Cytokines, leptin, and stress-induced thymic atrophy

Amanda L. Gruver and Gregory D. Sempowski

Departments of Pathology and Medicine and the Duke University Human Vaccine Institute, Duke University Medical Center, Durham, North Carolina, USA

Abstract: Thymopoiesis is essential for development and maintenance of a robust and healthy immune system. Acute thymic atrophy is a complication of many infections, environmental stressors, clinical preparative regimens, and cancer treatments used today. This undesirable sequela can decrease host ability to reconstitute the peripheral T cell repertoire and respond to new antigens. Currently, there are no treatments available to protect against acute thymic atrophy or accelerate recovery, thus leaving the immune system compromised during acute stress events. Several useful murine models are available for mechanistic studies of acute thymic atrophy, including a sepsis model of endotoxin-induced thymic involution. We have identified the IL-6 cytokine gene family members (i.e., leukemia inhibitory factor, IL-6, and oncostatin M) as thymosuppressive agents by the observation that they can acutely involute the thymus when injected into a young, healthy mouse. We have gone on to explore the role of thymosuppressive cytokines and specifically defined a corticosteroid-dependent mechanism of action for the leukemia inhibitory factor in acute thymic atrophy. We also have identified leptin as a novel, thymostimulatory agent that can protect against endotoxin-induced acute thymic atrophy. This review will highlight mechanisms of stress-induced thymic involution and focus on thymosuppressive agents involved in atrophy induction and thymostimulatory agents that may be exploited for therapeutic use. J. Leukoc. Biol. 84: 000–000; 2008.

Key Words: thymopoiesis · receptor · lipopolysaccharide

INTRODUCTION

The thymus is a specialized, central lymphoid organ located in the thorax above the heart. Postnatally, this bi-lobed organ is composed mostly of thymic epithelial space (TES), consisting of developing thymocytes and a supporting stromal thymic epithelial cell network [1, 2]. Adipocytes, peripheral lymphocytes, and fibroblasts are located in the surrounding perivascular space (PVS) [1, 2]. Histology of the thymus is marked by an outer capsule, a densely populated cortex filled with less-mature thymocytes, and an inner medullary region, where thymocytes will complete their self/nonself education and selection before being exported to the periphery. This process, known as thymopoiesis, ensures the establishment of central T cell tolerance in the host [3–5].

T cell development begins with the migration of bone marrow-derived, early thymic progenitor cells (Lin-CD44+e-Kit+IL-7Rα/β) to the thymus [6]. As these progenitors mature into thymocytes, they provide necessary cross-talk for the further development and maintenance of thymus stroma. Thymocyte interaction with thymic epithelial cells during fetal development establishes a robust and organized environment in which distinct cortical and medullary thymic compartments are formed to provide the architectural framework necessary for thymopoiesis and subsequent export of naïve T cells to the peripheral circulation [7].

Thymocyte developmental stages can readily be defined by the expression of the cell surface receptors CD4 and CD8. Maturing thymocytes begin as CD4-/CD8- double-negative populations, before up-regulating CD4 and CD8 to become double-positive (DP) thymocytes [8]. Over 90% of developing thymocytes will be of the DP phenotype. DP thymocytes undergo a rigorous selection process and eventually become CD4 single-positive (SP) or CD8 SP thymocytes, which exhibit MHC Class II or MHC Class I restriction, respectively [8]. Self-reactive cells failing negative selection are removed via apoptotic pathways, and mature, nonself-reactive SP thymocytes are exported to the periphery as antigenic, naïve Th cells (CD4) or cytotoxic T cells (CD8). These newly exported T cells are referred to as recent thymic emigrants (RTEs) [8].

In humans, monitoring thymus function is typically limited to noninvasive technology, including imaging of thymus size with chest-computed tomography or glucose-analog uptake with positron emission tomography (reviewed in Hudson et al. [9]). Peripheral monitoring of thymus output in humans is restricted to surrogate marker analysis of naïve T cell populations in the blood. Naïve T cells can be identified by flow cytometry-based immunophenotyping of CD45RA, the high molecular weight isoform of the tyrosine phosphatase CD45, and coexpression of CD62 ligand (CD62L; L-selectin) [10]. However, the identification of naïve T cells with this method can be complicated by memory T cells (typically CD45RO-positive), which revert to expressing CD45RA, or shedding CD62L by naïve cells after sample cryopreservation [11].

