovery of thymus cellularity after cyclosporine treatment have been reported (189). Similar effects of hormonal therapy in counteracting negative effects on the immune system due to disease may also be demonstrable (190). The ability of GH and IGF-I to enhance immune reconstitution after bone marrow transplantation in irradiated (180) or immunodeficient hosts (191) is an additional case in point. Again, the augmented immune reconstitution documented in rodents may be only one of the multiple system effects of these hormones, but the end result is beneficial.

A final context in which GH and/or IGF-I may be of benefit is during aging (192). GH and IGF-I levels decline with age (193–198), and it can be argued that this results in declines in multiple systems, including the immune system, that stress the organism and result in disease susceptibility. It may be advantageous to delay or reverse these declines, and this has provided a rationale for using GH and IGF-I to rejuvenate the involuted thymus.

F. Effects of GH and IGF-I on thymic involution

Multiple studies have conclusively demonstrated that administration of GH or IGF-I to aged rodents significantly increases thymic cellularity (161, 199–202). However, there appear to be limitations on the extent to which GH and IGF-I can stimulate thymopoiesis in old animals, because cell production is not restored after hormone administration to levels present in young animals. In all reported instances in the literature, treatment of mice or rats with exogenous GH or IGF-I alone failed to restore thymus cellularity to that present in young animals. Our own experience is that IGF-I administration approximately doubles thymocyte numbers, and consistent with previously reported results, this reflects a proportionate increase in the number of all CD4 and/or CD8 thymocyte subpopulations (161). This finding suggests that additional defects that accumulate during aging may contribute to thymic atrophy.

It has been reported that the number of bone marrow T cell precursors in old mice is reduced by approximately 40-fold and that these cells have reduced thymus colonization ability (203–207). In view of this, additional studies in which old mice were reconstituted with bone marrow cells from young donors in addition to receiving IGF-I were conducted in our laboratory. Under these conditions, thymus cellularity was consistently greater than in animals that received hormone or bone marrow cells alone. Results from *in vitro* studies using the fetal thymic organ culture system suggest that IGF-I acts by potentiating thymic colonization by bone marrow T cell precursors (161) and/or that the hormone affects some other event soon after thymus colonization (191, 208, 209).

Despite the focus on the thymus in these aging studies, it is again important to emphasize that the effects of GH and IGF-I are not thymus specific. The decline in circulating GH and IGF-I that occurs with age results in diminished size of multiple tissues such as muscle and bone. Increases in kidney size and overall weight gain of aging mice and an increase in lean body mass, skin thickness, and bone density in elderly men after GH or IGF-I administration also provide evidence for the multiorgan effects of these hormones (210).

V. Thyroid Hormones

Although the role of thyroid hormones in primary and secondary lymphopoiesis has recently been reviewed (211), in general their immune system effects have received comparatively less attention than PRL, GH, and IGF-I. Interest in thyroid hormones has recently been stimulated by complementary findings that primary B cell development is defective in hyt/hyt and TR$\alpha^{-/-}$ mice. As detailed below, only B lymphopoiesis, and not development in other hematopoietic lineages, is defective in the absence of thyroid hormone signaling. On the other hand, the hyt/hyt mouse studies suggest no requirement for thyroid hormones in secondary lymphocyte development.

A. hyt/hyt and TR$^{-/-}$ mice

An autosomal recessive mutation that causes hypothyroidism was identified in the mouse by Beamer and colleagues in 1981 (212). Mice with a mutation in the gene, named *hyt*, have retarded growth, infertility, and a hypoplastic thyroid gland with very low to undetectable serum T_4 levels (212–214). Subsequent studies (215, 216) demonstrated that a mutation in a single base, leading to the replacement of a highly conserved proline at amino acid position 556 with a leucine in the transmembrane domain IV of the G-linked TSH receptor, results in TSH hyporesponsiveness of thyroid epithelial cells.

