0163-769X/97/$03.00/0
Endocrine Reviews

Vol. 18, No. 2
Printed in U.S.A.

The Somatogenic Hormones and Insulin-Like Growth Factor-1: Stimulators of Lymphopoiesis and Immune Function

ROSS CLARK

Endocrinology Group, Genentech, Inc., South San Francisco, California 94080

Address reprint requests to: Ross Clark, Ph.D., Endocrinology Group, MS 37, Genentech Inc., 390 Pt San Bruno Boulevard, South San Francisco, California 94080.

I. Introduction

THE chief messenger systems between organs and tissues are the nervous, endocrine, and immune systems, which must be integrated on all levels to maintain homeostasis. The central hypothesis of this review is that the anabolic hormones (Fig. 1) GH, PRL, and the insulin-like growth factors (IGFs) that regulate whole body growth, metabolism, tissue repair, and cell survival also play an integrating role (Fig. 2) in the growth, maintenance, repair, and function of the immune system.

In the 1930s the mastery of hypophysectomy in the rat by Smith (1) was crucial to the discovery of the pituitary hormones. Smith (1) also found that "after the total ablation of the anterior hypophysis the thymus began to regress almost immediately." This discovery of a pituitary influence on the thymus caused a search for the causative factors (2, 3). Although there were reports that injections of pituitary extracts with somatotrophic activity stimulated thymic growth in rodents (4), this was not a consistent finding (3), possibly because the somatotrophic preparations used were impure and were sometimes contaminated with other pituitary hormones. More evidence for immunological activity of the somatogenic hormones came from the study of hypophysectomized rats (5), which showed a dramatic and continual age-related fall in both blood hemoglobin and white cell count, compared with the stable blood cell counts in normal animals, and a reduced antibody response to antigen that could be improved by GH and PRL treatment (6). The immunological activity of the pituitary was also revealed in studies of genetically hypopituitary rodents. The homozygous Snell-Bagg dwarf mouse was found to be deficient in GH, PRL, and thyroid hormones and to have an associated poorly developed immune system including a marked hypertrophy of the spleen and thymus, a progressive loss of small lymphocytes in the thymic cortex, and a decreased number of peripheral blood lymphocytes (7, 8). It is now established that the inhibition or stimulation of many hormone systems can affect immune responses (9). Such hormones fall into two classes. *In vivo*, GH, PRL, and thyroid hormones increase immune responses whereas ACTH, glucocorticoids, estrogen, progesterone, and androgens depress immune responses (10–17). This review marshals the evidence that, in addition, the insulin-like growth factors (IGF-1 and IGF-II) have an important role in stimulating lymphocyte production and function.

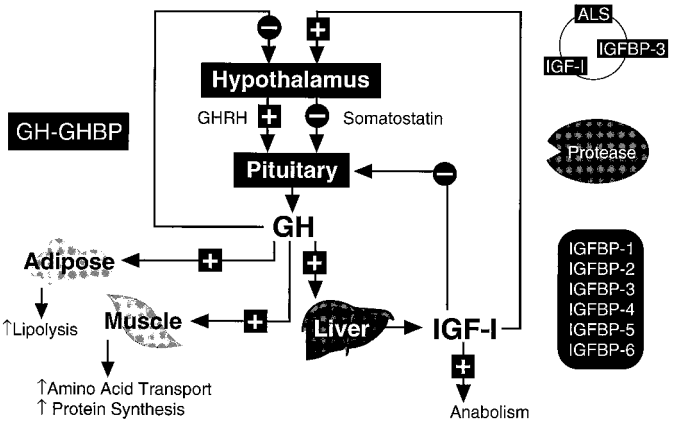

FIG. 1. The GH/IGF-I axis. This figure depicts the sites of production of GH and IGF-I, the feedback loops regulating their secretion, binding proteins, and main metabolic actions. The hypothalamic hormones GHRH and somatostatin control GH secretion from the pituitary. GH circulates in the blood, in part bound to a GH-binding protein (GHBP), to inhibit its own secretion and to stimulate the production of insulin-like growth factor 1 (IGF-1) in the liver. IGF-I circulates in the blood bound primarily to IGF-binding protein-3 (IGFBP-3), which is in turn complexed to a third protein, the acid-labile subunit (ALS), but a total of six IGFBPs are known. IGFBP-IGF complexes are subject to protease attack, which assists the dissociation of IGF-I. The actions of GH are exerted either directly or indirectly, via the generation of IGF-I.

To dissect the effects of GH, PRL, and IGF-I on the immune system, it is important to understand the relative importance of the mediating role of IGF-I generation to the effects of GH (Fig. 1). This is not a simple exercise. The history of the mechanism of action of GH is a long one, strewn with many theories. For example, fragments of GH were once proposed to mediate all the effects of GH (18). In 1953, Salter and Best (19) described body growth in hypophysectomized rats treated with insulin, a result that was not confirmed by others (20). Such data led to the hypothesis that insulin mediates many of the growth-promoting effects of GH (21). This hypothesis was proposed almost contemporaneously with the discovery, in the serum of hypophysectomized rats treated with GH, of a sulfation factor activity (22) that was different from insulin (23). The hormones with this activity were later renamed the somatomedins (24). Purification (25, 26) led to the finding that somatomedins and nonsuppressible insulin-like activities (27) were identical and they were then renamed (25, 26) IGF-I and IGF-II.

The finding that human IGF-I purified from serum caused significant whole body growth in the rat (28) seemingly confirmed that many, if not all, of the growth-promoting effects of GH were mediated via the systemic generation of IGF-I in the liver: the somatomedin theory of growth regulation (29). It was then discovered, using recombinant human IGF-I (rhIGF-1) and recombinant human GH (rhGH), that rhIGF-1 and rhGH have very distinct differential effects on the size of different body organs in the rat (30, 31). If the effects of GH were all mediated by IGF-I generation, then this would not be the case. Earlier it had been proposed (32) that some of the endocrine effects of GH were a result of its direct action on tissues (Fig. 1) rather than being indirect via the generation of IGF-I in the liver. The much greater effect of rhIGF-1 than of rhGH on the weight of the spleen and thymus in the rat (30, 31) was one of the first indications that IGF-I and GH had different growth- promoting activities *in vivo*. That IGF-I, GH, and PRL can have different activities on the lymphoid tissue will be discussed throughout this review. The review will also focus on the evidence for an autocrine or paracrine GH/PRL/IGF system in lymphoid tissues that produce PRL and GH, contain PRL and GH receptors (GHRs), express IGF-I, contain IGF-I receptors, and secrete IGF-binding proteins.

This review is timely in that rhIGF-1 is being tested in large clinical trials in several human diseases (33), and it is possible that the immunological activities of GH/IGF-I may be found useful in the treatment of immune-deficient states in humans (34). For related information not included in this review, particularly the effects of GH/IGF-I on other hematopoietic cells, several related recent reviews (9, 35–40) and a monograph (41) are available.

II. Local GH Axis in Lymphoid Tissue

A. Background: extrapituitary production?

Human GH is a protein of 191 amino acids produced and released by the anterior pituitary gland to circulate as an endocrine hormone (42). Until the placental GHs were discovered, GH was considered to be an exclusively pituitary hormone (42). It is now apparent that cells and tissues other than the pituitary and its somatotrophs also produce GH. Very recently, normal mammary tissue and mammary tumors, particularly under steroid stimulation, have been shown to produce surprisingly large amounts of GH (43, 44) that can be so large as to be detectable in blood.

This recent demonstration of GH production at an extrapituitary site may shed new light on the following discussion of the production of GH by lymphoid tissue. The total production of GH by extrapituitary tissues must be low compared with the production by the pituitary because hypophysectomy has such profound effects on body growth in a young animal. These findings, *i.e.* in some tissues GH is

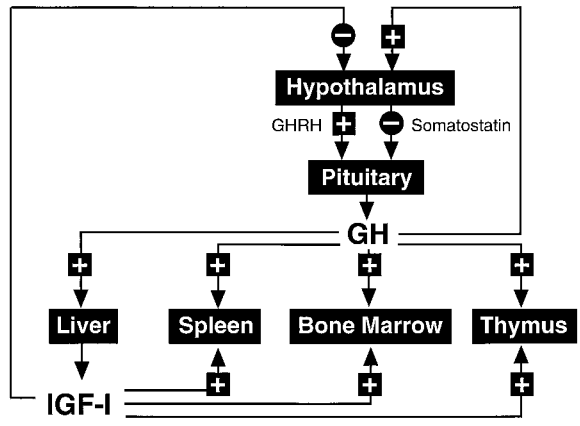

FIG. 2. Endocrine GH secreted by the pituitary stimulates the production of endocrine IGF-I by the liver and local IGF-I in many other tissues, including the stromal cells of hematopoietic tissues. GH and PRL also act directly on lymphocytes or their precursors in hematopoietic tissues such as bone marrow, spleen, and thymus.

produced locally and probably acts locally, reinforce the idea (32) that GH has many actions on tissues that are not mediated by endocrine IGF-I.

B. GH expression

The first evidence of GH production by lymphoid cells came from direct staining using fluorescent-labeled anti-GH antibodies (45) and showed that about 10% of unstimulated human peripheral blood mononuclear cells (PBMCs) were positive for GH, whereas after mitogen stimulation 20% were positive. This surprising result was then independently confirmed (46) and extended to show that GH mRNA is expressed in lymphocytes (47) and that anti-GH antisense oligonucleotides inhibit lymphocyte proliferation (48). A plaque assay confirmed that the GH produced by human PBMCs is biologically active GH (49). Two human cell lines, the B cell lymphoma line IM-9 (50) and the Burkitt lymphoma cell line sfRamos (51), have been shown to synthesize and release human GH (hGH). These results suggest that GH is synthesized *de novo* and perhaps continually secreted from lymphocytes. By *in situ* hybridization and immunocytochemistry, GH was shown to be expressed in human and rat bone marrow, spleen, thymus, lymph nodes, and in human tonsil (41). A recent study (52) of human tissues, using *in situ* hybridization and RT-PCR, has also shown GH mRNA in spleen, lymph node, tonsil, and thymus. The GH mRNA was present not only in lymphocytes but also in the supporting structures, *e.g.* in the thymus GH was expressed by epithelial cells and reticular cells (52). There are reports that granulocytes, rather than lymphocytes, are the main cell type producing GH in peripheral blood (41). In marked contrast, another study in the rat showed that although GH was expressed in developing lymphoid tissues, it was not expressed in adult tissues (53). The consensus of all the literature data, however, is that GH is expressed locally in lymphoid tissues (41).

C. GH regulation

The transcription of the GH, PRL, and TSH genes in the pituitary depends on the activity of the transcription factor Pit-1 (54). The presence of this transcription factor in lymphoid tissues, and its colocalization with GH and PRL, strongly supports the idea of regulated extrapituitary GH production in spleen, bone marrow, and thymus (41, 55).

GH production in the pituitary gland is directly regulated by the hypothalamus, which produces the inhibitory factor somatostatin and the stimulatory factor GHRH (56). GHRH peptide and mRNA have been detected in human lymphocytes (57). Somatostatin has been identified in lymphoid tissue (58) as has the somatostatin receptor (59). However, whether somatostatin and GHRH are involved in the local production of GH is less clear (60). Physiological concentrations of GHRH and somatostatin have been reported to have no effect on the secretion of GH from human lymphocytes (60). The effects of GHRH and somatostatin on the activity of cultured lymphocytes have produced conflicting data: various assays have reported stimulation (61), inhibition (61, 62), or no effect (62). It is possible that somatostatin has a direct effect on IGF-I production because in the liver somatostatin exerts a direct inhibitory effect on IGF-I generation (63). A recent review suggests that, compared with GHRH, there is more evidence for somatostatin having an immunemodulatory role (64). However, there seems to be no published data testing for a direct effect *in vitro* of somatostatin on IGF-I mRNA or peptide in lymphocytes. The regulation of local GH may be different from that in the pituitary because the addition of IGF-I to lymphocyte cultures has been reported not to affect their secretion of GH (65). The most convincing evidence of PRL/GH release from both T and B lymphocytes has been that seen after direct mitogen stimulation *in vitro* (66). Small amounts of GH (0.2–0.6 pg/well, measured by RIA) are released by nonstimulated human PBMCs, but after phytohemagglutinin (PHA, a T cell mitogen) or pokeweed mitogen (a B cell mitogen), a dose-related increase in GH to several picograms per well has been shown (60). It appears likely that the regulation of GH in the immune system differs from that in the endocrine system.

D. GHRs

The finding that GH is produced locally by lymphoid tissues has been given more of a functional significance by evidence that lymphocytes also express GHRs. GH binding was first detected on a human B cell lymphoma (IM-9) lymphocyte cell line (67) and subsequently identified (68) on human PBMCs. The development of specific monoclonal antibodies against the GHR allowed flow cytometry to confirm that GHRs are present on human IM-9 cells (69) and in human PBMC with the highest expression on B cells (70). The GHR on lymphocytes has been sequenced (71, 72) and found to be identical to the GHR cloned from liver (73). A more recent study confirmed that the hGH receptor is present on more than 90% of B lymphocytes and monocytes, but only variably present on T lymphocytes. B lymphocytes and monocytes had approximately 6000 GHRs per cell, and this number was not affected by a donor being GH-deficient (74).

E. GHR signaling

The purification and cloning of the GHR (73), the discovery of the dimerizing stochiometry of the GH-(GHR)2 complex (75), and the crystallization of this complex (76) have revolutionized understanding not only of GH but of the new family of the helix bundle peptide (HBP) cytokine receptors (40). The placing of the GH and PRL receptors in the family of hematopoietic cytokines (77), which includes erythropoietin, granulocyte-colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, and the interleukins, has provided a theoretical basis to the experimental results showing that GH and PRL have significant activity as hematopoietic cytokines (40).

Like other members of the HBP receptor superfamily, GH signals through the JAK kinase/STAT cascades. However, because many of these intracellular signaling mechanisms appear to share several receptor/ligand systems, it has been difficult to see how specificity is maintained (78). If signaling pathways do overlap, then the responses of lymphocytes to GH/PRL could be viewed as minor epiphenomena, with

other ligands or receptors mediating the same responses with more potency and specificity. However, mice with a disrupted STAT1 gene show defects in interferon signaling but normal responses to other cytokines, including GH (79). Such specificity was not predicted from *in vitro* studies and suggests that GH and PRL do have unique effects in lymphocytes.

GH action involves the sequential binding of an initial site on hGH (site 1) to a GHR molecule to form a GH/GHR monomer complex, followed by the binding of another GHR molecule to the second site on hGH (site 2), to form a receptor dimer complex (75). It is this complex that activates the JAK2/STAT cascades. GH analogs with mutations in the second binding site, but with an intact site 1, prevent dimer formation and can act as antagonists (80). Because the affinity of these mutant GH molecules for GHR remains high, and can be engineered to increase the affinity for site 1 selectively, highly potent hGH antagonists have been produced. A GH antagonist (80) is potentially a useful tool for elucidating the importance of GH to lymphoid tissues. Unfortunately the mutant hGH molecules that act as antagonists at the hGH receptor show no evidence of antagonist activity when administered to rats (81). The reason for the species specificity of hGH antagonists is unclear because native hGH binds the rat GHR with high affinity and stimulates body growth. A GH antagonist fully active in the rat is eagerly awaited because it would help explain the importance of endogenous GH, the importance of local GH production, and especially the importance of GH to lymphoid tissues.

III. GH Administration

A. Effects on the thymus

The thymus grows rapidly postnatally in mammals, peaks in size at around sexual maturity, and then slowly involutes with age (82). In humans, maximal thymic size is attained at puberty and then by 45–50 yr of age involutes so that only 5–10% of the cellular mass remains. In mice, maximal size (70 mg) is attained at puberty (6 weeks) while by 9 months of age the thymus has declined in weight to only 20 mg. Involution of the thymus with age was recognized (83) well before its immunological role (84). These changes in thymic structure and function follow the rising activity of the GH system, with serum IGF-I levels peaking at puberty and declining gradually with advancing age (85–87) suggestive of a causative relationship. The age-related decline in immune function is poorly explained. A portion of this decline could be related to the lack of activity, or resistance to the actions, of the anabolic hormones.

The first studies suggesting that treatment with GH could affect the thymus in nonrodents was a study in hypopituitary dwarf Weimaraner dogs (88). In follow-up studies, young, middle-aged, and aged dogs were treated with GH. Thymic growth was observed in middle-aged, but not in aged, dogs and the blood level of thymic hormone increased (89). The thymus glands of the treated dogs were described (90) as "resembling thymic tissue of young dogs." In the aged rat, the implantation of GH3 pituitary cells reversed age-related thymic atrophy and increased the number and function of T cells in the thymus (91). The next section discusses in more detail the effects in rodents of continuous infusions of hGH, which are very effective at stimulating thymic growth (92). It should be noted that GH3 cells may produce GH, but little PRL, *in vivo* (93), and their release of GH would be continuous, perhaps accounting for the clear effects reported above (91) on the thymus.

B. Pattern of GH exposure

In several tissues the pattern of GH administration or exposure can have major quantitative and qualitative tissue-specific effects (94, 95). For example, different patterns of endogenous GH exposure, best illustrated by differences in GH-secretory profiles in male and female rats, cause a number of sexually dimorphic responses (96). The administration of GH to rats either by daily injection (male pattern) or by continuous infusion (female pattern) can replicate these dimorphic responses, which include influencing hepatic enzymes (97), hepatic growth (98), and lipid metabolism (99). In mice that overexpress bovine GH, there is an enlargement of the internal organs, particularly of the spleen, which may be due to both the high levels of GH and the continuous pattern of GH exposure (100).

As described above, it is likely that GH is produced locally in lymphoid tissues. The production of this GH is probably by constitutive expression and release, giving a continuous pattern of local GH exposure. To explore this idea, GH was given by injection or infusion, and the effects on different body tissues were compared (98). It was found that in rats (Fig. 3A), injections of GH are much less potent than GH infusions at stimulating lymphoid tissue growth (98). Figure 3A shows that in hypophysectomized rats the spleen more than doubles in size after GH infusions, whereas the same doses of GH given by injection elicit no splenic growth. It has been stated that in mice, 20- to 40-fold higher doses of GH and PRL are needed by injection, compared with minipump infusion, to reverse corticosterone-induced suppression of splenic lymphocyte responses to mitogens (101). Recent data in young castrate and intact genetically obese pigs also suggest that treatment with porcine GH can affect thymic size and thymosin concentration in serum (102). When porcine GH was given by injection or by a slow release depot formulation, there was a greater effect on the thymic parameters of the slow release formulation (102). A different form of long-acting GH, *i.e.* that made by coupling polyethylene glycol (PEG) to hGH, has now been described (103). The administration by infrequent injection of PEG-rhGH to hypophysectomized rats also induced lymphoid organ overgrowth compared with daily injections of nonmodified rhGH. This difference was probably due to the more continuous pattern of GH exposure caused by the long plasma half-life of the PEG-rhGH (103).