1 Correspondence: Duke University Medical Center, 102 Research Dr., Rm. 1033, Global Health Research Building, Durham, NC 27710, USA. E-mail: gsm@duke.edu

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Given these complications, Douek et al. [12] developed a molecular assay that specifically identifies RTEs by targeting a DNA hyproduct of thymopoiesis. The majority of developing thymocytes rearranges their TCR α genes, which creates an episomal circle of the excised, intervening TCR δ locus. The TCR δ DNA forms what is called a signal joint TCR excision circle (sjTREC) [13]. Douek et al. [13] adapted a specialized, real-time PCR reaction to quantify this nonreplicating circle of DNA in naïve T cells. Thus, the frequency of molecular sjTREC in a given population of peripheral blood leukocytes is proportional to the degree of thymic output of naïve T cells.

The continuous output of naïve T cells from the thymus maintains the diversity of the peripheral T cell repertoire and allows for the increased ability of the host to respond to neo-antigens. Unfortunately, the thymus is sensitive to acute stress-induced atrophy and is often referred to as a “barometer of stress” for the body. Acute thymic atrophy can therefore contribute to the development of a less-diverse, oligoclonal peripheral T cell repertoire and constricted host immunity. In this review, we will discuss mechanisms of stress-induced thymic involution, small animal models used to study mechanisms of stress-induced thymic atrophy, thymosuppressive cytokines that negatively regulate thymopoiesis, and the role of the novel thymostimulatory agent leptin (Fig. 1).

STRESS-INDUCED THYMIC INVOLUTION

Stress disrupts the homeostatic balance of the immune system and causes acute thymic involution through physiologic conditions, such as malnutrition, emotional distress, or pregnancy, or pathological conditions, such as infection, disease, clinical cancer treatments, or preparative regimens, for bone marrow transplant. Stress-induced thymic involution is characterized by reduction in thymus size caused by acute loss of DP cortical thymocytes and reduced output of naïve T cells to the periphery [14]. Stress-induced thymic involution differs from chronic involution. Chronic, age-induced involution is characterized by a gradual expansion of PVS and reduction of TES capable of supporting thymopoiesis, whereas acute thymic involution (i.e., stress) is often followed by a spontaneous recovery period after the stressor is removed [1]. Mechanisms that drive acute thymic involution are poorly understood. Moreover, there are no treatments available to protect against acute thymic atrophy or accelerate recovery, thereby leaving the immune system compromised during acute stress events.

Acute thymic atrophy is a common problem associated with viral, bacterial, and fungal infections [15]. Of current concern in today’s society are viral infections, such as HIV/AIDS, in which robust thymus function would be greatly beneficial. Unfortunately, HIV-1 infection severely compromises thymus function by the substantial loss of developing thymocytes and thymic stromal cells needed to support thymopoiesis [16]. Complications from bacterial infections, many times acquired after surgery, result in acute sepsis, which is the 10th leading cause of death in the United States, according to the U.S. Center for Disease Control [17]. Sepsis causes massive lymphocyte apoptosis that impairs host T and B cell responses [18, 19] and causes severe thymic atrophy [14]. Critical players in sepsis are glucocorticoids (corticosterone in rodents, cortisol in humans) induced by activation of the hypothalamic-pituitary-adrenal (HPA) axis and acute, proinflammatory cytokine cascades [20].

Acute, stress-induced thymic atrophy is a complication from many environmental stressors as well, in which transient reduction in thymus function persists until the physiological stressor is removed. The effects of malnutrition, starvation, and alcoholism have a negative impact on human thymopoiesis [21–23]. Morphological studies, for example, on autopsy of patients suffering from alcoholism showed decreased size and cellularity of thymus compared with tissue from age-matched nonalcoholics [23]. Environmental stressors such as prolonged physical or emotional stress can activate the HPA axis to induce production of the stress hormone cortisol, causing abrupt thymus involution and a result in a drop in thymopoiesis [24].