Three laboratories have recently reported on the development of mice with targeted disruptions of the thyroid hormone receptor (TR). TRs are members of the nuclear hormone receptor family, and they share significant structural and functional similarities with receptors for the steroid hormones and fat-soluble vitamins (217). The TR is encoded by two genes, TRα and TRβ, and multiple isoforms of each are generated as a result of alternative mRNA processing (218). Targeted disruption of TRα1 (219), the entire TRα locus (220), and TRβ (221) genes has been performed (reviewed in Ref. 107). In addition, crosses between the TR$\alpha^{-/-}$ and TR$\beta^{-/-}$ mice have been made (222).

No lethal developmental defects were reported in any of these mice, although TR$\beta^{-/-}$ mice have auditory abnormalities (221) and TR$\alpha1^{-/-}$ mice have a reduced heart rate (219). Disruption of the entire TRα gene resulted in mice with decreased production of thyroid hormone, growth arrest, and delayed development of both the small intestine and bones (220). As discussed below, hematopoietic defects occur in TR$\alpha^{-/-}$ mice.

B. Primary lymphocyte development in hyt/hyt and $TR^{-/-}$ mice

The TR is expressed in thymocytes (223) and bone marrow cells (224). A recent analysis of TR expression in the stages of B lymphopoiesis depicted in Fig. 1 revealed that TRα1 and TRβ1 message is expressed at all stages, TRβ2 expression was not detected, and the dominant-negative TRα2 isoform was differentially expressed (224). The TR is also expressed by bone marrow stroma (our unpublished observation) and thymic epithelial cells (225), raising the possibility that direct and indirect effects of thyroid hormones on lymphopoiesis may occur.

1. Thymopoiesis. T cell production in the thymus of hyt/hyt mice is normal. Both thymus cellularity and the frequency of CD4- and/or CD8-expressing populations are comparable to values in their normal littermates (Fig. 3 and Ref. 47). One study suggested a decreased thymus size in hypothyroid mice (226), but if thymus cellularity is normalized to the reduced size of hyt/hyt mice, thymocyte numbers are not significantly different from those in normal littermates (47).

In contrast to the hyt/hyt mice, thymopoiesis in TR$\alpha^{-/-}$ mice is suppressed. Although thymocyte subsets are present in normal proportions, by approximately 3 weeks of age thymus cell numbers were drastically reduced in the knockout mice (227). To determine whether the decline in thymus cellularity was due to an intrinsic defect in thymocytes or to environmental influences, T3R$\alpha^{-/-}$ × Rag1$^{-/-}$ chimeric animals were produced. No Rag$^{-/-}$ lymphocytes develop in the chimeras, because the Rag gene defect prevents normal antigen receptor gene rearrangements. Instead, the lymphocytes that develop in the normal hematopoietic microenvironment provided by the Rag$^{-/-}$ mice are all derived from T3R$\alpha^{-/-}$ progenitors. In these chimeras, thymocyte cellularity was normal (227). One interpretation of these data is that thymocyte numbers are reduced in T3R$\alpha^{-/-}$ mice because of defects in the thymic environment. Because thymopoiesis is normal in hyt/hyt mice, one must propose that either the very low (<10% of normal) levels of thyroid hormone in hyt/hyt mice are sufficient to maintain normal thymic stromal cell physiology or that TRα has ligand-independent actions on thymic development. It is well established that TR binds to hormone response elements and represses transcription in the absence of ligand (228). Consequently, constitutive repression of gene expression in thyroid hormone-deficient animals may result in physiological consequences that are different from those in receptor-deficient mice because of the presence of unliganded receptors.

2. B lymphopoiesis. Murphy and colleagues (45) were the first to demonstrate that the frequency of B lineage cells was significantly reduced in the bone marrow of Snell dwarf mice, and this finding was confirmed in subsequent studies from our laboratory (46, 47). This defect is not related to a stress effect, because, as opposed to the thymic abnormalities, it is consistently observed in dw/dw mice that are housed apart from their normal littermates (46, 47).