Some of these differences between injected and infused GH could be due to continuous GH exposure, via hepatic stimulation, inducing higher serum IGF-I levels (Fig. 3B) than injections of GH (94, 99). These findings have been confirmed in monkeys where a depot form of hGH that chronically elevated blood GH levels gave higher serum IGF-I levels than did a comparable dose of GH given by daily

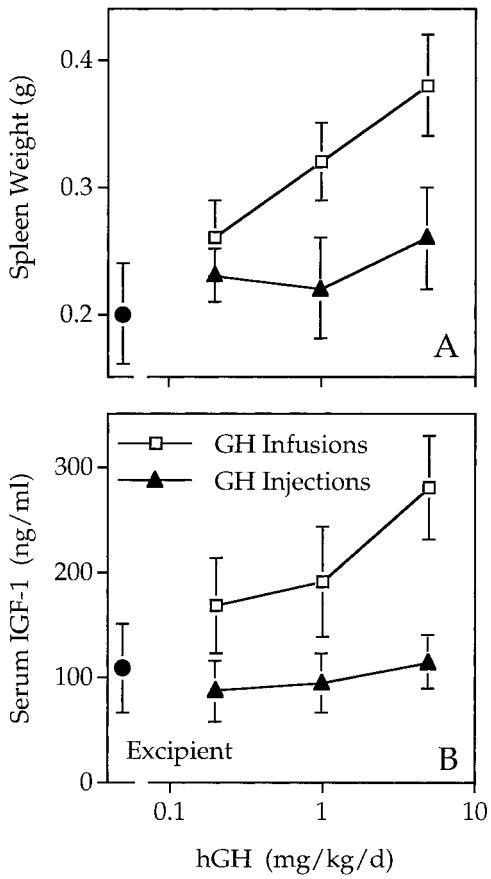

FIG. 3. Spleen weight (A) and serum IGF-I concentrations (B) in hypophysectomized rats treated with excipient, or three doses of hGH given by injection or infusion. At these doses, infusions, but not injections of hGH, increased serum IGF-I concentrations, perhaps explaining the selective effect of hGH infusions on spleen weight. Means and SDs are shown. [Derived from Ref. 98.]

injections (104). The higher concentrations of endocrine IGF-I (Fig. 3B) may be the cause of lymphoid tissue growth. There seems to be no comparative data on the local production of IGF-I in lymphoid tissues in response to injections or infusions of GH. Alternatively, it is possible that these pattern-dependent direct effects of GH on lymphoid tissues may not be mediated by IGF-I.

C. GH and immune function in humans

The strong evidence from studies in animals that the pituitary (3), GH (35), PRL (16), and IGF-I (36) affect hematopoietic and lymphoid tissues is generally accepted. However, the evidence in humans is perceived by recent reviewers as being unconvincing (39, 105). The most cited evidence (39, 105) is that GH-deficient children are not clinically immunodeficient and therefore replacement therapy with hGH would not be expected to have significant effects on immune function. This perception of a general lack of effect of GH deficiency or GH replacement treatment on immune function has been taken as evidence that the GH/IGF axis has a lesser effect on lymphoid tissues in humans (39). A lesson can be taken here from animal studies with other HBP cytokines, such as interleukin-6 (IL-6), which has a range of pleiotropic actions on T cell and B cell proliferation that it shares with IL-1, IL-2, IL-4, and IL-5 (106). The overexpression of IL-6 in mice has only a mild effect on B cells (107). Another example of the pleiotropic actions of cytokines is that disruption of the IL-2 gene allowed almost normal hematopoiesis (108). These cytokines do have important actions on the immune system, but the presence of multiple cytokines ensures that homeostasis is maintained. Therefore, the apparent lack of effect of an endocrine GH deficiency in humans should not be taken as evidence that GH has no effects on immune function in humans.

Human studies have concentrated on discovering the immunological phenotype of patients who lack pituitary GH. There has been almost no effort toward discovering whether these patients are deficient in local GH, local IGF peptides, or receptors or have a changed local IGF-binding protein (IGFBP) status. Even if pituitary GH is disturbed, it is likely that in many patients the local paracrine/autocrine axis in lymphoid tissue is intact. In GH-deficient humans it is possible that the GH produced locally in the immune system compensates for the lack of endocrine GH. The much higher endogenous levels of GH (96) in the rat, about 10-fold higher than in the human (85), would be expected to cause locally produced GH to be less important in the rat that in humans. In addition, rats, particularly female rats, have relatively high GH-binding protein levels that may act to enhance the activity of GH (109). This may help explain why in humans a deficiency in pituitary GH or endocrine IGF-I appears to have minor effects on immune function compared with the effect of such deficiencies in the rat. One group of patients who may be immunologically impaired are patients with defective GHR function who were once termed Laron dwarfs but are now described as having GH insensitivity syndrome (110). The largest cohort yet described, that localized in Ecuador, may have a significantly higher pediatric mortality (110), perhaps indicating an impaired immune system. A recent monograph includes a lengthy discussion of the relative importance of hormones to immunological status in rodents and humans (41). A proposal by these authors that should be considered, but for which there is little or no evidence, is that IGF-I expression is less dependent on GH in humans than in rodents (41) and that this may account for the apparent lack of effect on immune function of GH deficiency in humans. Another reason could be that IGF-II concentrations in adult rodents are very low compared with the concentrations in humans. Therefore, a deficiency of GH in rodents, leading to a fall in IGF-I concentrations, has a greater impact than in GH-deficient humans in whom the maintained IGF-II concentrations may preserve blood IGF concentrations and activity.

IV. PRL

A. PRL expression in lymphoid tissues

The first cytokines in the HBP family to be identified were GH and PRL. Because hGH, unlike rat GH, also binds with high affinity to PRL receptors, it was unclear, until human PRL was purified by Friesen and colleagues (111), whether humans had a separate PRL. PRL has always been viewed as

having a very broad range of activities (40); therefore the subsequent demonstration that it had effects on lymphoid tissue was not surprising. The discovery of PRL expression in lymphocytes was a surprise. A sensitive bioassay, based on immunostaining of Nb2 cells, showed PRL-like activity to be present in the culture fluid from concanavalin A (ConA)-activated murine splenocytes (112). ConA-stimulated thymidine incorporation was reduced by adding an antibody against PRL, suggesting that a PRL-like molecule was produced by the splenocytes and was essential for lymphocyte proliferation (112). Using an enzyme-linked immunoplaque assay, human PRL secretion was found after ConA or PHA stimulation of PBMCs, but not in unstimulated PBMCs (113). Murine and human T- and B cell mitogen proliferation has been shown to be inhibited by antibodies to PRL, due to a block in the G1 to GS transition in the cell cycle (114). *In situ* hybridization showed the presence of an mRNA in murine splenocytes that hybridized with a rat PRL cDNA probe (115). The PRL protein in human lymphocytes appears to be similar to pituitary PRL, *i.e.* the multiple forms present in the pituitary are also present in lymphocytes (116). The PRL gene is also expressed in rat thymus (117). As described above for GH, the extrapituitary production of PRL in lymphoid tissue is not unique; the PRL gene, like the GH gene (43, 44), also appears to be transcribed in the mammary gland (118).

B. PRL receptors

Lymphocytes not only produce PRL but also possess PRL receptors. The use of the rat Nb2 T cell line for bioassaying PRL clearly suggested the presence of PRL receptors on lymphoid cells (119). PRL binding on normal lymphocytes was first demonstrated on human T and B lymphocytes (120). Since then, biotinylated monoclonal antibodies against the human PRL receptor (121) have been used to show PRL receptors in the mouse (122), particularly on B cells, and the presence of receptors throughout human hematopoietic tissues including bone marrow and thymus. B cells were the most strongly labeled, while T cells showed an increased labeling upon activation (121). In the mouse and rat, PRL receptors are present in bone marrow, thymus, spleen, lymph nodes, and on peripheral blood lymphocytes (122), with receptor number increasing in a draining lymph node after foot pad immunization (123) and on T cells after ConA administration (124). In the thymus, PRL receptor number is greatest in the cortex and in thymic epithelial cells (125). Two forms of the PRL receptor, which differ in the length of their cytoplasmic domains, are present in lymphoid tissues in the mouse and rat (117). The PRL receptor can be detected by PRL binding, by antibodies, and by PCR in many lymphoid cell lines (both T and B cell) and hemopoietic cell lines (41). The signaling of PRL, especially in Nb2 cells, has been studied intensively and reviewed recently (40).

C. Administration of PRL, anti-PRL antibodies, or bromocriptine

Hypophysectomized rats are almost devoid of a primary antibody response after the injection of sheep red blood cells (126). Replacement with lactogenic hormones (40 μg/day) at the time of immunization restored antibody titers to those of a normal rat (126). After complete hypophysectomy an animal should be completely PRL-deficient if the pituitary were the only source of PRL. However, Nagy and Berczi (127) used the Nb2 cell bioassay to show that immediately after hypophysectomy rats have unexpectedly high blood PRL concentrations, 10–20% of normal, which then rise to 50% of normal 8 weeks after hypophysectomy. In normal rats, PRL had been described as having an effect on hematopoiesis; however, in the hypophysectomized rat, red cell count, although low, was compatible with life. It was therefore reasonable to conclude that PRL is of marginal importance to red cell biology. In an important set of experiments Nagy and Berczi (127) gave anti-PRL antibodies to hypophysectomized rats to neutralize this residual PRL. After the anti-PRL sera was given, severe anemia developed and all the animals were dead within 6 weeks (127). This is very compelling evidence that significant amounts of PRL are made by extrapituitary sources and that this local PRL has vital functions, especially for hematopoietic tissues. Comparable studies using anti-GH antibodies would be of great interest. The sensitivity of hypophysectomized rats to the lethal effects of estrogens also needs reevaluating (128).

In humans, the administration of hGH, as it binds to hGH and human PRL receptors, will activate both GH and PRL receptors and their signaling pathways. The apparent lack of effect of hGH administration in humans on immunological function may be due to a lack of selectivity. The administration in humans of hPRL, a specific hPRL receptor ligand, or V-gene hGH (42), a more specific hGH receptor ligand, may show different effects than hGH administration. The prospective availability of a GH antagonist (80, 81) provides the opportunity to antagonize both local and systemic hGH. Treatment with a GH antagonist may therefore cause a different immunological phenotype than a deficiency of pituitary GH.

Published data (66) describe the immunological effects of bromocriptine, a dopamine ergot alkaloid that inhibits the release of PRL from the pituitary. A bromocriptine-treated rat has been reported to show a reduced mixed lymphocyte reaction *in vitro* and a reduced graft-*vs*.-host reaction *in vivo* (66), presumably due to a suppression of pituitary PRL. This may not be the case. Bromocriptine appears to have direct effects *in vitro* on lymphocyte proliferation in the absence of added PRL (129, 130). Animal studies in transplantation (131) and a clinical trial using bromocriptine, in combination with cyclosporine, as an immunosuppressive regimen, have met with some success (132). The production of genetically engineered PRL-deficient mice or PRL receptor-deficient mice may help answer the question of the importance of PRL to lymphocytes and to immune function.

V. Insulin-Like Growth Factors

A. Background

The chief regulators of the IGF-I levels in blood are GH status (133) and nutrition (134, 135). GH and IGF-I, acting together, ensure ordered body growth and therefore are involved in complex interactions with most organ systems,

tissues, cell types, and also with many growth factors (133, 136). Due to these multiple effects, GH, PRL, and IGF-I can affect diverse physiological processes, including immune function, in many ways, both directly and indirectly (133, 136). The GH system regulates and coordinates whole body growth to ensure that different tissues grow in unison and are then maintained in an optimal proportion to the rest of the body. The immune system may be one such tissue. For example, GH and IGF-I stimulate cartilage growth and lengthen bones causing statural growth (136). By controlling the size of the bones in the growing animal, GH and IGF-I therefore indirectly control the volume of bone marrow and thus the production of hematopoietic cells (137). Recent evidence shows that IGF-I differs from insulin in that, at physiological concentrations, it also plays a direct and significant role in regulating hematopoiesis, especially lymphopoiesis and immune function (36).

B. IGF peptides

IGF-I and IGF-II, peptides of 70 and 67 amino acids, respectively, were named because of the similarity of their actions to that of insulin (138) and their chemistry to that of proinsulin (25, 26). A major difference between these hormones and insulin is that the IGF peptides are expressed almost ubiquitously (139–141). In adult humans, IGF-I and IGF-II are both present in large amounts in blood, as they are in fetal rodents, but in adult rodents IGF-II concentrations in blood are very low (142). It is unclear whether these differences between IGF-I and IGF-II status result in species-specific effects on immune function. IGF-I concentrations in blood are controlled by GH status; IGF-II levels are much more GH-independent (142). Exons 1 and 2 of the IGF-I gene contain two distinct promoters that give rise to IGF-I mRNAs containing either exon 1 or exon 2 (143). Exon 1 mRNA is the form in fetal tissue, whereas the exon 2 form appears postnatally when GH responsiveness is acquired (143). These different forms of IGF-I mRNA perhaps supply either GH-dependent endocrine IGF-I (exon 2) or local GH-independent paracrine or autocrine IGF-I (exon 1) (143). In myeloid cells, IGF-I transcripts have been found to be exclusively initiated within exon 1, characteristic of extrahepatic IGF-I mRNA (144). IGF mRNA and peptides are produced by myeloid cells, particularly by macrophages, in relatively large amounts (36, 144) and by human peripheral lymphocytes in small amounts (36, 144, 145). Bone marrow stromal cells also release IGF-I (146, 147) as do thymic epithelial cells (93), which can be stimulated by GH in culture (148). Therefore, there are ample data showing the local production of IGF-I in lymphoid tissues. However, there is scant evidence describing the regulation of this locally produced IGF-I or the relative importance to lymphoid tissues of local or endocrine IGF-I.

C. Regulation of lymphocyte IGF-I

Considerable amounts of IGF-I are regulated by GH-independent pathways, as seen by the presence of significant serum IGF-I levels in GHR-deficient humans (110) and sex-linked dwarf chickens (149). Treatment with GH increases IGF-I mRNA in many tissues (140), whereas in other tissues IGF-I generation is controlled by factors or hormones other than GH. For example, in the rat uterus IGF-I may be regulated chiefly by estrogen, rather than by GH (150). Cytokines other than GH affect IGF-I synthesis in lymphoid tissues, *e.g.* in macrophages tumor necrosis factor-α has been shown to regulate IGF-I production (151). Tumor necrosis factor-α and prostaglandin E_2 (PGE_2) stimulate IGF-I synthesis in macrophages by two separate pathways, with PGE_2 stimulating IGF-I synthesis through a cAMP/protein kinase A pathway (152). The colony-stimulating factors also induce the expression of IGF-I mRNA in macrophages (153), whereas the T cell-derived cytokine IFN-γ reduces macrophage IGF-I mRNA in a time- and dose-dependent manner (154).

It is therefore likely that lymphocytes are exposed to endocrine IGF-I from the circulation, their own autocrine IGF-I, and perhaps most importantly, in lymphoid organs and bone marrow, a third source of IGF-I from epithelial cells (148) and stromal cells (35). The proliferation of thymic epithelial cells can be stimulated *in vitro* by both hGH and IGF-I (155) and the effect of hGH blocked by either an anti-IGF-I or an anti-IGF-I receptor antibody (93). This effect of hGH may be via the PRL receptor, rather than the GHR, as rat and bovine GH (which do not bind to the PRL receptor) appear to be inactive in this model (156). However, *in vivo* in the mouse, although hGH and ovine GH exerted positive effects on thymic development, ovine PRL was described as having "the opposite effect of GH on the thymus" (157). Careful comparisons of GH and PRL activity using homologous systems are needed to discover their relative activities on lymphoid tissues. The effects of GH on the thymus may be due to local IGF-I generation (148), which would fit with the *in vivo* data of IGF-I administration having larger effects than GH on thymic growth (31). However, it should be remembered that GH and PRL may have effects, including on lymphoid tissues (158), that are not mediated via IGF-I generation.

Normal human PBMCs express very low amounts of the IGF peptides, which can be increased after mitogen stimulation (36, 144, 145). In contrast, macrophages are reported to produce much more IGF-I especially when they are differentiating to the mature phenotype (36). Human IM-9 lymphocytes (B lineage cells) also express IGF-I mRNA, but this message is not sensitive to treatment with hGH, despite evidence of GH-induced tyrosine phosphorylation (159). In contrast, transformed B cells (160) release IGF-I in response to GH. In human T lymphoblast cell lines, the stimulation of colony formation produced by GH may be mediated by IGF-I (161). The growth of T-acute lymphoblastic leukemic (ALL) cell lines can be slowed by antibodies against either IGF-I or the Type 1 IGF-I receptor, suggesting autocrine or paracrine activity of IGF-I in T-ALL cell lines (162).

D. IGF receptors on lymphocytes

The insulin receptor and the Type 1 IGF receptor are both tyrosine kinase receptors, have similar structures, and both bind insulin and the IGFs, albeit at lower affinities for the heterologous ligands (163). The receptors are so similar that their subunits are believed to be interchangeable so that they

can naturally form so-called hybrid receptors (163, 164). Both the IGFs and insulin therefore have similar powerful metabolic effects, but the Type 1 IGF receptor also possesses many of the differentiating and mitotic effects found for the ligand/receptor complexes of other tyrosine kinase receptors such as c-*kit*/KL and c-*fms*/colony-stimulating factor-1. Such receptors are important regulators of the differentiation of hematopoietic cells (165). As described above, the GH and PRL receptors are members of another family, the HBP family of cytokine receptors, which also regulate many processes in hematopoietic cells (40). As would be predicted from this discussion, the IGFs and the Type 1 IGF receptor have differentiating and mitogenic activities, including in hematopoietic cells (35, 133).

A third IGF receptor, termed the IGF-II receptor, is also the mannose-6-phosphate receptor, whose role in IGF biology is unclear (166) although it does not appear to transmit a direct intracellular signal (167). This receptor binds IGF-II with high affinity but binds IGF-I with about 100-fold less affinity (168). The IGF-II receptor may act mainly as a functional IGF-II "antagonist" to regulate local IGF-II cell exposure and may have tumor suppressor-like properties (167).