Additionally, many clinical situations arise in which stress-induced thymic atrophy is a complication of necessary treatment. Chemotherapy for cancer treatments, cyclosporine treatment for autoimmune conditions, as well as irradiation therapy and preparative regimens for bone marrow transplant decrease thymus function and contribute to delayed immune reconstitution [25–27]. Studies done with adult patients after cancer treatments or bone marrow transplant have shown minimal thymus function contributing to the peripheral T cell compart-

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**Fig. 1.** Model of stress-induced thymic atrophy and thymosuppressive and thymostimulatory mediators. LIF, Leukemia inhibitory factor; OSM, oncostatin M; KGF, keratinocyte growth factor; hGH, human growth hormone; TSLP, thymic stromal lymphopoietin.
ment with reconstitution resulting mainly from expansion of mature peripheral T cells (i.e., memory T cells) [25–27]. Evidence for thymic contribution in young patients after cancer treatment is slightly more promising, as documented by pediatric cases showing increased thymus size, increased CD45RA+ naïve T cells, and extensive peripheral T cell reconstitution after intensive chemotherapy [25]. Yet, even in these promising cases, there remains a significant delay in thymic function that leaves the patient vulnerable to opportunistic infections, underscoring the need to identify the mechanisms of stress-induced acute thymic atrophy and to develop therapeutics to block atrophy and/or enhance recovery.

MURINE MODELS OF ACUTE THYMUS INVOLUTION

As study of thymus function in humans is restricted to noninvasive approaches, which offer limited analysis of complex, intrathymic processes, murine models have proven particularly useful in the analysis of stress-induced thymic atrophy as well as during the recovery phase after the stressor is removed. Direct quantification of thymus function in mice can be performed upon necropsy. Total thymus cellularity, phenotypic analysis of developing thymocytes (CD3/CD4/CD8/CD44/CD25), and histological analysis of the thymus can be used to gain intricate knowledge of thymopoiesis. Moreover, we have adapted the molecular sjTREC real-time PCR assay for the mouse TCR sequences, allowing for quantification of murine TRECs (mTREC) in splenocytes, thymocytes, and whole thymus [28]. Using this assay of thymopoiesis, coupled with peripheral mouse markers of naïve T cells (CD45RB+, CD62L+, CD44+), investigators can now comprehensively monitor thymic function in models of stress-induced involution.

Several small animal models exist using various stressors capable of inducing acute thymic involution (Fig. 1). For example, stress from starvation [29] and physical restraint [30] increases glucocorticoid (corticosterone) levels, which mediate thymocyte apoptosis. Similarly, injection of synthetic corticosteroids, such as dexamethasone, can also cause acute thymus involution and have been used as a model system [31, 32]. Other murine stress-induced thymic atrophy models incorporate sex steroids such as progesterone and estrogen [32, 33] and testosterone [34]. γ-Irradiation can also induce acute thymic atrophy [32], reminiscent of clinical irradiation treatments. Viral infection models, such as rabies, measles, and hepatitis, also induce thymic atrophy [15], reminiscent of HIV-1 infection in humans [35].

As a model for bacterial sepsis, cecal ligation and puncture (CLP) or purified LPS injection can be used. CLP involves perforation of the intestines, a minor surgery performed under anesthesia, which releases infectious bacteria such as Escherichia coli to induce sepsis and subsequent acute thymic atrophy [36]. A noninfectious and reproducible model routinely used to study acute thymus involution is endotoxin or the LPS-induced acute thymic atrophy model. LPS is the endotoxin produced by gram-negative bacteria, such as E. coli. Purified LPS can be injected i.p. to induce sepsis and subsequent acute thymic atrophy without complications from surgery or active bacterial infection [14, 37, 38]. Mice treated with LPS (100 μg per mouse, i.p.) develop severe acute thymic atrophy that peaks within 3–5 days [37]. Thymic atrophy in the mouse can be characterized by loss of thymus weight, loss of DP thymocytes, and loss of mTREC/mg thymus. Using these measurements, we have reported that thymus weight, cellularity, and mTREC/mg thymus continues to decrease for up to 7 days after a single LPS challenge, which is then followed by a rebound in thymus function (Fig. 2) [37]. Using this model, we have defined the role of LIF as a thymosuppressive agent in stress-induced acute thymic atrophy, which will be reviewed below. We have also used this model to begin to understand the protective effects of the metabolic hormone leptin against LPS-induced acute thymic atrophy, which will also be discussed further.