The depressed frequency of B lineage cells in dw/dw mice was revealed to be due to the lack of normal thyroid hormone levels. Neither PRL, GH, nor IGF-I restored lymphopoiesis to normal in dw/dw mice, but T_4 administration completely corrected this deficiency (47). The dependence of B cell development on thyroid hormones has been confirmed in two additional genetic models. As was observed with dw/dw mice, B cell development in hyt/hyt mice is also suppressed and can be restored to normal with T_4 treatment (47). B cell development is also impaired in TR$\alpha^{-/-}$ mice (227). To define the precise stage of B cell development that was affected by the absence of thyroid hormone, bone marrow cells from dw/dw and hyt/hyt mice were stained with a panel of antibodies that allowed the frequency of pro-, pre-, and B cells to be determined. This analysis indicated that commitment of lympho-hematopoietic precursors to the B lineage is not impaired; instead, the defect in cell production occurs in cells already committed to B lymphopoiesis (47).

The ultimate production of B lymphocytes is a dynamic process involving cell differentiation, cell death, and cell proliferation, and the tempo of any of these events could be negatively impacted by the absence of thyroid hormones. While actions of thyroid hormones on the differentiation of B cell precursors cannot be excluded, such an effect seems unlikely, since all stages of B lymphopoiesis are present in hyt/hyt and dw/dw mice. Therefore, it is logical to propose that the B lineage deficiency in the absence of thyroid hormone is due to a decrease in survival or a lower rate of proliferation of committed B cell precursors.

Studies that investigated these possibilities revealed a requirement for thyroid hormones in pro-B cell proliferation, as the frequency of pro-B cells in the S-G_2/M phase of the cell cycle was lower in hyt/hyt mice than in their normal littermates (224). There was no effect of thyroid hormones on survival of B lineage cells. Further studies demonstrated that treatment of hyt/hyt mice with T_4 restored the frequency of cycling cells to normal levels. Interestingly, the depressed frequency of B lineage cells in TR$\alpha^{-/-}$ mice is also due to a pro-B cell proliferation defect (227). Thus, by analyzing mice with a defect in thyroid hormone secretion or a defect in TR expression, the same conclusion has been reached as to why B lymphopoiesis is depressed in mice that cannot respond to thyroid hormones.

Given the widespread distribution of the TR in lympho-hematopoetic cells, it is puzzling why B lineage cells have a specific requirement for thyroid hormone. T_3/T_4, like GH and IGF-I, are also anabolic hormones, yet GH and IGF-I do not play an obligate role during B cell development. Thyroid hormones have been shown to regulate the expression of proteins required for cell cycle progression (229), and it is possible that they play a similar role during B lymphopoiesis. Another possibility is that hyt/hyt mice may exhibit B lineage defects due to gene repression by unliganded TR. In the absence of thyroid hormones, the TR binds to regulatory sequences in specific genes and constitutively represses transcription (228). Thyroid hormones, unlike most other hormones, are secreted at relatively constant levels throughout postnatal life. Therefore, a dynamic equilibrium between gene repression and activation by unliganded and liganded receptors, respectively, must be maintained at a homeostatic level under normal conditions. Active gene repression under hypothyroid conditions may be an important pathological mechanism in many tissues, including the immune system.

However, it is unclear that this mechanism would explain the suppression of pro-B cell proliferation in TR$\alpha^{-/-}$ mice. A final possibility is that differential regulation of TR expression results in an intricate regulation of gene induction and/or repression. In support of this point are data demonstrating that the TRα2 form of the receptor is differentially expressed in the B lineage but not in myeloid lineages (Ref. 224 and our unpublished observations). This complex pattern of TR expression may contribute to the lineage-specific role for thyroid hormones in B cell development.

C. Secondary lymphocyte development in hyt/hyt mice

Both dw/dw and hyt/hyt mice exhibited a normal humoral response to T-dependent and T-independent antigens (59a). The response of hyt/hyt mice to LCMV and the day 6 (but not, as reviewed below, the day 2 response) response to LM are also comparable to those in their normal littermates (Ref. 59a and our unpublished observation). These results are consistent with the observation that the number and frequency of sIgM$^+$, CD4$^+$, and CD8$^+$ splenocytes are comparable to values observed in their normal littermates (47).