Twenty years ago the binding of insulin to resting (169) and activated (170) lymphocytes was an active area of research. This was followed by the discovery of IGF-I binding to human leukemic lymphoblasts (171), PBMC, resting and activated T cells (172, 173), and the cross-linking of IGF-I to activated T cells (172, 173). This strongly suggested the presence of IGF-I receptors on lymphocytes. The functional importance of these receptors to lymphocyte activation by mitogens was shown by the peak receptor number occurring at the same time as maximal thymidine incorporation (172). The use of two-color flow cytometry, staining with antibodies against the human Type 1 IGF-I receptor, the insulin receptor, and lymphocyte markers, showed that both IGF-I and insulin receptors are present on most monocytes and B lymphocytes, but on only 2% of T lymphocytes (174). Using similar techniques, IGF-I receptors were found in high numbers on monocytes, natural killer cells, and CD4+ cells, an intermediate number on CD8+ cells, and a relatively low number of receptors on B cells (175). Using flow cytometry and biotinylated des(1–3)IGF-1, IGF-I receptors were detected on rat T cells, B cells, and monocytes with the expression on resting CD4+ cells being greater than on CD8+ cells and increasing severalfold after ConA stimulation (176). Why there are discrepancies between these studies is unclear. In another study, T lymphocyte activation, by PHA or the OKT-3 monoclonal antibody (which binds to the CD-3 antigen of the T-cell receptor), led to peaks in IGF-I receptor mRNA after 20–60 h and IGF-I receptor content after 48–72 h (177). If the increased IGF receptor number caused by mitogens is physiologically relevant, the addition of IGF-I should increase proliferation caused by the mitogens. This has been shown for human peripheral lymphocytes (178) and thymocytes (179). Freshly isolated human peripheral lymphocytes have been shown to express IGF-I receptors by RT-PCR (145), and human T lymphoblast cell lines possess IGF-I receptors (180). Some of the differences between the data sets for IGF receptors on peripheral lymphocytes could be methodological, either due to differences between rodents and humans or to lymphocyte receptors being atypical IGF receptors. For example, it has been claimed that the majority of the IGF-I receptors on human IM-9 lymphocytes (B lineage cells) are atypical IGF-I receptors (150).

IGF-I receptors have also been identified on rodent (181) and human thymocytes (179, 182). The signal transduction in normal human thymocytes and T cells appears to be similar to that in other tissues, *e.g.* it involves the phosphorylation of insulin-receptor substrate-1 (IRS-1) (182). In human thymocytes, DNA synthesis can be stimulated directly by IGF-I *in vitro*, and DNA synthesis initiated by the mitogen PHA can be potentiated by adding physiological concentrations of IGF-I (179).

E. IGFBPs

The IGFs also differ from insulin in that *in vivo* they are bound (Fig. 1) to a family of at least six specific, soluble, high-affinity IGFBPs, termed IGFBPs 1–6, which are unrelated structurally to the IGF receptors or the insulin receptor (133). It is possible that novel IGFBPs remain to be discovered. In fact, mac25 (183) and PSF (184) or ESM-1 (185) show marked structural similarity to the IGFBPs. The binding proteins differ in their modes of regulation and perform a variety of functions. For example, the majority of the IGF in blood is bound to IGFBP-3 (Fig. 1), and this complex is bound by a third protein, the acid-labile subunit, to form a large stable 150-kDa complex (186). IGFBP-3 and acid-labile subunit are regulated primarily by GH, have a slow clearance from blood, and provide an accessible pool and reservoir of IGF in the blood (133). In contrast, IGFBP-1 concentrations in blood can change rapidly, are regulated by insulin, and may serve an acute metabolic role to bind and inactivate unbound IGF (187).

Many tissues and cell types secrete IGFBPs, including hematopoietic cells. By RT-PCR, normal human peripheral lymphocytes express mRNAs for the IGF-I receptor, the IGF-II receptor, IGFBP-2 and -3, but not the IGF peptides (145). After stimulation with PHA they express IGF-I, IGF-II, and IGFBP-4 and -5, in addition to IGFBP-2 and -3 (145). Ligand blotting of lymphocyte-conditioned media with labeled IGF-I revealed 34-, 43-, and 49-kDa IGFBPs. The addition of estrogen, progesterone, IGF-I, or GH did not affect secretion of IGFBPs by lymphocytes (145). IM-9 cell-conditioned medium has also been shown to contain a 30-kDa IGFBP (141). Murine stromal bone marrow cells, which support developing hematopoietic cells, not only produce IGF-I but also secrete IGFBPs (147). By ligand blotting (188), the most prominent IGFBPs were IGFBP-4 and IGFBP-5 whereas, by RNase protection assay, murine stromal cells expressed IGFBP-2 to IGFBP-6 mRNAs, with IGFBP-4, IGFBP-5, and IGFBP-6 mRNAs being predominant. These authors (188) suggest that IGFBPs 4–6 are released by stromal cells to modulate the hemopoietic response to IGFs. Sheep thymus cells also produce IGFBPs in culture, secretion is increased by mitogen stimulation, and medium from these cells also degrades recombinant human [^{125}I]IGFBP-3, suggesting IGFBP-3 protease production (189).

IGFBP-2 is the predominant IGFBP during fetal life (as IGFBP-3 is in the adult) and is expressed in a range of tumor

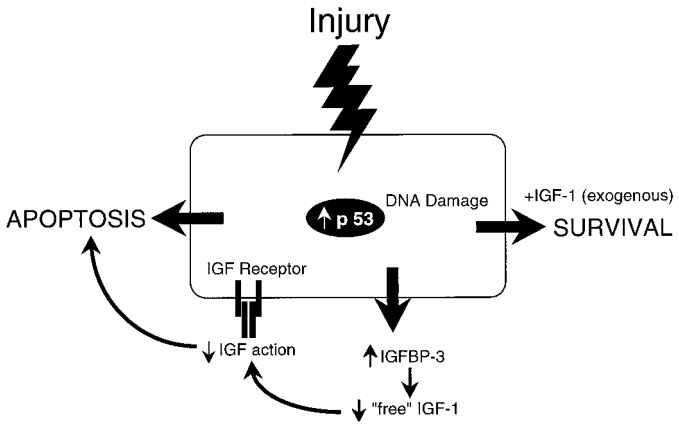

FIG. 4. Possible mechanism for the antiapoptotic effects of IGF-I and the apoptotic effects of IGFBP-3. Injury can induce the expression of the tumor suppression gene p53, which in turn may increase the expression of IGFBP-3. Because IGF-I is bound by IGFBP-3, this might prevent the activation of the IGF-I receptor and allow apoptosis to proceed. In contrast, exogenous IGF-I can allow cell survival.

cell lines (190). Significantly increased serum levels of IGFBP-2 have been detected in the sera from ALL and non-Hodgkin-lymphoma patients (191). At the time of diagnosis with leukemia, non-Hodgkin's lymphoma, or solid tumors the serum concentrations of IGF-I, IGF-II, and IGFBP-3 are very low in children (192). Such low concentrations of these proteins are normally seen only in patients with GH deficiency or during starvation (192). Somewhat surprisingly, IGFBP-2 levels were elevated (192), and it seems that the IGFBP-2 is produced by the tumor cells (193). Leukemic T cell lines, but not B cell lines, produce large amounts of IGFBP-2 and express mRNA for IGFBP-2, confirming data from the patients with tumors (193). The biological significance of this discovery is unclear.

Some evidence indicates that the IGFBPs regulate IGF action in lymphoid tissues. Preliminary data suggest that mice that are null for the IGFBP-2 gene show no gross phenotype except for a reduced spleen size, to 50% of normal (194). The above evidence, of IGFBP-2 production by T cells but not B cells, suggests that the distribution of lymphocyte subsets in these null mice may be altered. Overexpression of IGFBP-1 in transgenic mice has led to inconsistent effects on spleen size (195, 196), whereas overexpression of IGFBP-3 causes increased spleen size (197). An intriguing recent paper (198) shows that activation of the tumor suppressor gene p53, which induces apoptosis, stimulates IGFBP-3 expression. It is possible that the IGF system plays a role in the regulation of apoptosis (Fig. 4) via p53 stimulating IGFBP-3, leading to a local inhibition of IGF-I action with the induction of apoptosis. This provides a mechanism by which, in lymphoid tissue, locally produced IGFBPs, by regulating the availability of IGF-I, may help control cell division and survival.

VI. Actions of IGF-I

A. Bone marrow

This section will focus on the effects of IGF-I on B cell development, which is comparatively well characterized compared with its effects on other hematopoietic lineages. Hematopoiesis takes place in bone marrow in the intersinusoidal spaces of the medullary cavity with multiple cell types being in close association with the developing lymphocytes. For example, in long-term culture, the differentiation and growth of B cells require the presence of fibroblastic bone marrow stromal cells, which produce many growth factors (199). These stromal cells, which include macrophages, produce factors that act in a paracrine manner to regulate B cell lymphopoiesis (146). Factors affecting B cell development have been categorized by Dorshkind (199) as belonging to four categories. First, proliferation factors regulate developing B lineage cell growth, including IL-3 and IL-7. Second, proliferation cofactors synergize with cytokines that stimulate growth but have little intrinsic activity, including c-*kit* ligand and IGF-I. Third, differentiation factors potentiate B cell maturation and include IGF-I, c-*kit* ligand (Kl), IL-7, and ftl3 ligand. Fourth, negative regulators inhibit B cell development and include IL-1, IL-3, IL-4, interferons, and estrogens.

IGF-I has two of these major effects on B cell development (Fig. 5); it acts as a differentiation factor to potentiate pro-B to pre-B cell maturation (200), and it also acts as a B cell proliferation cofactor to synergize with IL-7 (201). The first indication that B cell differentiation factors exist came from clinical studies in infants with cyclic neutropenia in which the production of erythroid and myeloid cells oscillates. In the marrow of these children, pre-B cells also oscillate as does the presence in their urine of a factor that *in vitro* stimulates normal human marrow cells to generate pre-B cells (202, 203). The factor specifically affects differentiation as this occurs in the absence of proliferation. A bone marrow stromal cell line was found to release a similar activity. This activity was identified as IGF-I based on the use of anti-IGF-I antibodies, antisense to IGF-I, and that recombinant IGF-I could substitute for the activity (200). In the presence of IGF-I, pro-B cells mature to pre-B cells, as judged by their ability to proliferate in response to IL-7 (204). As described above, there is evidence that macrophages are a rich source of IGF-I and that bone marrow stromal cells also produce IGFBPs (147). The treatment of mice with rhIGF-1 confirmed these observations (Fig. 6) as it increased the number of pre-B and mature B cells

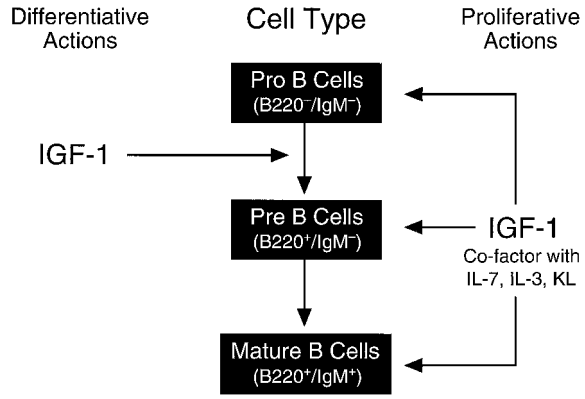

FIG. 5. The differentiative and proliferative actions of IGF-I during the stages of B cell development. IGF-I is unique in that by itself it stimulates the differentiation of Pro-B cells. IGF-I also acts as a proliferative cofactor throughout B cell development.

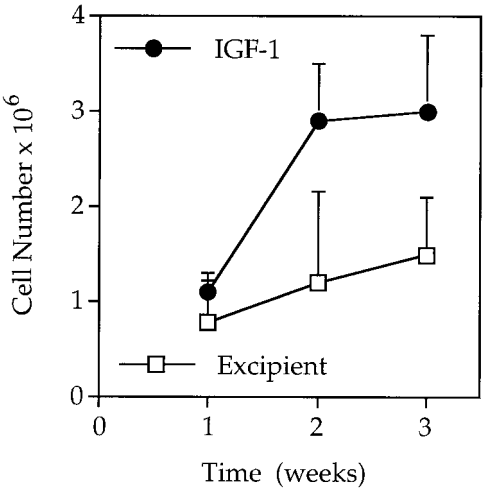

FIG. 6. Treatment with rhIGF-1 (4 mg/kg/day, sc infusion for 2 weeks) more than doubles the number of Pre-B cells (B220+, sIgM−) in the bone marrow of normal adult mice. Means and SDs are shown. [Derived from Ref. 205.]

in bone marrow (205). The mature B cell remains sensitive to IGF-I as immunoglobulin production is also stimulated by IGF-I *in vitro* and *in vivo* (206).

The activity of IGF-I as a cofactor (201) affecting IL-7-induced B cell proliferation is not unique. IGF-I acts as a cofactor in many situations. For example, traditional GH-like responses, such as the growth of the whole body, require optimal IGF-I levels for GH to produce maximal effects, and *vice versa* (99, 207). This has been amply shown in humans, in whom high dose IGF-I administration suppresses GH, leading to a loss of IGF-I efficacy unless GH is coadministered (33). In the periphery, IGF-I enhances the proliferative response of lymphocytes to mitogens (208). During or after immune system damage, which is commonly associated with a catabolic state, systemic and local IGF-I levels are likely to be low. Therefore, for optimal recovery, supplementing this co-factor seems logical to stimulate anabolism and immune reconstitution. There have, as yet, been few animal studies using IGF-I in combination with other growth factors, except for studies with IGF-I and GH (207). In the Snell dwarf mouse, treatment with bovine GH restores many measures of lymphocyte function, but pre-B cell numbers in bone marrow are not restored by bovine GH or ovine PRL (209). Further studies are needed in Snell mice to explain which hormones cause this B cell deficiency and thereby discover the factors that are important to normal B cell development.

The effects of IGF-I on T cell development are not as well characterized, although thymic T cell progenitors proliferate in response to IGF-I before they respond to any other known cytokine (210). It is also clear that thymic epithelial cells produce IGF-I, functional IGF-I receptors are present on thymocytes (181), and the administration of IGF-I to animals affects the number of T cells in the thymus (92). There is as yet no information on the thymic role of IGF-I in processes of positive or negative selection of thymocytes. It has been claimed that rhGH can induce significant migration of resting and activated human T cells (211). These authors speculate that, by directly altering their adhesive and migratory capacities, GH may play a role in normal lymphocyte recirculation. This finding is as yet unconfirmed and raises the question of the activity of IGF-I in these assays.

B. Effects on lymphoid organ size

An involvement of the somatomedins (IGFs) in the regulation of lymphoid organ growth was suggested when IGF-I was administered to hypophysectomized rats because it caused preferential thymic and splenic growth to a greater degree than did GH (31). Such studies were then extended to the mutant dw/dw rat, which showed a similar disproportionate growth of lymphoid tissue (30) whereas in normal aged 18-month-old rats, IGF-I stimulated thymic growth (Fig. 7) and increased lymphocyte numbers (212). Figure 8 shows the effect of 28 days treatment with rhIGF-1, rhGH, or rhIGF-1 plus rhGH on thymic architecture. It is clear (Fig. 7) that rhIGF-1, and to a lesser extent rhGH, stimulates thymic growth. Because the rat is not the preferred species for immunological studies, the mouse was selected as an experimental animal. At the time, there was very little information on effective doses or dosing regimens of IGF-I in the mouse apart from some early anabolic studies using somatomedin preparations in mutant dwarf mice (213) and isolated more recent studies with rhIGF-1, also in mutant mice (214). Instead of using GH-deficient animals, the effects of IGF-I were studied in normal, 9-month-old, retired breeder "middle-aged" male mice (92).

Older animals were chosen with the hope that their relatively impaired immune status could be improved by treatment with rhIGF-1, as had been seen in aged rats where GH3 cell implantation reversed age-related thymic atrophy (91). A range of doses of rhIGF-1 (0.25, 1, 4 mg/kg/day) were given by subcutaneous minipump infusion to avoid the need for the very frequent injections that were shown in growth studies to be most effective in mice. These doses doubled total serum IGF-I concentrations, induced a dose-related weight gain, and had minor effects on blood glucose, yet doubled the weight of both the thymus and spleen (92). Therefore, the effects of rhIGF-1 seen in GH-deficient rats could be duplicated in normal mice.

The administration of IGF-I has been shown to increase the size of lymphoid organs in several species. In rats and mice numerous studies report increases in lymphoid tissue mass with IGF-I administration (36). In 1-yr-old sheep, an 8-week regimen of three daily injections of rhIGF-1 (50 μg/kg) increased spleen weight by 40% (215). In the rabbit, cat, and dog similar effects of IGF-I have been observed (R. Clark, unpublished observations). In the rhesus monkey, IGF-I also expands lymphocyte numbers (216). This finding is discussed in more detail in *Section IX*. Lymphoid organ expansion has been reported in children with GH insensitivity who have been treated long-term with rhIGF-1 (110). This is the first direct confirmation that the immunological effects of administering IGF-I to animals are also present in humans.

Mice transgenic for GH or IGF-I have enlarged lymphoid organs (217). An effect of endogenous IGF-I on lymphoid tissue growth was presumed in a large study (218) where lines of mice were selected over many generations on the basis of high or low serum IGF-I levels. The high IGF-I line

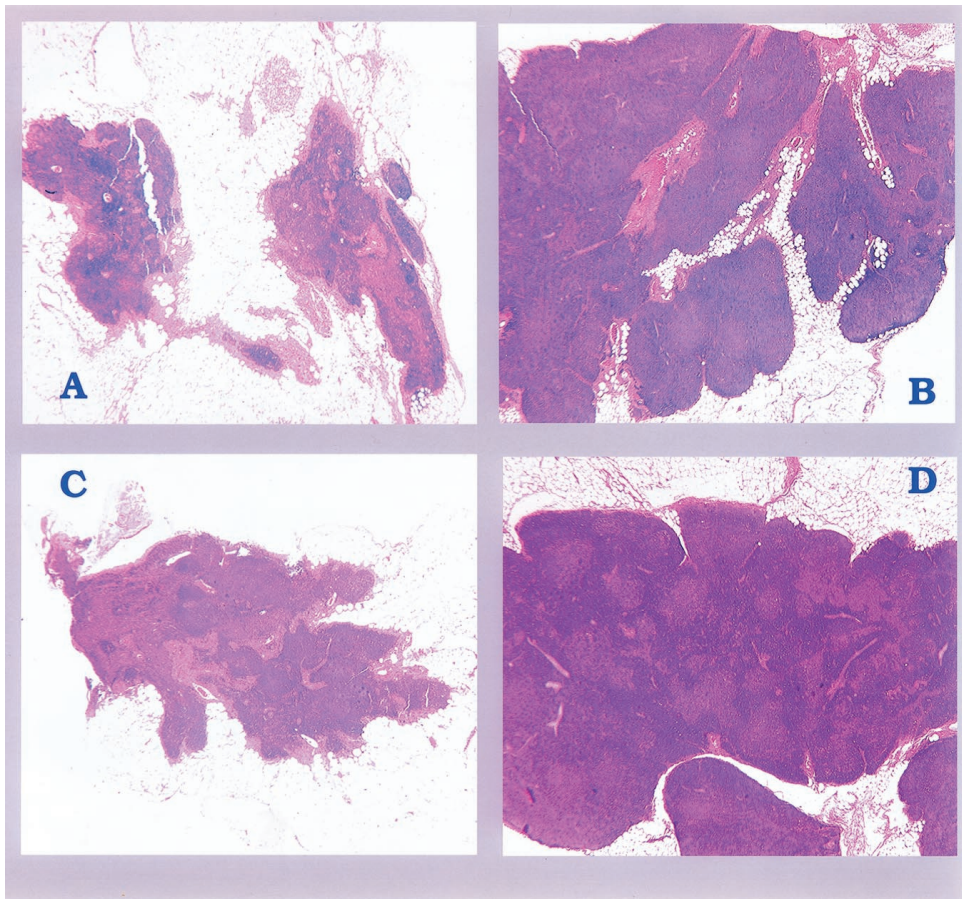

FIG. 7. Thymic histology in 18-month-old male rats treated for 4 weeks with either excipient (panel A), rhIGF-1 (panel B, 1.1 mg/rat/day, sc minipump infusion), rhGH (panel C, 1 mg/rat/day, daily sc injection), or rhIGF-1 plus rhGH (panel D). Formalin-fixed hematoxylin-eosin-stained sections. The hormone treatments, particularly rhIGF-1, caused a dramatic expansion and rejuvenation of the involuted thymus of aged rats [R. Clark, unpublished data].

had spleens 30% heavier than the low IGF-I line. Thymus weights were also greater in high-line than in the low-line mice, and developmental patterns of thymus weight closely paralleled those of circulating IGF-I (218).