THYMOSUPPRESSIVE AGENTS

Many lessons can be learned from the study of thymopoiesis across the lifespan and chronic age-induced thymic involution to facilitate our understanding of cytokine regulation in the setting of acute stress-induced thymic atrophy. We have reported that members of the IL-6 cytokine gene family are increased with age in human thymus tissue [39]. These studies led to the novel hypothesis that the key IL-6 cytokine gene family members, LIF, IL-6, and OSM, are thymosuppressive and play a key role in actively mediating thymus involution (Fig. 1). These cytokines are present in the thymic microenvironment and are produced by thymic epithelial cells, which are also capable of producing other immunoregulatory cytokines such as IL-1, IL-3, IL-7, and TGF-β [39–42]. Although the impact of the IL-6 cytokine family members as acute-phase response cytokines in sepsis has been well-established, the impact on thymus function has not been well-defined. Here, the role of these thymosuppressive agents during stress-induced thymic atrophy will be explored.

LIF, IL-6, and OSM signal through the common gp130 subunit to the IL-6R family, which is expressed ubiquitously on thymocytes [43] and on thymic epithelium [44, 45]. We have demonstrated that injection of pure, endotoxin-free, recombinant LIF, IL-6, or OSM into young, healthy mice induces acute thymic atrophy via loss of DP thymocytes within 3 days, similar to thymic atrophy induced by LPS [39]. LIF and IL-6 are potent, proinflammatory cytokines that play an integral role in inflammatory responses, such as is observed in LPS-induced acute thymus atrophy. Using the LPS mouse model, we have further defined the role of the IL-6 family members in being intermediaries in LPS-induced acute thymic atrophy. To that end, we have assessed whether blockade of the common signaling subunit gp130 would protect against thymic atrophy in the LPS model. We injected LPS alone or LPS with anti-gp130-neutralizing polyclonal antibodies and then assessed thymus atrophy by quantification of mTREC levels. We found anti-gp130 neutralization to provide protection against the loss of mTREC/mg thymus (Fig. 3), further indicating that the IL-6 cytokine family members are acutely thymosuppressive and play a negative role in thymus atrophy induced by LPS. In
addition, we have specifically investigated the role of LIF as a mediator of LPS-induced thymic involution, as pretreatment of mice with anti-LIF-neutralizing antibodies inhibited LPS-induced acute thymic involution by 52% [38]. Taken together, these data suggest that LIF, IL-6, and OSM are mediators of stress-induced thymic atrophy.

**LIF: mechanism of action**

We have defined the mechanism of action of LIF as a prototypical member of the thymosuppressive IL-6 cytokine gene family. LIF is a known activator of the HPA axis [46]; therefore, we determined if the effects of LIF were dependent on corticosteroid production. We found that LIF was unable to induce thymic atrophy when corticosterone production was blocked by the synthesis inhibitor metyrapone or by adrenalectomy, indicating that the mechanism of action of LIF was corticosteroid-dependent [38]. Thymic epithelial cells possess all of the enzymes necessary for steroidogenesis and produce small amounts of corticosterone, which at normal physiological levels, modulate thymocyte activation thresholds for proper positive and negative selection [47]. Therefore, to further delineate between intrathymic and systemic effects of LIF, we used fetal thymic organ culture (FTOC) to study the impact of LIF on thymus-produced corticosterone, without influence from systemic, adrenal-derived corticosterone (HPA axis). LIF treatment of FTOC was able to induce thymic involution, indicating that LIF can modulate thymopoiesis via an intrathymic mechanism. To test if this intrathymic mechanism was also corticosteroid-dependent, we treated FTOC with metyrapone, which blocked LIF-induced thymic involution [38]. These results demonstrated that LIF acts as a thymosuppressive cytokine via intrathymic and systemic mechanisms and may be an effector pathway of chronic and acute thymic involution [38].