Hypothyroid mice produce thyroid hormones at levels approximately 10% of normal (212–214), and it can be argued that these low concentrations are sufficient for normal effector cell function. However, the low levels of thyroid hormones in hyt/hyt mice were sufficient to cause repressed B lymphopoiesis. It is thus reasonable to assume that defects in the intensity or duration of the immune response would be observed in hyt/hyt mice if thyroid hormones played a critical role in antigen responsiveness.

D. Innate immunity in hyt/hyt mice

No hypothyroid mice succumbed to LM infection. However, as in dw/dw mice, the bacterial load of LM on day 2 post infection in hyt/hyt mice was significantly higher than in their normal littermates. Because the day 2 post infection response to LM is primarily mediated by macrophages and NK cells (62), these data suggest that normal concentrations of thyroid hormones may be required for optimal innate immunity. However, by day 6 post infection, the bacterial load of LM was comparable in hyt/hyt mice and their normal littermates (59a). Taken together, these results suggest that further studies to investigate the role of thyroid hormones in myeloid and NK cell function are warranted.

E. Potential actions of thyroid hormone on the immune system

Although thyroid hormones are required for normal B lymphopoiesis, whether or not they play a critical role in thymopoiesis is unclear in view of discordant data from hyt/hyt and TR$\alpha^{-/-}$ mice. Nevertheless, thyroid hormone effects on the thymus can occur (230), as T_4 administration to either Snell dwarf or normal mice increases the number of thymocytes (Fig. 4A). However, no effects on the proportion of CD4 and/or CD8 subsets is observed (Fig. 4B). It has been suggested that thyroid hormones stimulate thymocyte proliferation, although this does not appear to be due to direct growth effects (231). Because thyroid hormones directly induce GH synthesis in the anterior pituitary, it is possible that some of the stimulatory actions of T_4 administration are mediated through GH and IGF-I induction (232); however, it is not clear whether GH can be induced by thyroid hormones in dw/dw mice that have a defect in pit-1 gene expression.

Whereas the hyt/hyt mouse studies do not indicate an obligate role for thyroid hormones in lymphocyte function, thyroidectomy studies suggest that there may be instances in which hormonal effects are manifest. This procedure has been reported to depress humoral and cell-mediated immune responses (233–237) and lead to involution of spleen and lymph nodes (238, 239). In agreement with these results, blocking thyroid function with thiourea prevented the development of an antisheep red blood cell humoral response in birds (240). Upon release from the block, antibody synthesis resumed. On the other hand, there is no consensus on the ability of exogenous thyroid hormones to stimulate immune cell function; some studies have reported either no effects or depressed immune responses (235, 238–244).

No clear picture emerges regarding the effects of thyroid hormones on innate immunity. *In vivo* treatment of rodents with thyroid hormones can either inhibit or augment NK cell responses depending on the duration of treatment (245–251). It has also been reported that stimulatory effects of thyroid hormones on NK cell activity *in vitro* are due to the effect of thyroid hormones on increasing NK cell sensitivity to interferon (252). Given the failure of hyt/hyt mice to clear LM at the same rate as their normal littermates, further investigation of the effect of thyroid hormones on innate immunity is in order.

Taken together, these data suggest that in some instances thyroid hormones can influence lymphocyte function and innate immunity. As with PRL, GH, and IGF-I, the key issue is under what circumstances such potential effects are manifest. Additional studies to assess lymphocyte function in the absence of thyroid hormone need to be conducted. Chimeric animals generated by reconstitution of immunodeficient mice with bone marrow cells from TR$\alpha^{-/-}$ × TR$\beta^{-/-}$ mice might provide a mouse that could then be immunized to assess the role of thyroid hormones in lymphocyte function.

In view of these uncertainties, the potential for using thyroid hormones to alter immune function therapeutically is unclear. With regard to their role in primary B lymphopoiesis, it is relevant to note that B cell recovery after bone marrow transplantation is often delayed. If such patients were found to be hypothyroid, this might suggest the use of T_4 in these individuals. Thyroid hormone administration in selected cases of congenital hypothyroidism that result in severe immunodeficiency might also be indicated (253). The data in Fig. 4A showing effects of T_4 on increasing thymus cellularity also raise the possibility that, similar to GH and IGF-I, administration of T_3/T_4 could have beneficial effects on the aging thymus. The decline in the activity of the thyroid gland with aging (reviewed in Ref. 211) provides a rationale for this approach. However, variable effects of thyroid hormones on the immune system in old mice have been observed. For example, significant increases in the size of lymphoid organs in mice 15 months of age and older did not occur, and effects of thyroid hormones on augmenting the

immune response were not observed if mice were 20 months of age and older (251).