Compared with the ample data on the effects of IGF-I, the effects of IGF-II on lymphopoiesis are not as well studied (219, 220). IGF-II binds less well to the Type I IGF-receptor; therefore, higher concentrations are probably needed (although much less than insulin) to mimic the effects of IGF-I. However, there are reports, based on IGF-II transgenic mice, that IGF-II also has immune modulator properties (219, 220). In some situations, in some species, IGF-II may play a role in lymphoid tissue function. Adult humans, unlike rodents, have blood concentrations of IGF-II equal to or greater than those of IGF-I. The regulation of IGF-II production is an active area of research and has produced surprising findings. For example, IGF-II production may be controlled by a rapamycin-sensitive pathway (221).

C. Effect of in vivo treatment on lymphocyte number and in vitro function

In mice treated with rhIGF-1 for 7 or 14 days, analysis of the lymphocyte subsets showed that a large part of the increased spleen weight was due to a doubling in the number of both T- and B lymphocytes (92). The increased thymic mass was also due to a doubling in Thy 1-positive cells (T lymphocytes) and an increase in peanut agglutinin receptor binding (a marker for immature thymocytes), but there were no changes in Thy-1, CD4, or CD8 expression on single or double positive thymocytes. In the spleen, there was a preferential increase in the number or sIg+ cells (B-lymphocytes) compared with T lymphocytes (92). For the splenic T lymphocytes, the numbers of both CD4- and CD8-positive cells were increased. Peripheral lymph nodes were also increased in size and lymphocyte number but in peripheral blood lymphocyte number decreased by 20%, whereas neutrophil number increased. There were no changes in other blood cell numbers (92).

To test lymphocyte function, cells from spleen and lymph nodes were incubated *in vitro* with mitogens (92). After 14 days of treatment with rhIGF-1, the responses to ConA (T cells), lipopolysaccharide (LPS, B cells), and pokeweed mitogen (both T- and B cells) were increased 4-fold in spleen and doubled in lymph nodes. Cells from rhIGF-1-treated and control mice showed identical responses in a mixed lymphocyte response to allogenic splenocytes, suggesting unchanged antigen-specific T cell responses. To test B cell function, mice were immunized with dinitrophenyl-ovalbumin (DNP_2OA) and given a boost 35 days later and treated with rhIGF-1. When tested *in vitro*, splenocytes from IGF-treated mice had a doubling of their basal and antigen-stimulated immunoglobulin production. Therefore, there was clear evidence that treatment with rhIGF-1 increased both the number of lymphocytes and their function (92). In comparison, injections of hGH were much less potent and effective (143).

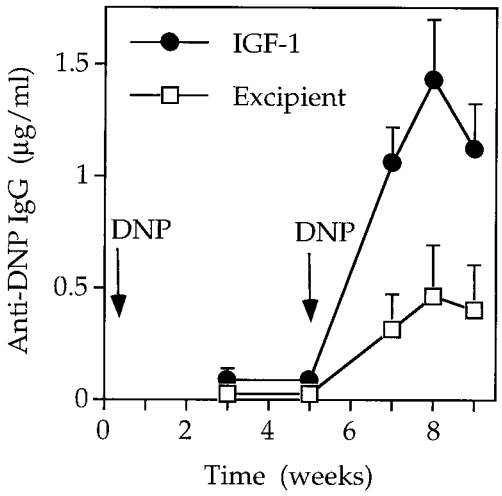

FIG. 8. Antibody production in mice immunized with 0.1 μg DNP_2OA at week 0, then boosted at week 5. In addition, the mice were treated with either excipient or rhIGF-1 (4 mg/kg/day) for 2 weeks after each immunization. Treatment with rhIGF-1 enhanced antibody production, especially after the secondary immunization. Means and SDs are shown. [Derived from Ref. 206.]

D. Functional effects of IGF-I in vivo: antibody responses

It was clear from the literature (222) and our own studies that B cells are preferentially responsive to rhIGF-1 and show an enhanced immunoglobulin production *in vitro* (92). To discover whether IGF-I enhanced immune function *in vivo*, immunization experiments (Fig. 8) were perfomed in retired breeder mice using DNP_2OA as the antigen (206). In the first experiments, mice were treated with rhIGF-1 for 14 days, commencing at the time of an antigen challenge, and showed a dramatically enhanced primary antibody response, as measured by the serum anti-DNP IgG concentration assayed by ELISA. This protocol was then repeated, but a secondary immunization was given after 21 days to test the effect of rhIGF-1 on the memory response to DNP_2OA (206). The anti-DNP IgG concentration at the peak of the secondary response was 4-fold higher in mice treated with rhIGF-1. A second 14-day course of rhIGF-1 treatment (begun 8 weeks after the first course), initiated when lymphoid organ cell numbers had returned to baseline, also increased lymphoid organ cell number. Lastly (Fig. 8), the ability of rhIGF-1 to enhance the antibody response to a suboptimal dose of antigen was tested (206). In this study the mice were treated with rhIGF-1 twice, at the primary immunization and then after 5 weeks at the secondary immunization. Primary and secondary responses to a suboptimal dose of antigen were greatly enhanced, reaching levels similar to those induced by an optimal antigen dose in excipient treated animals (206). In diabetic rats, IGF-I treatment did not improve the primary antibody response to an antigen challenge (223). It is unclear why this study in rats failed to show the effects on antibody generation seen in mice. However, the older literature (126) shows that in hypophysectomized rats, which have very suppressed antibody responses, there is a dramatic enhancement of antibody generation after treatment with GH.

E. Immune reconstitution

IGF-I treatment increases T and B cell number and improves antibody responses, suggesting that it might have a normal role in B and T cell function. To address the site(s) of action of IGF-I, mice were lethally irradiated and then reconstituted with a transplant of 10 million bone marrow cells from syngeneic donors (205). The catabolic effects of the radiation were attenuated by IGF-I with body weight loss reduced, spleen and thymus weight improved, and splenic T cell number and function improved 23 days after transplantation. Treatment with rhIGF-1 also doubled thymus weight, and thymic cell count tripled. In this model IGF-I increased the rate of peripheral lymphocyte repopulation by acting directly on bone marrow progenitors and by stimulating the entry of mature peripheral splenic lymphocytes into S phase of the cell cycle (205). After chemically destroying lymphoid tissues, similar restorative effects of rhIGF-1 have been shown in rats (224). In mice with severe combined immune deficiency (SCID), GH has also been shown to improve T cell engraftment after the transfer of human or murine cells (225). It is unclear whether IGF-I is involved in these effects of GH, although in our hands GH is much less effective than IGF-I at promoting immune reconstitution after bone marrow transplantation.

F. Mechanism of action: apoptosis

Programmed cell death (apoptosis) is fundamental to many levels of the immune system from the development of precursor cells in bone marrow, selection in the thymus, to deletion of mature cells in the periphery (226). How IGF-I expands B and T cell number is unclear (199); it could act positively to potentiate differentiation or it could act passively to enhance survival, *e.g.* by reducing apoptosis (Fig. 9). IGF-I has marked anti-apoptotic effects (227) in many tissues and cell types, which may be important in normal growth and differentiation, in tumor growth (228), and for the protection of tissues from damage. IGF-I has been especially impressive at protecting the kidney (229), heart (230), and brain (231) from damage after ischemic injury. This protec-

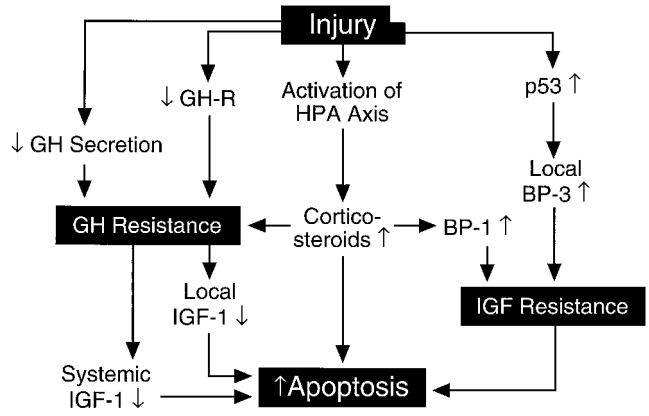

FIG. 9. A proposed scheme of how the GH/IGF-I axis might modulate apoptosis. An insult or injury induces GH resistance and IGF resistance, which adrenal steroids exacerbate. The inhibition of the effects of GH and IGF-I probably enhances the likelihood of damaged cells dying from apoptosis.

tion may, in part, involve anti-apoptotic mechanisms. The bulk of evidence for the identification of IGF-I and the Type I IGF receptor, as powerful inhibitors of apoptosis and survival factors for cells, comes from the field of tumor biology (228). Apoptosis is regulated by a rapidly growing array of families of signaling molecules; as a consequence, the pathway(s) affected by IGF-I will be described in the future. For example, in several interleukin 3 (IL-3)-dependent cell lines, IGF-I can prevent apoptosis after IL-3 withdrawal (232). In some cell types, even where it is a poor mitogen, IGF-I is the key antiapoptotic growth factor (227); therefore, it is reasonable to assume that IGF-I does signal through apoptotic pathways in lymphoid cells. There is, as yet, no direct published evidence that IGF-I increases lymphoid cell number in animals by inhibiting apoptosis, but a theoretical framework for such activity can be postulated, as illustrated in Fig. 9.

VII. IGF-I in Different Physiological States

A. IGF-I in pregnancy

It is well established (233) that the thymus atrophies when blood concentrations of estrogen rise. Conversely, thymic involution is delayed by castration. The involution of the thymus in pregnancy is dramatic. Gross thymic weight falls from about 40 mg to 10 mg by day 17 of pregnancy in the mouse, due to both cell death and the specific loss of cortical $CD4^+$ $CD8^+$ lymphocytes (234); while the cortex shrinks the medulla is rearranged. These changes probably contribute to the immune suppression of the mother to paternal and fetal antigens. Prevention of lactation causes thymic repopulation, which takes about 3 weeks (234). Clearly, pregnancy and lactation are physiological states in which the GH and IGF-I systems are very active, and these effects of steroids on the thymus may involve interactions with the GH and IGF systems. An interesting issue is the apparent immune reconstitution of the weanling Snell-Bagg mice if weaning is delayed from 21 to 30 days of age (235). Whether this effect is due to dwarf mice being particularly sensitive to stress at this age or is due to factors in milk is unknown. The administration of T_4 to Snell-Bagg mice has significant effects on lymphopoiesis (236), and recent discoveries in animals (237) and humans (238) show that hormones, *e.g.* T_4, can be absorbed from milk, suggesting that maternal influences on immune function persist beyond pregnancy and may also be hormonal in nature.

In pregnant mice the number of immature B-lymphocytes ($sIgM^+$, $sIgD^-$, heat-stable antigen, HSA ^{hi}) in bone marrow and spleen are reduced, but the number of mature B cells in peripheral sites is not affected. The number of immature B lymphocytes is also reduced in normal mice by estrogen treatment (239) while in hypogonadal mice the number of these cells is greatly elevated (240). Kincade *et al.* (233) state that for estrogens to affect B cell precursors stromal cells must be present, inferring that stromal factors mediate their effects. IGF-I has been shown to be produced by marrow stromal cells and to stimulate B cell development (200, 201, 205). In some systems, including bone, estrogens are known to inhibit the activity of GH (241), while in the uterus estrogens synergize with the ability of GH to stimulate IGF-I mRNA (242). In lymphoid tissues steroids may also modify the activity of the GH system. The reverse is also true, as GH can affect estrogen receptor (ER) levels (243). In adult female rats, hypophysectomy reduced hepatic ER levels 10-fold and treatment with PRL had no effect, but continuous infusion of GH to hypophysectomized animals tripled ER and doubled ER mRNA levels. These authors (243) state that GH is the most important hormone affecting ER protein levels. The interactions between the GH system and sex steroids in bone marrow stromal cells and their influence on B cell development may lead to a more general understanding of the molecular and signaling interactions between steroids and the GH system.

Therefore, at the level of the marrow, thymus, and spleen, reproduction and reproductive hormones have profound effects. It is likely that the GH and IGF-I systems are involved in these processes, which may help explain the remarkable inhibition of maternal immunity to fetal antigens and thus how the embryo escapes damage from the maternal immune system (233). Knowledge of these natural phenomena may aid in devising strategies for allogenic transplantation and also help to explain gender differences in autoimmune disease.

B. IGF-I in diabetes

The marked thymic atrophy in streptozotocin-diabetic rats can be reversed by treatment with insulin or IGF-I (223). In this situation, insulin probably corrects the thymic atrophy indirectly, by normalizing the metabolic derangements, including causing glucose uptake into tissues. However, treatment with IGF-I can restore thymic size without normalizing blood glucose (223). There is a growing interest in IGF-I as a glucose-regulatory hormone with a view to its use as a therapeutic agent in diabetes (138, 244). The use in Type I diabetes of IGF-I, a potentially immunologically active molecule that has even been implicated in the development of this disease (245), should proceed with caution. However, a recent study (246) shows that the treatment of nonobese diabetic mice (NOD) with IGF-I has protective, rather than deleterious, effects. Nondiabetic mice received 7 million activated T cells from diabetic NOD mice, and 12 of 21 became diabetic; only six of 24 mice treated with IGF-I became diabetic and the IGF-I-treated mice retained 49% of their islets intact while in the control mice only 1.6% of the islets were intact. These authors state (246) that IGF-I has protective effects in autoimmune diabetes and that this opens new preventive strategies in human Type I diabetes. Careful repetition of these important experiments is needed as are experiments in animal models of other autoimmune diseases to discover whether this is a disease-specific effect of IGF-I or whether IGF-I has therapeutic potential in a range of autoimmune diseases.

C. IGF-I in gastrointestinal disorders

GH and IGF-I affect the growth and function of the gastrointestinal tract in animals (247, 248) and in humans (249). For example, after 80% gut resection in rats, treatment with IGF-I can cause the remaining gut to hypertrophy and to

show an increased function (247). After a 50% burn in rats, gut mucosal atrophy and increased permeability were associated with a 89% incidence of bacterial translocation to mesenteric lymph nodes (250). However, if the rats were treated with IGF-I (3 mg/kg/day, sc, minipump), gut mucosal weight increased, and the incidence of bacterial translocation was reduced to 30% (250). These beneficial effects of IGF-I treatment on gut structure in burned rats have been confirmed (251). IGF-I may inhibit bacterial translocation by affecting both gut integrity and the gut's immune response. In a rat model of cecal ligation and sepsis, combined with total parenteral nutrition, IGF-I (4 mg/kg/day for 3 days) increased gut metabolism, reduced mucosal atrophy, and reduced the hepatic portal blood endotoxin concentrations (252). The authors conclude that IGF-I may play a role in maintaining gut barrier function in sepsis (252). A recent report (253) tested the efficacy of GH and IGF-I in a murine model of sepsis. Normal female mice were pretreated (three times a day, sc) with rhIGF-1 (2.4 or 24 mg/kg/day), rhGH (0.48 and 4.8 mg/kg/day), or excipient for 6 days, then challenged with *Escherichia coli* (1×10^8 units, ip). IGF-I and GH significantly prolonged survival, reduced bacterial counts in the peritoneum, and suppressed cytokine production. The authors conclude that GH and IGF-I improve host defenses via immunomodulation in murine sepsis (253). However, in another model of septic shock, the opposite result has been reported (254). Normal rats were pretreated for 3 days with an infusion of rhGH (192 μg/day, sc, minipump) or excipient and then given endotoxin LPS (5 mg/kg). The infusion of rhGH in this study (254) potentiated endotoxinemia, as measured by liver and kidney enzymes and metabolites in blood 14 h later. Why this study produced such discordant results is unclear. The evidence therefore suggests that IGF-I may play a role in maintaining gut structure and function and that treatment with IGF-I may be useful therapeutically to improve gut function when it is compromised.

A reason why IGF-I status may be important in sepsis is that bacterial endotoxins reduce blood IGF-I levels (255, 256). The intravenous administration of LPS to rats dramatically decreases serum IGF-I to 50% of normal in only 4 h due to a direct effect of LPS on the liver (256). If IGF-I is important to gut barrier function, such an immediate effect of endotoxin on blood IGF-I concentrations would further exacerbate gut failure and allow even more bacterial translocation. There is much to understand in this promising new area of research, including the effects of IGF-I on the largest lymphoid system in the body, that of the gut.

D. IGF-I action in polycythemia vera

It is interesting and instructive to consider recent developments regarding the effects of IGF-I on erythropoiesis. Erythroid cell number is primarily regulated by erythropoietin but is impacted by many other growth factors. For example, hypophysectomized rats show low blood cell counts (3) for erythroid, myeloid, and lymphoid cells, and there is a deep literature showing effects of both GH, PRL, and IGF-I on all hematopoietic lineages (15, 35, 137, 257–259). However, mice with disrupted IGF peptides or IGF-receptors have normal erythropoiesis (260, 261). The significance of IGF-I in hematopoiesis has also been questioned (262), chiefly on the basis of IGF receptor-positive bone marrow cells lacking clonable hematopoietic progenitor cells (262). These authors (262) also refer to the lack of effect of GH deficiency on blood morphology. Recent findings (263, 264) in polycythemia vera (PV) will renew interest in the role of IGF-I in hematopoiesis. PV is a chronic myeloproliferative disease of a deregulated clonal expansion of a pluripotent stem cell giving rise to granulocytosis, thrombocytosis, and erythrocytosis (265), which is not erythropoietin dependent. Correa *et al.* (263) developed a serum-free system culture system for circulating erythroid progenitors and discovered that PV cells are 100-fold more sensitive to IGF-I. An antibody against the IGF-I receptor blocked IGF-I stimulation (263). Why the IGF-I receptor in PV is supersensitive to ligand is unknown. These unexpected findings should be seen as instructive in terms of the role of IGF-I in lymphopoiesis and disease. It is likely that diseases or deficiencies of the immune system will be found that are caused by aberrations in the local GH, PRL, and IGF axes in hematopoietic cells. What is recognized by the endocrinologist as systemic GH, PRL, or IGF deficiency or excess may be of little importance to the hematologist to whom the paracrine or autocrine mechanisms regulating the same molecules or their receptors may be more relevant.