**LIF, IL-6, and OSM: other considerations**

The mechanism of action of IL-6 and OSM induction of acute thymic atrophy has yet to be elucidated. Reports, however, of IL-6 cytokine gene family deficiency can provide insight into the function of these cytokines in the thymus and perhaps hint at the mechanism causing stress-induced thymic atrophy. IL-6 appears to play an endogenous role in thymocyte proliferation, as IL-6 can stimulate thymocyte proliferation in vitro [48, 49], and thymus cellularity of IL-6-deficient mice is reduced by 20–40% [50]. These impacts on thymocyte proliferation are
reminiscent of the impacts seen with LIF-deficient mice, which have decreased thymocyte proliferation in response to mitogens, although LIF-deficient mice have normal thymic cellularity [51]. These observations are in conflict with the proposed role of IL-6 cytokine gene family members as thymosuppressors, suggesting that these cytokines may have a positive role at physiological levels yet be thymosuppressive at higher concentrations, similar to what is seen with endogenous corticosteroid levels in the thymus [31, 52]. Details of thymic function have not been reported for OSM-deficient nor OSM receptor (OSMR)-deficient mice, although defects in bone marrow hematopoiesis have been shown to occur in OSMR-deficient mice [53].

Alternatively, overexpression studies using transgenic animals or transplant of cells producing these cytokines suggest an active role in thymic atrophy. Metcalf and Gearing [54, 55] injected mice with a myeloid progenitor cell line that overproduced LIF, causing excess serum LIF and acute atrophy of the thymus, as well as other tissues such as pancreas and adipose tissue. Studies done in transgenic mice that overexpress LIF specifically in T cells showed a preferential loss of cortical thymus epithelium along with cortical (DP thymocytes) with infiltration of B cells [56]. Instead of normal T cell development occurring in the thymus, thymocytes developed extrathymically in lymph nodes [56]. Similar to excess LIF, excess OSM (i.e., OSM-transgenic mice) caused a similar phenotype with acute thymic atrophy and extrathymic T cell development in lymph nodes [57-59]. The thymi of these OSM transgenic mice showed a dramatic decrease in DP thymocytes that were almost completely gone by 10 weeks of age [57]. The epithelial network of the thymus was disrupted severely, with a preferential loss of cortical epithelium, and an expansion of the capsule layer infiltrated with mature, activated T cells (CD25+CD69+CD44hi) and B cells, leaving the thymus reminiscent of a secondary, lymphoid-like tissue [57]. Although little attention has been paid in the literature to the thymus of IL-6-transgenic mice, it has been reported that plasma cells prolifereate and infiltrate the thymus in IL-6-transgenic mice [60, 61], making B cell hyperplasia of the thymus a common characteristic. TSLP has been shown to enhance proliferation of thymocyte progenitors [62], and KGF has been noted for the ability to enhance thymopoiesis and immune reconstitution in chemotherapy-induced thymic atrophy [63, 64]. Other factors, such as hGH [65] and IL-7 can enhance thymus reconstitution in bone marrow transplant models [66, 67], known to be complicated by acute thymic atrophy. However, there is little evidence for the ability of these growth factors/cytokines to protect against viral or bacterial acute stress-induced thymic atrophy models (i.e., LPS-induced acute thymic atrophy). One study has reported that adenoviral delivery of the anti-inflammatory cytokine IL-10 can lessen the degree of sepsis-induced thymus atrophy [68, 69]. The effects were only significant, however, when the viral vector was delivered intrathymically. These observations are great for understanding the mechanism but not useful therapeutically. Recently, we and others [29, 37] have shown that leptin, the peptide hormone best known for its role in modulating metabolism, confers protection in the setting of LPS-induced thymic atrophy and in the setting of starvation. Following is a discussion of the novel role of leptin as a thymostimulatory agent in the setting of stress-induced thymic atrophy.

Leptin: more than a satiety hormone

Leptin is the 16-kDa product of the obese (ob) gene, which was named for the obese phenotype of leptin-deficient ob/ob mutant mice [70]. Leptin is primarily produced by adipocytes and has many roles in the neuroendocrine and reproductive systems [71]. Leptin is best known for its role in satiety and feeding behavior. Leptin signals through the leptin receptor (OBR), the product of the diabetes (db) gene, named for the diabetic phenotype of leptin receptor-deficient db/db mutant mice [72]. Leptin has structural similarities to class I cytokines (i.e., IL-6 cytokine gene family), and its receptor has structural similarities to the class I cytokine receptor gp130 subunit [73]. For this reason, leptin has been cross-classified as a cytokine.