VI. Synthesis

The aim of this review has been to summarize recent results obtained from analyzing mice with genetic defects in the expression of PRL, GH, IGF-I, and thyroid hormones or their receptors and to reevaluate relevant aspects of the endocrine-immune literature in view of these new findings. The picture that emerges is that, with the exception of a role for thyroid hormones in primary B lymphopoiesis, these hormones are not obligate immunoregulatory factors (Table 3), although they may have important pharmacological or homeostatic effects.

It is conceivable that PRL, GH, IGF-I, or thyroid hormones could play a role in lymphocyte development and function that was not detected because the range of assays employed was too limited or not sensitive enough. Thus, it is possible that some developmental event is impaired or there is a pathogen to which the hormone-deficient mice could not respond. It is also difficult to exclude the possibility that these hormones may have effects on immune responses in nonstressed, healthy animals that are compensated by the overlapping actions of other hormones or cytokines (254). However, defects in lymphocyte development and humoral or cell-mediated immunity have been documented in mice deficient in the expression of IL-2 (255, 256), IL-4 (257, 258), and IL-7 (259), or the IL-2α (260), IL-2β (261), and IL-7α (262) receptors even though other cytokines or cytokine signaling pathways may compensate in part for their absence (254). The assays used to assess immunity in the hormone-deficient mice are the same as those used to reveal subtle defects, such as in failure to produce Igs of a particular isotype, in cytokine/cytokine receptor knockout mice, and these systems did reveal subtle defects in innate immunity in hyt/hyt and dw/dw mice.

These points aside, there is little evidence that PRL, GH, IGF-I, or thyroid hormones are required for primary lymphopoiesis or for B and T cells to generate humoral or cell-mediated immune responses. However, the data indicate that there are circumstances under which one or more of these hormones can stimulate immune cell development and function. As emphasized throughout this review, their nonobligate role in immunity can be reconciled with their potential augmentation of the immune system if one considers that their primary role is to counteract negative immunoregulatory signals.

While in the short term, the latter signals can be beneficial to the organism, over the long term they can have adverse effects on cells and tissues. Thus, it is fundamentally important to down-regulate the stress response before this occurs, and one way in which this might be done is for hormones to counteract the negative effects of stress mediators such as the glucocorticoids. The concept that hormones might counteract negative immunoregulatory signals generated during immune responses or due to stress is not particularly novel to this manuscript (122, 123, 263). However, it is important to emphasize again that these proposed stress-adaptive hormonal actions are not immune system specific. On the contrary, as consistently emphasized in this article, PRL, GH, IGF-I, and thyroid hormones also have systemic actions that affect the growth, survival, or other functions of cells in multiple tissues. These inferences are consistent with the wide distribution of PRL, GH, IGF-I, and thyroid hormone receptors on cells in most tissues of the body (18, 20, 27, 71). The systemic effects of these hormones contrast with the more restricted actions of immune-specific cytokines, such as IL-2, IL-4, IL-7, and IL-15. The discrete and dramatic actions of immunoregulatory cytokines are a consequence of their receptors being largely restricted to immune cells and their secretion being limited to a local environment in which the cytokines act primarily through autocrine and paracrine mechanisms.

The limited role of these hormones as homeostatic moderators of negative immunoregulation can be appreciated by recognizing that none of them has been shown to cause a consistent immune hyperstimulation, even in conditions where they are massively hypersecreted, such as in prolactinoma or acromegaly. There is some evidence that autoimmune diseases may be exacerbated by PRL hypersecretion (131), but these effects are very subtle compared with the dramatic effects of hyperprolactinemia on primary target tissues such as the reproductive system. In contrast, continuous hypersecretion of immunoregulatory cytokines, such as IL-7 (264), produces definitive syndromes of hypercellularity, consistent with their known actions on specific hematopoietic lineages.