VIII. GH/IGF-I as Antistress Hormones

For efficient homeostatic regulation, most physiological systems are regulated by inhibitors and stimulators. This section will explore the hypothesis that, physiologically, the adrenal steroids are the major immunosuppressive hormones while the somatogenic hormones are a major counterbalancing immunostimulatory system (266). It is also proposed (Fig. 9) that, in situations of extreme stress or where the immune system is damaged, the somatogenic hormones also have a repair function.

As described above, IGF-I is "insulin-like" in that it is sensitive to nutritional status. During the lifespan in most species, and certainly in most of mankind, periods of starvation are common, and so hormonal systems have evolved to manage this state. Some of the effects of undernutrition may be mediated by IGF-I (135). In nutritional stress or injury (Fig. 9) the hypothalamic-pituitary-adrenal axis is activated so that glucocorticoid production rises leading to many changes in the GH/IGF axis. A GH resistant state is induced so that local and systemic concentrations of IGF-I fall, which is exacerbated by a fall in systemic IGFBP-3 because of reduced production and increased protease activity (Fig. 1). The amount of IGFBP-1 and IGFBP-2 in the circulation rises (133), and the induction of p53 may increase local BP-3 concentrations so that local IGF resistance also occurs. This combination of GH and IGF resistance is associated with anabolism being suppressed and subsequently bone marrow depletion and perhaps immune function being compromised. It is unknown whether IGF-I can be used to assist in the protection of the immune system during undernutrition. Replacement of IGF-I during undernutrition can, at best, only partly protect against these deleterious effects. However, the

anabolic hormones have a clear role in recovery from stress and undernutrition and in reconstituting the immune system, as shown by studies in radiated mice (205). It is unclear whether pretreatment with IGF-I would protect lymphoid tissues from the damage caused by radiation or other stresses, as it does in other damaged tissues such as kidney (229), heart (230), and brain (231).

There are animal studies suggesting that anabolic hormones can counteract some of the anticatabolic and immunosuppressive effects of administered glucocorticoids. There is evidence that GH, either given by injection or endogenously elevated by stress, can in the rat partly reverse the leukopenia (267) and the reduced antibody levels (268) caused by steroids. More recent studies (101) in mice have shown that rhGH, ovine PRL, or bovine GH (24 μg/day, sc osmotic pump) all reversed corticosterone (50 μg/day)-induced suppression of spleen lymphocyte responses to T cell mitogens. In contrast, the thymic atrophy caused by corticosterone was not reversed by rhGH. These authors (101) also show a suppression of liver PRL receptor levels by corticosterone, their recovery by the lactogenic hormones, and a relationship with the immune responses observed. They propose a similar effect of corticosterone on lymphocyte PRL receptors. In a more recent study in C57/Bl/6J mice, the coadministration of rhGH (0.8, 4, 8 IU/kg/day) with prednisolone (10 mg/kg/day, ip, for 10 days) was found to prevent the reduction in thymic and splenic weight and cell number caused by the steroid (269). Whether this is a practical treatment must be balanced against the diabetogenic effects of high-dose GH exacerbating the metabolic risks (insulin resistance, hyperglycemia, hypertriglyceridemia) of glucocorticoid treatment (270). On the other hand, IGF-I treatment in humans, because it suppresses GH production (271) and has insulin-like rather than diabetogenic effects when given in combination with prednisone (272), should have fewer adverse metabolic effects.

In the rat, the whole body weight loss caused by dexamethasone (20 μg/rat/day, by sc minipump) was partly inhibited by cotreatment with IGF-I for 7 days (247). In these studies, IGF-I could restore spleen weight almost back to normal, but the thymus did not regrow on a gross weight basis (247). These reports of differences between GH and IGF-I in their ability to reduce thymic involution in rodents are worthy of further investigation.

Lymphopoiesis may be regulated normally by local IGF-I, whereas for the task of repopulating marrow after damage it may be necessary to access the larger pool of IGF-I present in blood. Systemic IGF-I may be required in a damaged marrow or thymus as the supporting stromal cells, which produce IGF-I locally, will be damaged reducing the supply of locally produced IGF-I. Therefore, in different situations either endocrine or local IGF-I production may have differing contributions to lymphopoiesis. Studies using transgenic and IGF-I and IGF receptor null mice (260, 261, 273) will help shed light on the relative contribution of locally produced IGF-I and endocrine IGF-I in the regulation of immune function. Mice with tissue-specific expression of IGF-I crossed on to an IGF-I null background may help establish the relative importance of IGF-I of local and endocrine origins not only for lymphopoiesis but for its effects in general.

IX. Therapeutic Potential

A recent study (216) suggests that the immunological effects of GH or IGF-I treatment in rodents may also be present in primates. In aged (16- to 20-yr-old) rhesus macaque monkeys, infusions of rhGH and rhIGF-1 for 7 weeks affected the phenotype of lymphocytes in blood, spleen, and lymph nodes as measured by flow cytometry (Fig. 10). Quite different effects of treatment were seen in the blood compared with the peripheral lymphoid organs. In blood, the percent CD4 cell count and the CD4/CD8 ratio fell with rhIGF-1 treatment but were normalized by rhGH plus rhIGF-1. In the spleen (Fig. 10) combination treatment almost tripled the percent CD4 cells and more than doubled the CD4/CD8 ratio (216). This paradox of differential effects on lymphocyte populations in different body compartments may be due to the anabolic hormones affecting lymphocyte trafficking as rhGH and rhIGF-1 appear to cause lymphocytes to accumulate in lymphoid organs at the expense of lymphocyte numbers in the circulation (92, 205). One implication of this effect is that in humans, where it is only practical to sample blood lymphocytes, the activities of rhGH and rhIGF-1 may be difficult to detect. These observations in primates make it more likely that rhIGF-1 will prove useful in humans to improve immune function, especially after damage to the immune system or in immune senescence in the elderly. Growth factors are used in humans to restore hematopoietic cells after radiation, chemotherapy, or bone marrow transplantation. At present, no growth factor therapy is available that would speed the slow and incomplete recovery of lymphopoiesis. As a consequence, infections remain a major long-term problem even after the most successful bone marrow transplantation regimens have been used (274).

The importance of the effects of IGF-I on the thymus has assumed a more interesting dimension with the recent publications by Gress *et al.* (275, 276). There is an increased incidence of opportunistic infections after intensive cancer chemotherapy; therefore, immune incompetence may be a dose-limiting toxicity for high dose chemotherapy (275). In

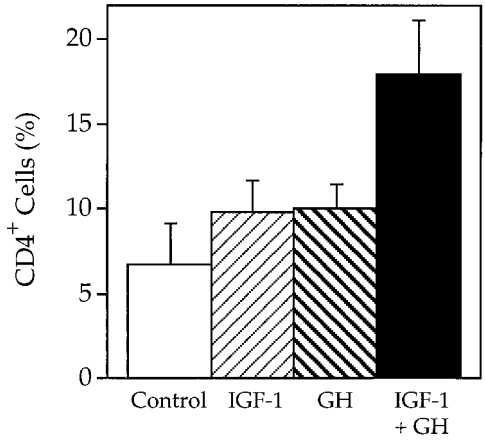

FIG. 10. CD4+ T lymphocytes in the spleen of adult rhesus macaques treated by sc minipump infusion with excipient, rhIGF-1 (120 μg/kg/day), hGH (100 μg/kg/day), or rhIGF-1 plus hGH for 7 weeks. The combination of rhIGF-1 and hGH almost tripled the percentage of splenic cells that were CD4 positive. Means and SEs are shown. [Derived from Ref. 216.]

children, the recovery of CD4+ T cells, after treatment with chemotherapeutic agents, occurs primarily by a thymus-dependent pathway (276). With advancing age, the contribution of this pathway declines rapidly. The degree of immune reconstitution after damage is directly related to the age of the patient and, more importantly, the amount of residual thymic function (276). Therefore it is possible that if thymic growth and function could be stimulated by growth factors, such as rhIGF-1 or rhGH, then lymphocyte function might be more rapidly restored after chemotherapy, especially in adults. In the setting of combined chemotherapy and bone marrow transplantation, treatment with growth factors may be doubly valuable. The effect of such growth factors on tumor growth needs to be addressed, although initial short-term studies with IGF-I suggest that tumor growth is not enhanced (277).

GH is being tested as an anabolic treatment for patients with AIDS-associated wasting (278). In other experiments, testing IGF-I and the combination of IGF-I and GH as a therapy for AIDS wasting, there was some evidence of a beneficial effect on body composition (279). These studies have been relatively short-term; appropriate dosing regimens or dose levels in humans are not established; and measures of lymphocyte number and function, if made at all, have been based on blood cell counts. As stated above, the idea that peripheral blood lymphocyte numbers and ratios provide useful information about immune status has been questioned (280) as only a few percent of the lymphocytes in the body are circulating in blood, with the majority of the cells being in the lymphoid organs. This problem is inherent to the use of anabolic hormones that can double lymphoid organ size while having minor effects on blood cell counts. Measuring the immunological effects of IGF-I in human studies will be difficult. In rodent or monkey studies, lymphocyte number and function can be measured directly in lymphoid organs removed when the animals are killed; measuring potential benefits solely by studying effects on the number or function of the lymphocytes in the peripheral blood is more difficult.

Much is to be learned in this fascinating area of IGF research. For example, data from animal studies suggest that GH and IGF-I can protect against bacterial infections (252, 253). Although GH has been reported to augment human immunodeficiency virus growth (281), an intriguing recent paper indicates that IGF-I may directly inhibit human immunodeficiency virus replication *in vitro* (282). Mice given the antiviral drug azidothymidine (AZT) showed significant myelotoxic effects, which treatment with rhIGF-1 reversed as measured by splenic and bone marrow progenitor cell content and blood cell counts (283). The thymic atrophy caused by AZT in mice can also be reversed by treatment with rhIGF-1 (284). Similar effects of GH have been reported when it is given with AZT (285). The use of IGF-I in some human diseases with an immunological component is also bought into question by its immunological activities. For example, the hypomyelination in the IGF-I null mouse (286) and the re-myelination in damaged tissue in animal models of multiple sclerosis (287) suggest that IGF-I might be useful for the treatment for multiple sclerosis. However, the possible benefits of IGF-I on oligodendrocytes must be balanced against the possible adverse effects of IGF-I enhancing immune function and so stimulating the underlying immunological disease.

X. Conclusions

Aging, stress, and nutrition affect blood concentrations of the anabolic hormones GH, PRL, and IGF-I, which in turn modulate immune function. Recent studies show that IGF-I plays an important role in the maturation of lymphocytes in bone marrow and assists their function in the periphery. In rodents, treatment with IGF-I can restore age-related thymic involution, increase lymphocyte number and activity and improve the reduced antibody response to an antigen challenge, and accelerate lymphoid reconstitution after radiation and bone marrow transplantation. IGF-I may act on lymphoid tissues via its potent anti-apoptotic effects. Perhaps the anabolic hormones have a dual role in regulating lymphopoiesis. First, in the well-fed, nonstressed state, normal bone marrow may utilize the IGFs as cofactors for ongoing lymphopoiesis. Second, during stress, IGF may be protective from tissue damage, but if damage occurs it may also help restore a damaged immune system. For these latter effects there may be a requirement for endocrine IGF-I. These results imply that IGF-I may be useful as a therapeutic in immunodeficient states.

Acknowledgments

Dr. Paula Jardieu is thanked for her help and advice on matters immunological and for the long and fruitful research collaboration that led to much of the data and many of the ideas in this review. Long-standing collaborations with Dr. Kenneth Dorshkind (University of California, Riverside) and Dr. I. C. A. F. Robinson (Medical Research Council, London) have also helped develop ideas, stimulated experiments, and aided data interpretation. The excellent research support at Genentech is acknowledged, especially that of Deborah Mortensen, Joseph Strasser, Wesley Won, Yan Hui Ma, Winnie Werther, Kim Robbins, Susan McCabe, and Dan Tumas.

References

1. **Smith P** 1930 The effect of hypophysectomy upon the involution of the thymus in the rat. Anat Rec 47:119–143
2. **Nagy E, Berczi I** 1978 Immunodeficiency in hypophysectomized rats. Acta Endocrinol (Copenh) 89:530–537
3. **Berczi I, Nagy E** 1991 Effects of hypophysectomy on immune function. In: Ader R, Felten DL, Cohen N (eds) Psychoneuroimmunology II. Academic Press, New York, pp 339–375
4. **Lundin PM** 1958 Anterior pituitary gland and lymphoid tissue growth. Acta Endocrinol (Copenh) 28[Suppl 40]:
5. **Everitt AV, Cavanagh LM** 1965 The aging process in the hypophysectomised rat. Gerontologia 11:198–207
6. **Berczi I, Nagy E, Kovacs K, Horvath E** 1981 Regulation of humoral immunity in rats by pituitary hormones. Acta Endocrinol (Copenh) 98:506–513
7. **Pierpaoli W, Baroni C, Fabris N, Sorkin E** 1969 Hormones and immunological capacity. II. Reconstitution of antibody production in hormonally deficient mice by somatotropic hormone, thyrotropic hormone, and thyroxin. Immunology 16:217–230
8. **Fabris N, Pieropaoli W, Sorkin E** 1972 Lymphocytes, hormones and ageing. Nature 240:557–559
9. **Besedovsky HO, Rey AD** 1996 Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev 17:64–102

10. **Grossman CJ** 1984 Regulation of the immune system by sex steroids. Endocr Rev 5:435–455
11. **Bateman A, Singh A, Kral T, Solomon S** 1989 The immune-hypothalamic-pituitary-adrenal axis. Endocr Rev 10:92–112
12. **Munck A, Guyre PM** 1991 Glucocorticoids and immune function. In: Ader R, Felten D, Cohen N (eds) Psychoneuroimmunology. Academic Press, New York, pp 447–513
13. **McCruden AB, Stimson WH** 1991 Sex hormones and immune function. In: Ader R, Felten DL, Cohen N (eds) Psychoneuroimmunology. Academic Press, New York, pp 475–493
14. **Berton EW, Bryant HU, Holaday JW** 1991 Prolactin and immune function. In: Ader R, Felten DL, Cohen N (eds) Psychoneuroimmunology. Academic Press, New York, pp 403–428
15. **Kelley KW** 1991 Growth hormone in immunobiology. In: Ader R, Felten DL, Cohen N (eds) Psychoneuroimmunology. Academic Press, New York, pp 377–402
16. **Berczi I** 1994 The role of the growth and lactogenic hormone family in immune function. Neuroimmunomodulation 1:201–216
17. **Fabris N, Mocchegani E, Mariotti S, Pacini F, Pinchera A** 1989 Thyroid-thymus interactions during development and aging. Horm Res 31:85–89
18. **Zimmet P, Ng F, Bornstein J, Taft P** 1972 Somantin (In-G), cataglykin (Ac-G), and glucose homeostasis in man. Horm Metab Res 4:309–310
19. **Salter J, Best GH** 1953 Insulin as a growth hormone. Br Med J 2:353–356
20. **Wagner EM, Scow RO** 1957 Effect of insulin on growth in force-fed hypophysectomized rats. Endocrinology 61:419–425
21. **Young FG** 1967 Insulin and the action of growth hormone. In: Pecile A, Muller EE (eds) Growth Hormone: Proceedings of the First International Symposium. Excerpta Medica Foundation, Amsterdam, pp 139–152
22. **Salmon JWD, Daughaday WH** 1957 A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage *in vitro*. J Lab Clin Med 68:825–836
23. **Salmon JWD, Duvall MR** 1970 *In vitro* stimulation of leucine incorporation into muscle and cartilage protein by a serum fraction with sulfation factor activity: differentiation of effects from those of growth hormone and insulin. Endocrinology 87:1168–1180
24. **Daughaday WH, Hall K, Raben MS, Salmon WD, van den Brande JL, VanWyk JJ** 1972 Somatomedin: proposed designation for sulfation factor. Nature 235:107–108
25. **Rinderknecht E, Humbel RE** 1978 The amino sequence of human insulin-like growth factor I and its structural homology with proinsulin. J Biol Chem 253:2769–2776
26. **Rinderknecht E, Humbel RE** 1978 Primary structure of human insulin-like growth factor II. FEBS Lett 89:283–286
27. **Froesch ER, Muller WA, Burgi H** 1966 Nonsuppressible insulin-like activity of human serum. II Biological properties of plasma extracts with nonsuppressible insulin-like activity. Biochim Biophys Acta 121:360–374
28. **Schoenle E, Zapf J, Humbel RE, Froesch ER** 1982 Insulin-like growth factor I stimulates growth in hypophysectomized rats. Nature 296:252–253
29. **Zapf J, Hunziker EB** 1994 The somatomedin hypothesis revisited: differential effects of growth hormone and IGF-I on skeletal growth of the rat *in vivo*. In: Baxter RC, Gluckman PD, Rosenfeld RG (eds) The Insulin-like Growth Factors and Their Regulatory Proteins. Elsevier, Amsterdam, pp 381–391
30. **Skottner A, Clark RG, Fryklund L, Robinson ICAF** 1989 Growth responses in a mutant dwarf rat to human growth hormone and recombinant human insulin-like growth factor I. Endocrinology 124:2519–2526
31. **Guler H, Zapf J, Scheiwiller E, Froesch E** 1988 Recombinant human insulin-like growth factor 1 stimulates growth and has distinct effects on organ size in hypophysectomized rats. Proc Natl Acad Sci USA 85:4889–4893
32. **Greene H, Morikawa M, Nixon T** 1985 A dual effector theory of growth hormone action. Differentiation 29:195–198
33. **Underwood LE, Clemmons DR** 1994 Prospects of therapy with IGF-1 for catabolic conditions. In: Baxter RC, Gluckman PD, Rosenfeld RG (eds) The Insulin-like Growth Factors and Their Regulatory Proteins. Elsevier, Amsterdam, pp 449–456
34. **Murphy WJ, Rui H, Longo DL** 1995 Effects of growth hormone and prolactin immune development and function. Life Sci 57:1–14
35. **Kelley KW, Arkins S, Li YM** 1994 Role of growth hormone and insulin-like growth factor I in immunoregulation. In: Bercu BB, Walker RF (eds) Growth Hormone II: Basic and Clinical Aspects. Springer-Verlag, New York, pp 87–103
36. **Kelley KW, Arkins S** 1994 A role for IGF-1 during myeloid cell growth and differentiation. In: Baxter RC, Gluckman PD, Rosenfeld RG (eds) The Insulin-like Growth Factors and Their Regulatory Proteins. Elsevier, Amsterdam, pp 315–327
37. **Weigent DA, Blalock JE** 1994 Production and function of growth hormone in the immune system. In: Bercu BB, Walker RF (eds) Growth Hormone II: Basic and Clinical Aspects. Springer-Verlag, New York, pp 104–115
38. **Fabris N, Mocchegiani E** 1994 Immunomodulating role of growth hormone. In: Bercu BB, Walker RF (eds) Growth Hormone II: Basic and Clinical Aspects. Springer-Verlag, New York, pp 116–130
39. **Auernhammer CJ, Strasburger CJ** 1995 Effects of growth hormone and insulin-like growth factor on the immune system. Eur J Endocrinol 133:635–645
40. **Horseman ND, Yu-Lee L-Y** 1994 Transcriptional regulation by the helix-bundle peptide hormones: growth hormone, prolactin, and hematopoietic cytokines. Endocr Rev 15:627–649
41. **Hooghe-Peters EL, Hooghe R (eds)** 1995 Growth hormone, prolactin, and IGF-1 as lymphohemopoietic cytokines. RG Landes Company, Austin, TX, p 262
42. **Baumann G** 1991 Growth hormone heterogeneity: genes, isohormone variants and binding proteins. Endocr Rev 12:434–449
43. **Mol JA, van Garderen E, Selman PJ, Wolfswinkel J, Rijinberk A, Rutteman GR** 1995 Growth hormone mRNA in mammary gland tumors of dogs and cats. J Clin Invest 95:2028–2034
44. **Mol JA, Henzen-Logmans SC, Hageman P, Misdorp W** 1995 Expression of the gene encoding growth hormone in the human mammary gland. J Clin Endocrinol Metab 80:3094–3096
45. **Weigent DA, Baxter JB, Wear WE, Smith LR, Smith LR, Bost KL, Blalock JE** 1988 Production of immunoreactive growth hormone by mononuclear leukocytes. FASEB J 2:2812–2818
46. **Hattori N, Shimatsu A, Sugita M, Kumagai S, Imura H** 1990 Immunoreactive growth hormone (GH) secretion by human lymphocytes: augmented release by exogenous GH. Biochem Biophys Res Commun 168:396–401
47. **Weigent DA, Riley JE, Galin FS, LeBoeuf RD, Blalock JE** 1991 Detection of GH and GHRH-related messenger RNA in rat lymphocytes by the polymerase chain reaction. Proc Soc Exp Biol Med 198:643–648
48. **Weigent DA, LeBoeuf RD, Blalock JE** 1991 An anti-sense oligonucleotide to growth hormone mRNA inhibits lymphocyte proliferation. Endocrinology 128:2053–2057
49. **Varma S, Sabharwal P, Sheridan JF, Malarkey WB** 1993 Growth hormone secretion by human peripheral blood mononuclear cells detected by an enzyme-linked immunoplaque assay. J Clin Endocrinol Metab 76:49–53
50. **Kao TL, Harbour D, Meyer IWJ** 1992 Immunoreactive growth hormone production by cultured lymphocytes. Ann NY Acad Sci 650:179–181
51. **Lytras A, Quan N, Vrontakis ME, Shaw JE, Cattini PA, Friesen HG** 1993 Growth hormone expression in human Burkitt lymphoma serum-free ramos cell line. Endocrinology 132:620–628
52. **Wu H, Devi R, Malarkey WB** 1996 Localization of growth hormone messenger ribonucleic acid in the human immune system: a clinical research center study. J Clin Endocrinol Metab 81:1278–1282
53. **Binder G, Revskoy S, Gupta D** 1994 *In vivo* growth hormone gene expression in neonatal rat thymus and bone marrow. J Endocrinol 140:137–143
54. **Theill LE, Karin M** 1993 Transcriptional control of GH expression and anterior pituitary development. Endocr Rev 14:670–689
55. **Delhase M, Vergani P, Malur A, Hooghe-Peters EL, Hooghe RJ** 1993 The transcription factor Pit-1/GHF-1 is expressed in hemopoietic and lymphoid tissues. Eur J Immunol 23:951–955
56. **Frohman LA, Downs TA, Chomcynski P** 1992 Regulation of growth hormone secretion. Front Neuroendocrinol 13:344–405
57. **Stephanou A, Knight RA, Lightman SL** 1991 Production of a