A role for leptin in modulating immunity was first speculated by the observance of suppressed cell-mediated immunity, chronic thymic atrophy, and decreased numbers of lymphocytes in ob/ob and db/db mice [29, 74, 75]. The impact of leptin on cells of the immune system continues to be an emerging area of investigation, with several functions of leptin in innate and adaptive immunity already identified (reviewed in Lam and Lu [76]). Leptin influences many cells important to innate immunity, such as dendritic cells, macrophages, neutrophils, and NK cells [76]. Leptin also modulates CD4 Th cell cytokine production, favoring a Th response versus Th2 response [76], and preferentially promotes naïve T cell proliferation and inhibits memory T cell proliferation [77]. Leptin also plays a role in autoimmune disorders (reviewed in Matarese et al. [78]). Autoimmune dysregulation is enhanced by Th1-type immune responses, and the elevated serum leptin levels in patients with autoimmune diseases are thought to increase autoimmune severity [78]. Leptin-deficient ob/ob mice are protected in experimental autoimmune encephalitis, a model for multiple sclerosis [79]. More recently, leptin has been found to constrain the proliferation of T regulatory cells, thymic-derived cells charged with maintaining peripheral tolerance [80]. These studies show that leptin is a pleiotropic mediator in a wide-range of biological systems (i.e., neuroendocrine, immune). The emerging role of leptin in modulation of thymopoesis and thymic involution will now be discussed.

THYMOSTIMULATORY AGENTS

Now, we will turn our attention to cytokines that can positively impact the thymus during stress-induced involution. Many thymostimulatory agents show promise as therapeutics to boost T cell reconstitution in clinical settings of stress-induced thymic involution (i.e., irradiation and chemotherapy; Fig. 1). Stromal tissue growth factors such as KGF and TSLP can impact thymopoiesis. TSLP has been shown to enhance proliferation of thymocyte progenitors [62], and KGF has been noted for the ability to enhance thymopoiesis and immune reconstiution in chemotherapy-induced thymic atrophy [63, 64]. Other factors, such as hGH [65] and IL-7 can enhance thymus reconstitution in bone marrow transplant models [66, 67], known to be complicated by acute thymic atrophy. However, there is little evidence for the ability of these growth factors/cytokines to protect against viral or bacterial acute stress-induced thymic atrophy models (i.e., LPS-induced acute thymic atrophy). One study has reported that adenoviral delivery of the anti-inflammatory cytokine IL-10 can lessen the degree of sepsis-induced thymus atrophy [68, 69]. The effects were only significant, however, when the viral vector was delivered intrathymically. These observations are great for understanding the mechanism but not useful therapeutically. Recently, we and others [29, 37] have shown that leptin, the peptide hormone best known for its role in modulating metabolism, confers protection in the setting of LPS-induced thymic atrophy and in the setting of starvation. Following is a discussion of the novel role of leptin as a thymostimulatory agent in the setting of stress-induced thymic atrophy.

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Leptin in normal thymopoiesis?

Thymus atrophy associated with leptin deficiency or leptin receptor deficiency has long been an indicator that leptin may play a role in normal thymopoiesis. Leptin-deficient \( \text{ob/ob} \) mice and leptin receptor-deficient \( \text{db/db} \) mice, however, have many other complications, such as severe obesity, metabolic abnormalities, and hypercorticosteronemia [81]. These abnormalities suggest the possibility that thymic atrophy from leptin deficiency could be a result of the stress seen from metabolic and hormonal dysregulation, as opposed to direct effects in the thymus. An elegant study by Palmer et al. [82] has shed light on this issue by addressing the following questions: Does leptin receptor deficiency result in a cellular defect of lymphocytes or thymic stromal cells, and is thymic atrophy simply mediated by the metabolic defects of leptin receptor-deficient mice, such as elevated corticosterone levels?