The introductory sections of this review summarized contradictions in the dw/dw mouse literature and noted that reconciliation of these inconsistencies could provide important insights into understanding hormonal effects on the

TABLE 3. Status of primary and secondary lymphoid development in hormone-deficient mice

Strain	Lymphocyte development		Immune response		Innate immunity
	B	T	Humoral	Cell-mediated	
dw/dw	Depressed	Normal	Normal	Normal?	Depressed
PRL$^{-/-}$	Normal	Normal	Normal	Normal	Normal
PRLR$^{-/-}$	Normal	Normal	Normal	Normal	Normal
lit/lit	Normal	Normal	Normal	Normal	Normal
IGF-I$^{-/-}$	Normal	Normal	ND	ND	ND
hyt/hyt	Depressed	Normal	Normal	Normal	Depressed
TR$\alpha^{-/-}$	Depressed	Depressed?	ND	ND	ND

Normal T cell responses in dw/dw, PRL$^{-/-}$, lit/lit, and hyt/hyt mice based on ability to respond to a T cell-dependent antigen. Data based on results from Refs. 46, 47, 59a, 90, 99, and 227. ND, Not determined.

immune system. In the context of the stress adaptation hypothesis, a compelling case can be made that dw/dw mice that exhibited immunodeficiency were under severe stress due to disease and/or the conditions under which they were maintained. Thus, as discussed in *Section II.D*, those dw/dw mice that exhibited primary or secondary lymphopoietic defects had a reduced life span, were weaned earlier than optimal for that strain, or were housed under conditions that subjected them to psychogenic or metabolic stresses.

The hypothesis that PRL, GH, IGF-I, and/or thyroid hormones are principally antistress mediators and that their effects are not specific for the immune system in no way diminishes their potential clinical utility. In fact, since most clinical circumstances that manifest an immunosuppressed state are characterized by deficits in many nonimmune tissues, hormones that can ameliorate defects systemically might be more useful than cytokines that act on restricted cell populations. Administration of one or more hormones may be useful in increasing blood cell production and function after traumatic injury, myeloablative treatments, or reconstitution after bone marrow transplantation. Aging may be considered in the context of stress as well. A decline in immune and other systems occurs concomitantly with a deficiency in the production of various hormones, and hormone therapy may be of benefit in their reversal.

It is hoped that the points raised in this article will result in a better understanding of PRL, GH, IGF-I, and thyroid hormone actions on the immune system and stimulate additional experimentation in animal models in which genetic manipulation can be applied, and in human experiments. In view of the current or anticipated uses of these hormones in humans, the information obtained from such studies may be of great value in the design of strategies for their therapeutic use.

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Announcement: Recent Progress in Hormone Research

The Publications Committee of The Endocrine Society decided last year that, despite the discontinuation of the Recent Progress in Hormone Research meeting, the annual proceedings journal of the same name will be maintained due to its continuing popularity. Articles will be invited, many of which will be drawn from The Endocrine Society's Annual Meeting.

Although the articles published in *Recent Progress in Hormone Research* (RPHR) differ significantly from those published in *Endocrine Reviews*, the Publications Committee has taken the extra step of ensuring the most effective coordination of editorial office efforts by linking RPHR with *Endocrine Reviews.* The RPHR Editor will work with the Editor-in-Chief of *Endocrine Reviews* to identify and solicit the most appropriate articles for RPHR and will use the *Endocrine Reviews* editorial office as a base for correspondence. In addition, the RPHR Editor will use the *Endocrine Reviews* Editorial and Advisory Boards for consultation.

Anthony R. Means has been appointed the first RPHR Editor under this structure. His appointment is for three years, after which time the *Endocrine Reviews* Editor-in-Chief will have the option of re-appointing him for another three years or recommending another individual.

Any questions, comments, or recommendations regarding this new structure for RPHR are encouraged and should be sent to Marc Caron (endoreviews@endo-society.org) or Tony Means (tony.means@duke.edu).