growth hormone-releasing hormone-like peptide and its mRNA by human lymphocytes. Neuroendocrinology 53:628–633
58. **Aguila MC, Dess WL, Haensly WE, McCann SM** 1991 Evidence that somatostatin is localized and synthesized in lymphoid organs. Proc Natl Acad Sci USA 88:11485–11489
59. **Bhathena SJ, Louie J, Schechter GP, Redman S, Wahl L, Recant L** 1981 Identification of human mononuclear leukocytes bearing receptors for somatostatin and glucagon. Diabetes 30:127–131
60. **Hattori N, Ikekubo K, Ishihara T, Moridera K, Hino M, Kurahachi H** 1994 Spontaneous growth hormone (GH) secretion by unstimulated human lymphocytes and the effects of GH-releasing hormone and somatostatin. J Clin Endocrinol Metab 79:1678–1680
61. **Valtora A, Moretta A, Maccario R, Bozzola M, Severi F** 1991 Influence of growth hormone-releasing hormone (GHRH) on phytohemaglutinin-induced lymphocyte activation: comparison of two synthetic forms. Thymus 18:51–59
62. **Zelazowski P, Dohler KD, Stepien H, Pawilowski M** 1989 Effect of growth hormone releasing hormone on human peripheral blood leukocyte chemotaxis and migration in normal subjects. Neuroendocrinology 50:236–239
63. **Serri O, Brazeau P, Kachra Z, Posner B** 1992 Octreotide inhibits insulin-like growth factor-I hepatic gene expression in the hypophysectomized rat: evidence for a direct and indirect mechanism of action. Endocrinology 130:1816–1821
64. **Campbell RM, Scanes CG** 1995 Endocrine peptides "moonlighting" as immune modulators: roles for somatostatin and GH-releasing factor. J Endocrinol 147:383–396
65. **Hattori H, Shimomura K, Ishihara T, Moridera K, Hino M, Ikekubo K** 1993 Growth hormone (GH) secretion from human lymphocytes is up-regulated by GH but not by insulin-like growth factor-I. J Clin Endocrinol Metab 76:937–939
66. **Hiestand PC, Mekler P, Nordmann R, Grieder A, Permongkol C** 1986 Prolactin as a modulator of lymphocyte responsiveness provides a possible mechanism of action for cyclosporine. Proc Natl Acad Sci USA 83:2599–2603
67. **Lesniak MA, Gorden P, Roth J, Gavin Jr I** 1974 Binding of ^{125}I human growth hormone to specific receptors in human cultured lymphocytes. J Biol Chem 249:1661–1667
68. **Kiess W, Butenandt O** 1985 Specific growth hormone receptors on human peripheral mononuclear cells: reexpression, identification and characterization. J Clin Endocrinol Metab 60:740–746
69. **Barnard R, Bundensen PG, Rylatt DB, Waters MJ** 1985 Evidence from the use of monoclonal antibody probes for structural heterogeneity of the growth hormone receptor. Biochem J 231:459–468
70. **Badolato R, Bond HM, Valerio G, Petrella A, Morrone G, Waters MJ, Venuta S, Tenore A** 1994 Differential expression of surface membrane growth hormone receptor on human peripheral blood lymphocytes detected by dual fluorochrome flow. J Clin Endocrinol Metab 79:984–990
71. **Hansen B, Hijorth S, Welinder B, Skriver L, De Meyts P** 1993 The growth hormone (hGH)-binding protein cloned from human IM-9 lymphocytes modulates the down-regulation of hGH receptors by 22- and 20-kilodalton human hGH in IM-9 lymphocytes and the biological effects of the hormone in Nb2 lymphoma cells. Endocrinology 133:2809–2817
72. **Mercado M, Da Vila N, McLeod JF, Baumann G** 1994 Distribution of growth hormone receptor messenger ribonucleic acid containing and lacking exon 3 in human tissues. J Clin Endocrinol Metab 78:731–735
73. **Leung DW, Spencer SA, Cachianes G, Waters MJ, Wood WI** 1987 Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature 330:537–543
74. **Rapaport R, Sills IN, Green L, Barrett P, Labus J, Skuza KA, Chartoff A, Goode L, Stene M, Petersen BH** 1995 Detection of human growth hormone receptors on IM-9 cells and peripheral blood mononuclear cell subsets by flow cytometry: correlation with growth hormone-binding protein levels. J Clin Endocrinol Metab 80:2612–2619
75. **Cunningham BC, Ultsch M, de Vos A, Mulkerrin M, Clauser K, Wells J** 1991 Dimerization of the extracellular domain of the human growth hormone receptor by a single hormone molecule. Science 254:821–825
76. **de Vos A, Ultsch M, Kossiakoff AA** 1992 Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. Science 255:307–312
77. **Bazan JF** 1989 A novel family of growth factor receptors: a common binding domain in the growth hormone, prolactin, erythropoietin and IL-6 receptors and the p75 IL-2 receptor B-chain. Biochem Biophys Res Commun 164:788–795
78. **Schindler C, Darnell JEJ** 1995 Transcriptional responses to polypeptide ligands: the JAK/STAT pathway. Annu Rev Biochem 64:621–651
79. **Meraz MA, While JM, Sheehan KCF, Bach EA, Rodig SJ, Dighe AS, Kaplan DH, Riley JK, Greenlund AC, Campbell D, Carver-Moore K, DuBois RN, Clark R, Auget M, Schreiber RD** 1996 Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell 84:431–442
80. **Fuh G, Cunningham BC, Fukunaga R, Nagata S, Goeddel DV, Wells JA** 1992 Rational design of potent antagonists to the human growth hormone receptor. Science 256:1677–1680
81. **Mode A, Tollet P, Wells T, Carmignac DF, Clark RG, Chen WY, Kopchick JJ, Robinson ICAF** 1996 The human GH (hGH) antagonist G120RhGH does not antagonize GH in the rat, but has paradoxical agonist activity, probably via the PRL receptor. Endocrinology 137:447–454
82. **Hirokawa K, Makinodan T** 1975 Thymus involution: effect on T cell differentiation. J Immunol 6:1659–1664
83. **Boyd E** 1932 The weight of the thymus in health and disease. Am J Dis Child 43:1162–1214
84. **Miller JFAP** 1961 The immunological role of the thymus. Lancet 2:748–749
85. **Corpas E, Harman S, Blackman M** 1993 Human growth hormone and aging. Endocr Rev 14:20–39
86. **Juul A, Bang P, Hertel N, Main K, Dalgaard P, Jorgenson K, Muller J, Hall K, Skakkebaek NE** 1994 Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size and body mass index. J Clin Endocrinol Metab 78:744–752
87. **Juul A, Main K, Blum W, Lindholm J, Ranke M, Skakkebaek NE** 1994 The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. Clin Endocrinol (Oxf) 41:85–93
88. **Roth JA, Kaeberle ML, Grier RL, Hopper JG, Spiegel HE, McAllister HA** 1984 Improvement in clinical condition and thymus morphological features associated with growth hormone treatment of immunodeficient dwarf dogs. Am J Vet Res 45:1151–1155
89. **Goff BL, Roth JA, Arp LH, Incefy GS** 1987 Growth hormone treatment stimulates thymulin production in aged dogs. Clin Exp Immunol 68:580–587
90. **Monroe WE, Roth JA, Grier RL, Arp LH, Naylor PH** 1987 Effects of growth hormone on the adult canine thymus. Thymus 9:173–187
91. **Kelley KW, Brief S, Westly HJ, Novakofski J, Bechtel PJ, Simon J, Walker EB** 1986 GH3 pituitary adenoma cells can reverse thymic aging in rats. Proc Natl Acad Sci USA 83:5663–5667
92. **Clark R, Strasser J, McCabe S, Robbins K, Jardieu P** 1993 Insulin-like growth factor-1 stimulation of lymphopoiesis. J Clin Invest 92:540–548
93. **Timsit J, Savino W, Safieh B, Chanson P, Gagnerault MC, Bach JF** 1992 Growth hormone and insulin-like growth factor-I stimulate hormonal function and proliferation of thymic epithelial cells. J Clin Endocrinol Metab 75:183–188
94. **Maiter D, Underwood LE, Maes M, Davenport ML, Ketelslegers JM** 1988 Different effects of intermittent and continuous growth hormone (GH) administration on serum somatomedin-C/insulin-like growth factor I and liver GH receptors in hypophysectomized rats. Endocrinology 123:1053–1059
95. **Tauber MT, Du PH, SallerinCaute B, Rochiccioli P, Bastide R** 1993 Differential regulation of serum growth hormone (GH)-binding protein during continuous infusion *vs.* daily injection of recombinant human GH in GH-deficient children. J Clin Endocrinol Metab 76:1135–1139
96. **Clark RG, Robinson ICAF** 1987 The control and significance of the secretory pattern of growth hormone in the rat. In: Isaksson O,

Binder K, Hall K, Hokfelt B (eds) Growth Hormone Basic and Clinical Aspects. Elsevier, Amsterdam, pp 109–128
97. **Mode A, Norstedt G, Simic B, Eneroth P, Gustafsson JA** 1981 Continuous infusion of growth hormone feminizes hepatic steroid metabolism in the rat. Endocrinology 108:2103–2108
98. **Clark RG, Mortensen DL, Carlsson L, Carmignac D, Robinson ICAF** 1995 Growth responses to patterned GH delivery. Endocrine 3:717–723
99. **Clark RG, Mortensen DL, Carlsson LMS, Carlsson B, Carmignac D, Robinson ICAF** 1996 The obese growth hormone (GH)-deficient dwarf rat: body fat responses to patterned delivery of GH and insulin-like growth factor-I. Endocrinology 137:1904–1912
100. **Cecim M, Bartke A, Yun JG, Wagner TE** 1993 Growth allometry of transgenic mice expressing the mouse metallothionein-I/bovine growth hormone gene. Transgene 1:125–132
101. **Bernton E, Dave J** 1991 Prolactin, growth hormone and immune homeostasis. In: Meltzer MS, Mantovani A (eds) Cellular and Cytokine Networks in Tissue Immunity. Wiley-Liss, New York, pp 69–75
102. **Wise T, Klindt J, Buonomo FC, Yen JT** 1994 Effects of porcine somatotropin on thymic weight, thymosin alpha 1, and thymosin beta4 in gilts and barrows. In: Bercu BB, Walker RF (eds) Growth Hormone II: Basic and Clinical Aspects. Springer-Verlag, New York, pp 317–327
103. **Clark RG, Olsen K, Fuh G, Marian M, Mortensen DL, Teshima G, Chang S, Chu H, Muuku V, Canova-Davis E, Somers T, Cronin M, Winkler M, Wells J** 1996 Long-acting growth hormones produced by conjugation with polyethylene glycol. J Biol Chem 271: 21969–21977
104. **Johnson OL, Cleland JL, Lee HJ, Charnis M, Duenas E, Jaworowicz W, Shepard D, Shahazamani A, Jones AJS, Putney SD** 1996 A month-long effect from a single injection of microencapsulated human growth hormone. Nature Medicine 2:795–799
105. **Adler WH, Nagel JE, Harman SM, Corpas E, Blackman MR** 1995 Growth hormone and immune function in the elderly. In: Blackman MR, Roth J, Harman SM, Shapiro JR (eds) GHRH, GH and IGF-1: Basic and Clinical Advances. Springer-Verlag, New York, pp 277–290
106. **Hirano T** 1991 Interleukin-6. In: Thomson AW (eds) The Cytokine Handbook. Harcourt Brace Jovanovich, San Diego, CA, pp 169–190
107. **Woodroofe C, Muller W, Ruther U** 1992 Long-term consequences of interleukin-2 overexpression in transgenic mice. DNA Cell Biol 11:587–592
108. **Kundig TM, Schorle H, Bachmann MF, Hengartner H, Zinkernagel RM, Horak I** 1993 Immune responses in interleukin-2-deficient mice. Science 262:1059–1061
109. **Clark RG, Mortensen DL, Carlsson LMS, Spencer SA, McKay P, Mulkerrin M, Moore J, Cunningham BC** 1996 Recombinant human growth hormone (GH)-binding protein enhances the growth-promoting activity of human GH in the rat. Endocrinology 137: 4308–4315
110. **Rosenfeld RG, Rosenblom AL, Guevara-Aguirre J** 1994 Growth hormone (GH) insensitivity due to primary GH receptor deficiency. Endocr Rev 15:369–390
111. **Hwang P, Guyda H, Friesen H** 1972 Purification of human prolactin. J Biol Chem 247:1955–1958
112. **Montgomery DW, Zukoski CF, Shah GN, Buckley AR, Pacholczyk T, Russell DH** 1987 Concanavalin A-stimulated murine splenocytes produce a factor with prolactin-like bioactivity and immunoreactivity. Biochem Biophys Res Commun 145:692–698
113. **Sabharwal P, Glaser R, Lafuse W, Varma S, Liu Q, Arkins S, Kooijman R, Kutz L, Kelley KW, Malarkey WB** 1992 Prolactin synthesized and secreted by human peripheral blood mononuclear cells: an autocrine growth factor for lymphoproliferation. Proc Natl Acad Sci USA 89:7713–7716
114. **Hartmann DP, Holaday JW, Bernton EW** 1989 Inhibition of lymphocyte proliferation by antibodies to prolactin. FASEB J 3:2194–2202
115. **Shah GN, Laird II HE, Russell DH** 1991 Identification and characterization of a prolactin-like polypeptide synthesized by mitogen-stimulated murine lymphocytes. Int Immunol 3:297–304
116. **Pellegrini I, Lebrun JJ, Ali S, Kelly PA** 1992 Expression of prolactin and its receptor in human lymphoid cells. Mol Endocrinol 6:1023–1031
117. **Touraine P, Leite de Moraes MC, Dardenne M, Kelly PA** 1994 Expression of short and long forms of prolactin receptor in murine lymphoid tissues. Mol Cell Endocrinol 104:183–190
118. **Le Provost F, Leroux C, Martin P, Gaye P, Dijane J** 1994 Prolactin gene expression in ovine and caprine mammary gland. Neuroendocrinology 60:305–313
119. **Tanaka T, Shiu RPC, Gout PW, Beer CT, Noble RL, Friesen HG** 1980 A new sensitive and specific bioassay for lactogenic hormones: measurement of prolactin and growth hormone in human serum. J Clin Endocrinol Metab 51:1058–1063
120. **Russell DH, Kibler R, Matrisian L, Larson DF, Poulos B, Magun BE** 1985 Prolactin receptors on human T and B lymphocytes: antagonism of prolactin binding by cyclosporine. J Immunol 134: 3027–3031
121. **Dardenne M, Leite de Moraes MC, Kelly PA, Gagnerault MC** 1994 Prolactin receptor expression in human hematopoietic tissues analyzed by flow cytometry. Endocrinology 134:2108–2114
122. **Gagnerault MC, Touraine P, Savino W, Kelly PA, Dardenne M** 1993 Expression of prolactin receptors in murine lymphoid cells in normal and autoimmune situations. J Immunol 150:5673–5681
123. **Koh CY, Phillips JT** 1993 Prolactin receptor expression by lymphoid tissues in normal and immunized rats. Mol Cell Endocrinol 92:R21–R25
124. **Gala RR, Shevach EM** 1993 Identification by analytical flow cytometry of prolactin receptors on immunocompetent cell populations in the mouse. Endocrinology 133:1617–1623
125. **Dardenne M, Kelly PA, Bach JF, Savino W** 1991 Identification and functional activity of prolactin receptors in thymic epithelial cells. Proc Natl Acad Sci USA 88:9700–9704
126. **Nagy E, Berczi I, Friesen HG** 1983 Regulation of immunity in rats by lactogenic hormones. Acta Endocrinol (Copenh) 102:351–357
127. **Nagy E, Berczi I** 1991 Hypophysectomized rats depend on residual prolactin for survival. Endocrinology 128:2776–2784
128. **Paesi FGS, de Jong SE** 1954 Growth inhibition by estrogen in hypophysectomized rats. Acta Physiol Pharmacol 3:227–231
129. **Morikawa K, Oseko F, Morikawa S** 1993 Immunosuppressive property of bromocriptine on human B lymphocyte function *in vitro*. Clin Exp Immunol 93:200–205
130. **Morikawa K, Oseko F, Morikawa S** 1995 Immunosuppressive activity of bromocriptine on human T lymphocyte function *in vitro*. Clin Exp Immunol 95:514–518
131. **Wilner ML, Ettenger RB, Koyle MA, Rosenthal JT** 1990 The effect of hypoprolactinemia alone an in combination with cyclosporine on allograft rejection. Transplantation 49:264–267
132. **Carrier M, Wild J, Pelletier LC, Copeland JG** 1990 Bromocriptine as an adjuvant to cyclosporine immunosuppression after heart transplantation. Ann Thorac Surg 49:129–132
133. **Jones JI, Clemmons DR** 1995 Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16:3–34
134. **Straus DS, Hayden JM, Marten NW** 1994 Molecular mechanisms for nutritional regulation of genes for IGF-I, IGF-binding proteins and other plasma proteins. In: Baxter RC, Gluckman PD, Rosenfeld RG (eds) The Insulin-like Growth Factors and Their Regulatory Proteins. Elsevier, Amsterdam, pp 33–41
135. **Oster MH, Fielder P, Levin N, Cronin MJ** 1995 Adaptation of the growth hormone and insulin-like growth factor-I axis to chronic and severe calorie or protein malnutrition. J Clin Invest 95:2258–2265
136. **Isaksson OGP, Lindahl A, Nilsson A, Isgaard J** 1987 Cellular mechanism(s) for the stimulatory effect of growth hormone on longitudinal bone growth. In: Isaksson O, Binder C, Hall K, Hokfelt B (eds) Growth Hormone Basic and Clinical Aspects. Excerpta Medica, Amsterdam, pp 307–320
137. **Kurtz A, Matter R, Eckardt KU, Zapf J** 1990 Erythropoiesis, serum erythropoietin, and serum IGF-I in rats during accelerated growth. Acta Endocrinol (Copenh) 122:323–328
138. **Froesch ER, Hussain M** 1994 Recombinant human insulin-like growth factor-I: a therapeutic challenge for diabetes mellitus. Diabetologia 37: S179–S185
139. **Han VK, D'Ercole AJ, Lund PK** 1987 Cellular localization of so-