Palmer et al. [82] conducted a series of experiments using bone marrow chimeras (BMC) between \( \text{db/db} \) mice and their lean heterozygous littermates (\( \text{db/+} \)). \( \text{db/db} \) mice lack the long leptin receptor isoforms capable of full intracellular signaling [83]. Normal mice potentially have leptin receptors on stromal cells and/or on hematopoietic cells. \( \text{db/db} \) mice lack the potential expression of the long signaling leptin receptor isoform on stromal cells or lymphocytes. \( \text{db/db} \) mice also exhibit complicated metabolic defects, such as elevated corticosterone, and have chronic thymic atrophy. They constructed \( \text{db/db} \rightarrow \text{db/+} \) BMC, which potentially have leptin receptors on thymus stroma but not thymocytes [82]. These chimeras also do not have the environmental abnormalities of \( \text{db/db} \) mice. \( \text{db/db} \rightarrow \text{db/+} \) BMC did not exhibit thymic atrophy, suggesting that there were no cell autonomous defects in thymocytes. \( \text{db/+} \rightarrow \text{db/db} \) BMC were also made, which could have leptin receptors on thymocytes but not thymus stroma and have the metabolic abnormalities (i.e., elevated corticosterone levels) of \( \text{db/db} \) mice. Thymus atrophy was seen in these mice, thus suggesting a cellular defect of the thymus stroma or an environmental effect mediated by corticosterone and/or other metabolic factors was the cause of the observed thymus atrophy. To distinguish between these later two scenarios, BMC from \( \text{db/db}, \text{db/+}, \text{and C57BL/6} \) wild-type mice were transplanted into wild-type recipient mice. \( \text{db/db} \) BMC were equally capable of undergoing thymopoiesis, thereby indicating that there are no inherent cellular defects in thymic stromal cells.

Contrary to current dogma, Palmer’s study [82] suggested that environmental factors play a large role in the chronic thymic atrophy in \( \text{db/db} \) mice, in which corticosterone is a likely mediator. These data also rule out a cellular defect of thymocytes or thymic stromal cells to develop in a normal environment, further suggesting that leptin does not play a significant role in normal thymopoiesis. In agreement with these observations, we have found that exogenous leptin treatment is unable to boost thymus cellularity of healthy, wild-type mice [37].

Leptin protects against stress-induced thymic atrophy

In contrast to having no effect in a normal, unstressed mouse, leptin has been reported to be thymostimulatory in settings of thymus stress. We have recently shown leptin to have therapeutic potential in the LPS stress model of acute thymic atrophy [37]. In this model, thymic atrophy was induced and peaked within 3–5 days (Fig. 2). We found leptin was able to significantly blunt the LPS-induced thymus weight loss at 1 and 7 days after LPS challenge (Fig. 4A), with a trend toward increased cellularity. We also found leptin to completely protect against the LPS-induced loss of mTREC/mg thymus [37]. These data demonstrated the thymostimulatory effects of leptin by protecting against loss of thymopoiesis in the setting of LPS-induced stress.

**Fig. 4.** Leptin protected against LPS-induced thymic atrophy and modulated the systemic response to LPS. Female BALB/c mice were simultaneously administered (i.p.) saline or leptin (1 μg/g body weight) and challenged with saline or \( \text{E. coli} \) LPS (100 μg/mouse). Mice were killed on Days 1, 7, and 14 to monitor thymopoiesis (n = 5). Mean thymus weight Day 7 (A). Serum corticosterone levels were determined 4 h post-treatment (B). Serum IL-1β cytokine levels (C) and serum IL-12p70 cytokine levels (D). *, \( P < 0.05 \), compared with LPS-treated controls [37].
involution. This protective effect was consistent with other models of stress-induced acute thymic atrophy, such as starvation.

Starvation-induced acute thymus atrophy is characterized by elevated corticosterone levels and fluctuations in glucose availability, which contribute to thymocyte apoptosis [29]. Bloom and colleagues [29] have shown that acute starvation in this model causes severe and preferential loss of DP thymocytes and that leptin was able to completely protect against the loss of thymus weight and cellularity. Taken together, these two studies identified leptin as a potential therapeutic for protection from stress-induced acute thymic atrophy.