matomedin (insulin-like growth factor) messenger RNA in the human fetus. Science 236:193–197
140. **Gosteli-Peter MA, Winterhalter KH, Schmid C, Froesch ER, Zapf J** 1994 Expression and regulation of insulin-like growth factor-I (IGF-I) and IGF-binding protein messenger ribonucleic acid levels in tissues of hypophysectomized rats infused with IGF-I and growth hormone. Endocrinology 135:2558–2567
141. **Sussenbach JS, Steenbergh PH, Holthuizen P** 1995 Structure and expression of the human insulin-like growth factor genes. Growth Regul 2:1–9
142. **Daughaday WH, Rotwein P** 1989 Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures. Endocr Rev 10:68–91
143. **Rotwein P, Thomas MJ, Gronowski AM, Bichell DP, Kikuchi K** 1994 The somatomedin hypothesis revisited: early events in growth hormone action. In: Baxter RC, Gluckman PD, Rosenfeld RG (eds) The Insulin-like Growth Factors and Their Regulatory Proteins. Elsevier, Amsterdam, pp 13–21
144. **Arkins S, Rebeiz N, Biragyn A, Reese DL, Kelley KW** 1993 Murine macrophages express abundant insulin-like growth factor-I class I Ea and Eb transcripts. Endocrinology 133:2334–2343
145. **Nyman T, Pekonen F** 1993 The expression of insulin-like growth factors and their binding proteins in normal human lymphocytes. Acta Endocrinol (Copenh) 128:168–172
146. **Dorshkind K** 1990 Regulation of hemopoiesis by bone marrow stromal cells and their products. Annu Rev Immunol 8:111–137
147. **Abboud SL, Bethel CR, Aron SL** 1991 Secretion of insulin-like growth factor I and insulin-like growth factor-binding proteins by murine bone marrow stromal cells. J Clin Invest 88:470–475
148. **Yamada M, Hato F, Kinoshita Y, Tominaga K, Tsuji Y** 1994 The indirect participation of growth hormone in the thymocyte proliferation system. Cell Mol Biol 40:111–121
149. **Tanaka M, Hayashida Y, Sakaguchi K, Ohkubo T, Wakita M, Hoshino S, Nakashima K** 1996 Growth hormone-independent expression of insulin-like growth factor I messenger ribonucleic acid in extrahepatic tissues of the chicken. Endocrinology 137:30–34
150. **Murphy LJ, Friesen HG** 1988 Differential effects of estrogens and growth hormone on uterine and hepatic insulin-like growth factor I gene expression in the ovariectomized hypophysectomized rat. Endocrinology 122:325–332
151. **Noble PW, Lake FR, Henson PM, Riches DWH** 1993 Hyaluronate activation of CD44 induces insulin-like growth factor-1 expression by a tumor necrosis factor-alpha dependent mechanism in murine macrophages. J Clin Invest 91:2368–2377
152. **Fournier T, Riches DW, Winston BW, Rose DM, Young SK, Noble PW, Lake FR, Henson PM** 1995 Divergence in macrophage insulin-like growth factor-I (IGF-I) synthesis induced by TNF-alpha and prostaglandin E2. J Immunol 155:2123–2133
153. **Arkins S, Rebeiz N, Brunke-Reese DL, Minshall C, Kelley KW** 1995 The colony-stimulating factors induce expression of insulin-like growth factor-I messenger ribonucleic acid during hematopoiesis. Endocrinology 136:1153–1160
154. **Arkins S, Rebeiz N, Brunke-Reese DL, Biragyn A, Kelley KW** 1995 Interferon-gamma inhibits macrophage insulin-like growth factor-I synthesis at the transcriptional level. Mol Endocrinol 9:350–360
155. **Tsuji Y, Kinoshita Y, Hato F, Tominaga K, Yoshida K** 1994 The *in vitro* proliferation of thymus epithelial cells stimulated with growth hormone and insulin-like growth factor-I. Cell Mol Biol 40:1135–1142
156. **Dardenne M, Savino W, Gagnerault M-C, Itoh T, Bach J-F** 1989 Neuroendocrine control of thymic hormonal production. I. Prolactin stimulates in vivo and in vitro the production of thymulin and murine thymic epithelial cells. Endocrinology 125:3–12
157. **Murphy WJ, Durum SK, Longo DL** 1992 Role of neuroendocrine hormones in murine T cell development: growth hormone exerts thymopoietic effects *in vivo*. J Immunol 149:3851–3857
158. **Berczi I, Nagy E, de Toledo SM, Matusik RJ, Friesen HG** 1991 Pituitary hormones regulate c-myc and DNA synthesis in lymphoid tissue. J Immunol 146:2201–2206
159. **Clayton PE, Day RN, Silva CM, Hellmann P, Day KH, Thorner MO** 1994 Growth hormone induces tyrosine phosphorylation but does not alter insulin-like growth factor-I gene exprression in human IM-9 lymphocytes. J Mol Endocrinol 13:127–136
160. **Merrimee TJ, Grant MB, Broder CM, Cavalli-Sforza LL** 1989 Insulin-like growth factor secretion by human B-lymphocytes: a comparison of cells from normal and pygmy subjects. J Clin Endocrinol Metab 69:978–984
161. **Geffner ME, Bersch N, Lippe BM, Rosenfeld RG, Hintz RL, Golde DW** 1990 Growth hormone mediates the growth of T-lymphoblast cell lines via locally generated insulin-like growth factor I. J Clin Endocrinol Metab 71:464–469
162. **Baier TG, Jenne EW, Blum W, Schonberg D, Hartmann KK** 1992 Influence of antibodies against IGF-I, insulin or their receptors on proliferation of human acute lymphoblastic leukemia cell lines. Leuk Res 16:807–814
163. **Siddle K, Soos MA, Field CE, Nave BT** 1994 Hybrid and atypical insulin/insulin-like growth factor-I receptors. Horm Res 41[Suppl 2]:56–65
164. **LeRoith D, Werner H, Beitner-Johnson D, Roberts CTJ** 1995 Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 143–163
165. **Nicola NA** 1989 Hemopoietic cell growth factors and their receptors. Annu Rev Biochem 58:45–77
166. **Nissley P, Lopaczynski W** 1991 Insulin-like growth factor receptors. Growth Factors 5:29–49
167. **Ellis MJC, Leav BA, Yang Z, Rasmussen A, Pearce A, Zweibel JA, Lippman ME, Cullen KJ** 1996 Affinity for the insulin-like growth factor-II (IGF-II) receptor inhibits autocrine IGF-II activity in MCF-7 breast cancer cells. Mol Endocrinol 10:286–297
168. **Nissley SP, Kiess W** 1991 Binding of insulin-like growth factor-II (IGF-II) and lysosomal enzymes to the IGF-II/mannose-6-phosphate receptor. In: Spencer EM (ed) Modern Concepts of Insulin-like Growth Factors. Elsevier, Amsterdam, pp 419–430
169. **Gavin JR, Buell DN, Roth J** 1972 Water soluble insulin receptors form human lymphocytes. Science 178:168–169
170. **Helderman JH, Strom TB** 1978 Specific insulin binding site on T and B lymphocytes as a marker for cell activation. Nature 274:62–63
171. **Lee PD, Rosenfeld RG, Hintz RL, Smith SD** 1986 Characterization of insulin, insulin-like growth factors I and II, and growth hormone receptors on human leukemic lymphoblasts. J Clin Endocrinol Metab 62:28–35
172. **Kozak RW, Haskell JF, Greenstein LA, Rechler MM, Waldmann TA, Nissley SP** 1987 Type I and II insulin-like growth factor receptors on human phytohemagglutinin-activated T lymphocytes. Cell Immunol 109:318–331
173. **Tapson VF, Boni-Schnetzler M, Pilch PF, Center DM, Berman JS** 1988 Structural and functional characterization of the human T lymphocyte receptor for insulin-like growth factor I *in vitro*. J Clin Invest 82:950–957
174. **Stuart CA, Meehan RT, Neale LS, Cintron NM, Furlanetto RW** 1991 Insulin-like growth factor-I binds selectively to human peripheral blood monocytes and B-lymphocytes. J Clin Endocrinol Metab 72:1117–1122
175. **Kooijman R, Willems M, De Haas CJ, Rijkers GT, Schuurmans AL, Van Buul-Offers SC, Heijnen CJ, Zegers BJ** 1992 Expression of type I insulin-like growth factor receptors on human peripheral blood mononuclear cells. Endocrinology 131:2244–2250
176. **Xu X, Mardell C, Xian CJ, Zola H, Read LC** 1995 Expression of functional insulin-like growth factor-1 receptor on lymphoid cell subsets of rats. Immunology 85:394–399
177. **Hartmann KK, Baier TG, Papa V, Kronenwett M, Brown EJ, Goldfine ID, Rosenthal SM** 1992 A monoclonal antibody to the T-cell receptor increases IGF-I receptor content in normal T-lymphocytes: comparison with phytohemagglutinin. J Cell Biochem 48:81–85
178. **Schillaci R, Ribaudo CM, Rondinone CM, Roldan A** 1994 Role of insulin-like growth factor-1 on the kinetics of human lymphocytes stimulation in serum-free culture medium. Immunol Cell Biol 72: 300–305
179. **Kooijman R, Scholtens LE, Rijkers GT, Zegers BJ** 1995 Type I insulin-like growth factor receptor expression in different developmental stages of human thymocytes. J Endocrinol 147:203–209
180. **Hattori Y, Vera JC, Rivas CI, Bersch N, Bailey RC, Geffner ME, Golde DW** 1996 Decreased insulin-like growth factor I receptor

expression and function in immortalized African Pygmy T cells. Endocrinology 81:2257–2263
181. **Verland S, Gammeltoft S** 1989 Functional receptors for insulin-like growth factors I and II in rat thymocytes and mouse thyoma cells. Mol Cell Endocrinol 67:207–216
182. **Kooijman R, Lauf JJ, Kappers AC, Rijkers G** 1995 Insulin-like growth factor induces phosphorylation of immunoreactive insulin receptor substrate and its association with phosphatidylinositol-3 kinase in human thymocytes. J Exp Med 182:593–597
183. **Swisshelm K, Ryan K, Tsuchiya K, Sager R** 1995 Enhanced expression of an insulin growth factor-like binding protein (mac25) in senescent human mammary epithelial cells and induced expression with retinoic acid. Proc Natl Acad Sci USA 92:4472–4476
184. **Yamauchi T, Umeda F, Masakado M, Isaji M, Mizushima S, Nawata H** 1994 Purification and molecular cloning of prostacyclin-stimulating factor from serum-free conditioned medium of human diploid fibroblast cells. Biochem J 303:591–598
185. **Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J, Fiers W, Devos R, Tonnel AB** 1996 ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. J Biol Chem 271:20458–20464
186. **Baxter RC, Dai J, Holman S, Lewitt MS** 1994 Determinants of complex formation between IGFBP-3 and the acid-labile subunit. In: Baxter RC, Gluckman PD, Rosenfeld RG (eds) The Insulin-like Growth Factors and Their Regulatory Proteins. Elsevier, Amsterdam, pp 227–235
187. **Cox GN, McDermott MJ, Merkel E, Stroh CA, Ko S, Squires CH, Gleason TM, Russell D** 1994 Recombinant human-insulin-like growth factor (IGF)-binding protein-1 inhibits somatic growth stimulated by IGF-1 and growth hormone in hypophysectomized rats. Endocrinology 135:1913–1920
188. **Grellier P, Yee D, Gonzalez M, Abboud SL** 1995 Characterization of insulin-like growth factor binding proteins (IGFBP) and regulation of IGFBP-4 in bone marrow stromal cells. Br J Haematol 90:249–257
189. **Tonner E, Beattie J, Flint DJ** 1995 Production of insulin-like growth factor binding protein (IGFBP), IGFBP-3 protease, and expression of IGF-I receptors by cells of the sheep immune system. Eur J Endocrinol 132:118–122
190. **Schwander J, Mary JL** 1993 The RIA for IGFBP-2 in man: a meagre catch? Growth Regul 3:104–108
191. **Blum WF, Horn N, Kratzsch J, Jorgensen JO, Juul A, Teale D, Mohnike K, Ranke MB** 1993 Clinical studies of IGFBP-2 by radioimmunoassay. Growth Regul 3:100–104
192. **Mohnike K, Kluba U, Blum WF, Aumann V, Vorwerk P, Mittler U** 1995 Serum concentrations of insulin-like growth factors (IGF)-I and IGF-II and IGF binding proteins (IGFBP)-2 and IGFBP-3 in 49 children with ALL, NHL or solid tumors [German]. Klin Padiatr 207:225–229
193. **Wimmer K, Biemer E, Elmlinger MW, Dannecker L, Blum WL, Ranke MB, Dannecker GE** 1995 IGFBP-2 is differentially expressed in leukemic T- and B-cell lines. Program of the 3rd International Symposium on IGFBPs. Tubingen, Germany, pp
194. **Pintar JE, Schuller A, Cerro JA, Czick M, Grewal A, Green B** 1995 Genetic ablation of IGFBP-2 suggests functional redundancy in the IGFBP family. Prog Growth Factor Res 6:437–445
195. **Dai Z, Xing Y, Boney CM, Clemmons DR, D'Ercole AJ** 1994 Human Insulin-like growth factor-binding protein-1 (hIGFBP-1) in transgenic mice: characterization and insights into the regulation of IGFBP-1 expression. Endocrinology 135:1316–1327
196. **Rajkumar K, Barron D, Lewitt MS, Murphy LJ** 1995 Growth retardation and hyperglycemia in insulin-like growth factor binding protein-1 transgenic mice. Endocrinology 136:4029–4034
197. **Murphy L, Rajkumar K, Molnar P** 1995 Phenotypic manifestations of insulin-like growth factor binding protein-1 (IGFBP-1) and IGFBP-3 overexpression in transgenic mice. Prog Growth Factor Res 6:425–432
198. **Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Selzinger BR, Kley N** 1995 Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature 377:646–649
199. **Dorshkind K** 1996 Growth and differentiation factors that regulate B lymphocyte development. In: Herzenberg LA, Weir DM (eds) Handbook of Experimental Immunology. Blackwell Science, Cambridge MA, in press
200. **Landreth K, Narayanan R, Dorshkind K** 1992 Insulin-like growth factor-I regulates pro-B-cell differentiation. Blood 80:1–6
201. **Gibson LF, Piktel D, Landreth KS** 1993 Insulin-like growth factor-1 potentiates expansion of interleukin-7-dependent pro-B cells. Blood 82:3005–3011
202. **Landreth KS, Englehard D, Beare MH, Kincade PW, Kapoor N, Good RA** 1985 Regulation of human B lymphopoiesis; effect of a urinary activity associated with cyclic neutropenia. J Immunol 134: 2305–2309
203. **Kincade PW** 1987 Experimental models for understanding B lymphocyte formation. Adv Immunol 41:181–267
204. **Funk P, Kincade PW, Witte PL** 1994 Native associations of early hematopoietic stem cells and stromal cells involved in cell aggregates. Blood 83:361–369
205. **Jardieu P, Clark R, Mortensen D, Dorshkind K** 1994 In vivo administration of insulin-like growth factor-1 stimulates primary B lymphopoiesis and enhances lymphocyte recovery after bone marrow transplantation. J Immunol 152:4320–4327
206. **Robbins K, McCabe S, Scheiner T, Strasser J, Clark R, Jardieu P** 1994 Immunological effects of insulin-like growth factor-I: enhancement immunoglobulin synthesis. Clin Exp Immunol 95:337–342
207. **Clark R, Mortensen DL, Carlsson L** 1995 Insulin-like growth factor-I and growth hormone (GH) have distinct and overlapping anabolic effects in GH-deficient rats. Endocrine 3:297–304
208. **Geffner ME, Bersch N, Golde DW** 1992 Insulin and IGF-I stimulate normal and virally transformed T-lymphocyte cell growth *in vitro*. Brain Behav Immun 6:377–386
209. **Murphy WJ, Durum SK, Anver MR, Longo DL** 1992 Immunologic and hematologic effects of neuroendocrine hormones. Studies on DW/J dwarf mice. J Immunol 148:3799–3805
210. **Gjerset RA, Yeargin J, Volkman SK, Vila V, Arya J, Haas M** 1990 Insulin-like growth factor-I supports proliferation of autocrine thymic lymphoma cells with a pre-T cell phenotype. J Immunol 145: 3497–3501
211. **Taub DD, Tsarfaty G, Lloyd AR, Durum SK, Longo DL, Murphy WJ** 1994 Growth hormone promotes human T cell adhesion and migration to both human and murine matrix proteins *in vitro* and directly promotes xenogeneic. J Clin Invest 94:293–300
212. **Clark RG, Mortensen DL, Figari I, Jardieu P,** Anabolic and immunologic effects of IGF-1 and GH given alone and together to aged rats. Program of the 76th Annual Meeting of The Endocrine Society, Anaheim, CA, 1994, p 296
213. **van Buul-Offers SC, Ueda I, Van den Brande JL** 1986 Biosynthetic somatomedin C (SM-C/IGF-I) increases the length and weight of Snell dwarf mice. Pediatr Res 20:825–827
214. **Woodall SM, Breier BH, O'Sullivan U, Gluckman PD** 1991 The effect of the frequency of subcutaneous insulin-like growth factor-1 administration on weight gain in growth hormone deficient mice. Horm Metab Res 23:581–584
215. **Cottam YH, Blair HT, Gallaher BW, Purchas RW, Breier BH, McCutcheon SN, Gluckman PD** 1992 Body growth, carcass composition, and endocrine changes in lambs chronically treated with recombinantly derived insulin-like growth factor-I. Endocrinology 130:2924–2930
216. **LeRoith D, Yanowski J, Kaldjian EP, Jaffe ES, LeRoith T, Purdue K, Cooper BD, Pyle R, Adler W** 1996 The effects of growth hormone and insulin-like growth factor I on the immune system of aged female monkeys. Endocrinology 137:1071–1079
217. **D'Ercole J** 1993 Expression of insulin-like growth factor-1 in transgenic mice. Ann NY Acad Sci 692:149–160
218. **Siddiqui RA, McCutcheon SN, Blair HT, Mackenzie DD, Morel PC, Breier BH, Gluckman PD** 1992 Growth allometry of organs, muscles and bones in mice from lines divergently selected on the basis of plasma insulin-like growth factor-I. Growth Dev Aging 56:53–60
219. **Kooijman R, van Buul-Offers SC, Scholtens LE, Schuurman HJ, van den Brande LJ, Zegers BJ** 1995 T cell development in insulin-like growth factor-II transgenic mice. J Immunol 154:5736–5745
220. **van Buul-Offers SC, de Haan K, Reijnen-Gresnigt MG, Meinsma D, Jansen M, Oei SL, Bonte EJ, Sussenbach JS, Van den Brande**

JL 1995 Overexpression of human insulin-like growth factor-II in transgenic mice causes increased growth of the thymus. J Endocrinol 144:491–502
221. **Nielsen FC, Ostergaard L, Nielsen J, Christiansen J** 1995 Growth-dependent translation of IGF-II mRNA by a rapamycin-sensitive pathway. Nature 377:358–362
222. **Kimata H, Yoshida A** 1994 Effect of growth hormone and insulin-like growth factor-I on immunoglobulin production by and growth of human B-cells. J Clin Endocrinol Metab 78:635–641
223. **Binz K, Joller P, Froesch P, Zapf J, Froesch ER** 1990 Repopulation of the atrophied thymus in diabetic rats by insulin-like growth factor I. Proc Natl Acad Sci USA 87:3690–3694
224. **Beschorner WE, Divic J, Pulido H, Yao X, Kenworthy P, Bruce G** 1991 Enhancement of thymic recovery after cyclosporine by recombinant human growth hormone and insulin-like growth factor I. Transplantation 52:879–884
225. **Murphy WJ, Durum SK, Longo DL** 1992 Human growth hormone promotes engraftment of murine or human T cells in severe combined immunodeficient mice. Proc Natl Acad Sci USA 89:4481–4485
226. **Krammer P, Behrmann I, Daniel P, Dhein J, Debatin K-M** 1994 Regulation of apoptosis in the immune system. Curr Opin Immunol 6:279–289
227. **Harrington EA, Bennett MR, Fanidi A, Evan GI** 1994 CMyc-induced apoptosis in fibroblasts is inhibited by specific cytokines. EMBO J 13:3286–3295
228. **Baserga R** 1995 The insulin-like growth factor receptor: a key to tumor growth? Cancer Res 55:249–252
229. **Hammerman MR, Miller SB** 1994 Therapeutic use of growth factors in renal failure. J Am Soc Nephrol 5:1–11
230. **Buerke M, Murohara T, Skurk C, Nuss C, Tomaselli K, Lefer AM** 1995 Cardioprotective effect of insulin-like growth factor I in myocardial ischemia followed by reperfusion. Proc Natl Acad Sci USA 92:8031–8035
231. **Guan J, Williams C, Gunning M, Mallard C, Gluckman P** 1993 The effects of IGF-1 treatment after hypoxic-ischemic brain injury in adult rats. J Cereb Blood Flow Metab 13:609–616
232. **Rodriguez-Tarduchy G, Collins MKL, Garcia I, Lopez-Rivas A** 1992 Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells. J Immunol 149:535–540
233. **Kincade PW, Medina KL, Smithson G, Scott DC** 1994 Pregnancy: a clue to normal regulation of B lymphopoiesis. Immumol Today 15:539–544
234. **Clarke AG, Kendall MD** 1994 The thymus in pregnancy: the interplay of neural, endocrine and immune influences. Immunol Today 15:545–551
235. **Cross RJ, Bryson JS, Roszman TL** 1992 Immunologic disparity in the hypopituitary dwarf mouse. J Immunol 148:1347–1352
236. **Montecino-Rodriguez E, Clark RG, Johnson A, Collins L, Dorshkind K** 1996 Defective B cell development in Snell Dwarf (dw/dw) mice can be corrected by thyroxine treatment. J Immunol 157:3334–3340
237. **Slebodzinski AB, Lipczak W, Brzezinska-Slebodzinska E** 1995 Peroral administration of triiodothyronine (T_3) affects absorption of immunolactoglobulins in calves. Reprod Nutr Dev 35:387–393
238. **Phillips DI, Barker DJ, Osmond C** 1993 Infant feeding, fetal growth and adult thyroid function. Acta Endocrinol (Copenh) 129:134–138
239. **Medina KL, Kincade PW** 1994 Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. Proc Natl Acad Sci USA 91:5382–5386
240. **Smithson G, Beamer WG, Shultz KL, Christianson SW, Shultz LD, Kincade PW** 1994 Increased B lymphopoiesis in genetically sex steroid-deficient hypogonadal (hpg) mice. J Exp Med 180:717–720
241. **Holder AT, Clark RG, Preece MA** 1983 Prolonged oestrogen treatment inhibits growth hormone induced growth in hypopituitary dwarf mice. J Endocrinol 96:451–456
242. **Ruan W, Catanese V, Wieczorek R, Feldman M, Kleinberg DL** 1995 Estradiol enhances the stimulatory effect of insulin-like growth factor-I (IGF-I) on mammary development and growth hormone-induced IGF-1 messenger ribonucleic acid. Endocrinology 136:1296–1302
243. **Freyschuss B, Sahlin L, Masironi B, Eriksson H** 1994 The hormonal regulation of the oestrogen receptor in rat liver: an interplay involving growth hormone, thyroid hormones and glucocorticoids. J Endocrinol 142:285–298
244. **Cusi K, DeFronzo RA** 1995 Treatment of NIDDM, IDDM, and other insulin-resistant states with IGF-I. Diabetes Rev 3:206–236
245. **Polychronakos C, Giannoukakis N, Deal CL** 1995 Imprinting of IGF2, insulin-dependent diabetes, immune function, and apoptosis: a hypothesis. Dev Genet 17:253–262
246. **Bergerot I, Fabien N, Maguer V, Thivolet C** 1995 Insulin-like growth factor-1 (IGF-1) protects NOD mice from insulitis and diabetes. Clin Exp Immunol 102:335–340
247. **Ballard FJ, Tomas FM, Read LC, Knowles SE, Owens PC, Lemmey AB, Martin AA, Wells JRE, Wallace JC, Francis GL** 1991 Effects of IGF-1 and IGF analogs on growth during catabolic states in rats. In: Spencer EM (ed) Modern Concepts of Insulin-like Growth Factors. Elsevier, Amsterdam, pp 617–627
248. **Vanderhoof JA, McCusker RH, Clark RG, Mohammadpour H, Blackwood DJ, Harty RF, Park JH** 1992 Truncated and native insulin-like growth factor-1 enhance mucosal adaptation after jejunoileal resection. Gastroenterology 102:1949–1956
249. **Ziegler TR, Leader I** 1994 Adjunctive human growth hormone therapy in nutrition support: potential to limit septic complications in intensive care unit patients. Semin Respir Infect 9:240–247
250. **Huang KF, Chung DH, Herndon DN** 1993 Insulin-like growth factor 1 (IGF-1) reduces gut atrophy and bacterial translocation after severe burn injury. Arch Surg 128:47–53
251. **Morishima Y, Tashiro T, Takagi K, Yamamori H, Mashima Y, Nakajima N** 1995 Effects of insulin-like growth factor-I in burned rats. [Japanese]. J Jpn Surg Soc 96:72–79
252. **Chen K, Okuma T, Okamura K, Tabira Y, Kaneko H, Miyauchi Y** 1995 Insulin-like growth factor-I prevents gut atrophy and maintains intestinal integrity in septic rats. J Parenter Enteral Nutr 19:119–124
253. **Inoue T, Saito H, Fukushima R, Inaba T, Lin M-S, Fukatsu K, Muto T** 1995 Growth hormone and insulin-like growth factor I enhance host defence in a murine model of sepsis. Arch Surg 130:1115–1122
254. **Liao W, Rudling M, Angelin B** 1996 Growth hormone potentiates the *in vivo* biological activities of endotoxin in the rat. Eur J Clin Invest 26:254–258
255. **Elsasser TH, Caperna TJ, Rumsey TS** 1995 Endotoxin administration decreases plasma insulin-like growth factor (IGF)-I and IGF-binding protein-2 in Angus x Hereford steers independent of changes in nuritional intake. J Endocrinol 144:109–117
256. **Fan J, Char D, Kolasa AJ, Pan W, Maitra SR, Patlak CS, Spolarics Z, Gelato MC, Lang CH** 1995 Alterations in hepatic production and peripheral clearance of IGF-I after endotoxin. Am J Physiol 269:E33–E42
257. **Kurtz A, Jelkmann W, Bauer C** 1982 A new candidate for the regulation of erythropoiesis: insulin-like growth factor I. FEBS Lett 149:105–108
258. **Claustres M, Chatelian P, Sultan C** 1987 Insulin-like growth factor I stimulates human erythroid colony formation *in vitro*. J Clin Endocrinol Metab 65:78–82
259. **Kurtz A, Zapf J, Eckardt K-U, Clemons G, Froesch RE** 1988 Insulin-like growth factor I stimulates erythropoiesis in hypophysectomized rats. Proc Natl Acad Sci USA 85:7825–7829
260. **Liu J-P, Baker J, Perkins AS, Robertson EJ, Efstratiadis A** 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (IGF-1) and type-1 IGF receptor (IGFR). Cell 75:59–72
261. **Baker J, Liu J-P, Robertson EJ, Efstratiadis A** 1993 Role of insulin-like growth factors in embryonic and post-natal growth. Cell 75:73–82
262. **Ratajczak MZ, Kuczynski WI, Onodera K, Moore J, Ratajczak J, Kregenow DA, DeReil K, Gerwitz AM** 1994 A reappraisal of the role of insulin-like growth factor I in the regulation of human hematopoiesis. J Clin Invest 94:320–327
263. **Correa PN, Eskinazi D, Axelrad AA** 1994 Circulating erythroid progenitors in polycythemia vera are hypersensitive to insulin-like growth factor-1 *in vitro*: studies in an improved serum-free medium. Blood 83:99–112
264. **Mirza AM, Correa PN, Axelrad AA** 1995 Increased basal and

induced tyrosine phosphorylation of the insulin-like growth factor I receptor beta subunit in circulating mononuclear cells of patients with polycythemia vera. Blood 86:877–882
265. **Prchal JT, Prchal JF** 1994 Evolving understanding of the cellular defect in *Polycythemia vera*: implications for its clinical diagnosis and molecular pathophysiology (Editorial). Blood 83:1–4
266. **Kelley KW, Dantzer R** 1991 Growth hormone and prolactin as natural antagonists of glucocorticoids in immunoregulation. In: Plotnikoff N, Murgo A, Faith R, Wybran J (eds) Stress and Immunity. CRC Press, Boca Raton, FL, pp 433–452
267. **Chatterton JRT, Murray CT, Hellman L** 1973 Endocrine effects on leukocytopoiesis in the rat. Evidence for growth hormone secretion as the leukocytopoietic stimulus following acute cortisol-induced lymphocytopenia. Endocrinology 92:775–787
268. **Hayashida T, Li CH** 1957 The infuence of adrenocorticotropic and growth hormones on antibody formation. J Exp Med 105:93–98
269. **Franco P, Marelli O, Lattuada D, Locatelli V, Cocchi D, Muller E** 1990 Influence of growth hormone on the immunosuppressive effect of prednisolone in mice. Acta Endocrinol (Copenh) 123:339–344
270. **Underwood LE** 1988 Assessment of the risks of treatment with human growth hormone. In: Bercu B (ed) Basic and Clinical Aspects of Growth Hormone. Plenum Press, New York, pp 357–366
271. **Hartman ML, Clayton PE, Johnson ML, Celniker A, Perlman AJ, Alberti KGMM, Thorner MO** 1993 A low-dose euglycemic infusion of recombinant human insulin-like growth factor-1 rapidly suppresses fasting-enhanced pulsatile growth hormone secretion in humans. J Clin Invest 91:2453–2462
272. **Mauras N, Beaufrere B** 1995 Recombinant human insulin-like growth factor-I enhances whole body protein anabolism and significantly diminishes the protein catabolic effects of prednison in humans without a diabetogenic effect. J Clin Endocrinol Metab 80:869–874
273. **Powell-Braxton L, Warburton C, Gillett N** 1994 Genetically engineered IGF-I deficient mice. In: Baxter RC, Gluckman PD, Rosenfeld RG (eds) The Insulin-like Growth Factors and Their Regulatory Proteins. Elsevier, Amsterdam, pp 247–252
274. **Sullivan KM, Mori M, Sanders J, Siadak M, Witherspoon RP, Anansetti C, Appelbaum FR, Bensinger W, Bowden R, Buckner CD, Clark J, Crawford S, Deeg HJ, Doney K, Flowers M, Hansen J, Loughran T, Martin P, McDonald G, Pepe M, Petersen FB, Schuening F, Stewart P, Storb R** 1992 Late complications of allogenic and autologous marrow transplantation. Bone Marrow Transplant 10:127–134
275. **Mackall CL, Fleisher TA, Brown MR, Magrath IT, Shad AT, Horowitz ME, Wexler LH, Adde MA, McClure LL, Gress RE** 1994 Lymphocyte depletion during treatment with intensive chemotherapy for cancer. Blood 84:2221–2228
276. **Mackall CL, Fleisher TA, Brown MR, Andrich MP, Chen CC, Feuerstein IM, Horowitz ME, Magrath IT, Shad AT, Steinberg SM, Gress RE** 1995 Age thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. N Engl J Med 332:143–149
277. **Dong YL, Fleming RY, Huang KF, Herndon DN, Yan TZ, Waymack JP** 1993 Effect of insulin-like growth factor 1 on host response to tumor. J Surg Oncol 53:121–127
278. **Mulligan K, Grunfeld C, Hellerstein MK, Neese RA, Schambelan M** 1995 Effects of recombinant human growth hormone on body composition and metabolism in HIV-associated wasting. In: Blackman MR, Roth J, Harman SM, Shapiro JR (eds) GHRH, GH and IGF-1: Basic and Clinical Advances. Springer-Verlag, New York, pp 75–91
279. **Waters DL, Hardy KJ, Danska J, Nickell D, Qualls C, Jackson L, Gesundheit N, Watson D, Nightingale S, Koster F, Schade DS,** A double-blind placebo-controlled randomized clinical trial of growth hormone and IGF-I in AIDS wasting. Program of the 76th Annual Meeting of The Endocrine Society, Anaheim, CA, 1994 (Abstract 17)
280. **Westermann J, Pabst R** 1990 Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system? Immunol Today 11: 406–410
281. **Laurence J, Grimison B, Gonenne A** 1992 Effect of recombinant human growth hormone on acute and chronic human immunodeficiency virus infection *in vitro*. Blood 79:467–472
282. **Germinario R, DeSantis T, Wainberg M** 1995 Insulin-like growth factor 1 and insulin inhibit HIV type 1 replication in cultured cells. AIDS Res Hum Retroviruses 11:555–561
283. **Tsarfaty G, Longo DL, Murphy WJ** 1994 Human insulin-like growth factor I exerts hematopoietic growth-promoting effects after *in vivo* administration. Exp Hematol 22:1273–1277
284. **Clark RG, Jardieu P** 1994 Anabolic and immunologic activity of recombinant human insulin-like growth factor-I (rhIGF-I) in AZT-treated mice. Growth Regul 4:123 (Abstract)
285. **Murphy WJ, Tsarfaty G, Longo D** 1992 Growth hormone exerts hematopoietic growth-promoting effects *in vivo* and partially counteracts the myelosuppressive effects of azidothymidine. Blood 80: 1443–1447
286. **Beck KD, Powell-Braxton L, Widmer HR, Valverde J, Hefti F** 1995 IGF-1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. Neuron 14:717–730
287. **Yao DL, Liu X, Hudson LD, Webster HD** 1995 Insulin-like growth factor I treatment reduces demyelination and up-regulates gene expression of myelin-related proteins in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA 92:6190–6194