**Leptin: mechanisms of action**

The mechanism of action in which leptin mediates protective effects on thymus is just beginning to be understood. Leptin has been shown to have systemic effects that may in part mediate the protection from LPS-induced acute thymic atrophy (Fig. 4) [37]. Leptin inhibited the peak systemic corticosterone response to LPS (Fig. 4B) and had a significant effect in blunting 10/23 measured serum cytokines induced 0–12 h post-LPS challenge [37]. Leptin modulated the proinflammatory cytokine cascade in two distinct ways, as represented by responses of IL-1β and IL-12p70 [37]. There was no change in peak IL-1β levels, but a significant acceleration to baseline was observed (Fig. 4C). IL-12p70, however, had a reduced peak response in addition to an accelerated resolve to baseline (Fig. 4D). These data demonstrated a systemic, anti-inflammatory role for leptin in this LPS model of stress-induced thymic involution. The extent of these systemic effects, however, does not fully explain the degree of protection seen with leptin treatment in the model, thus suggesting additional mechanisms need to be elucidated within the thymus.

Studies have indicated that thymocytes themselves are indeed responsive to the effects of leptin, as in vitro treatment of isolated thymocytes with leptin can completely inhibit dexamethasone-induced apoptosis [29]. These data suggested that the leptin receptor may be expressed on thymocytes. In the periphery, leptin receptor expression has been confirmed on human CD4+ T cells [84], but leptin receptor distribution in the thymus is currently unknown. Recent studies have found leptin mRNA transcripts present in whole thymus tissue samples [82, 85], indicating that the leptin receptor is expressed within the thymus in lymphoid and/or stromal compartments. These leptin mRNA transcript levels were not diminished by hydrocortisone depletion of DP thymocytes, indicating that leptin receptor expression exists and may be more abundant in the stromal compartment of the thymus [82]. At the protein level, leptin receptor expression has also been confirmed in whole thymus tissue [82], although full characterization of leptin receptor expression to distinguish between lymphoid and thymic stroma has yet to be performed. Identification of compartmental expression of the leptin receptor will provide valuable insight into possible intrathymic mechanisms of leptin in the protection against stress-induced acute thymic atrophy.

**SUMMARY AND FUTURE CONSIDERATIONS**

Ongoing thymopoiesis is essential for the development and maintenance of a robust and healthy immune system. Acute thymic atrophy is a complication of many infections, environmental stressors, clinical preparative regimens, and cancer treatments used today. Thymic involution associated with stress is a balance between suppressive and stimulatory agents (Fig. 1). The induction of thymic atrophy and the delay in thymus recovery underscore the need to identify the mechanisms that drive stress-induced acute thymic involution and the need to develop therapeutics to block atrophy and/or enhance thymus recovery during acute stress events.

We and others have developed useful models in which to study the mediators of thymic atrophy, as well as potential therapeutic targets to protect against thymic atrophy. Using isolated cell culture systems, FTOC, and whole animal models, we have functionally defined the mechanism by which LIF is a critical intermediate in transmitting the atrophic signals of bacterial endotoxin to the thymus via systemic and intrathymic corticosteroids. LIF exemplifies the emerging concept that members of the IL-6 cytokine gene family are thymosuppressive and may be common mediators of thymic atrophy, resulting in loss of thymic output and damage to the thymic microenvironment.

The severe degree of change in the thymic stromal environment observed in the presence of excess IL-6 cytokine family members supports a hypothesis in which these cytokines mediate their thymosuppressive effects via the thymic stroma. The intrathymic production of corticosteroids induced by LIF is a specific example of a stromal-mediated response. Recently, Marino et al. [86] described the unresponsiveness of DP thymocytes to IL-6 in vitro. This is consistent with the observation that thymocytes do not acquire IL-6Rα, necessary for IL-6 signaling, until the CD4 SP or CD8 SP stage [43, 86]. The absence of receptor activation in thymocytes supports the hypothesis of a stromal-mediated effect, given that IL-6Rα is expressed on thymic epithelial cells [45]. It is also evident that the normal effects of these cytokines may be mediated through stromal mechanisms. Ablation of STAT3, an intracellular signaling molecule involved in the activation of many IL-6 family cytokine receptors, specifically from thymic epithelial cells, results in severe thymic atrophy caused by loss of thymic epithelial cells as well as thymocytes [87]. These studies suggest more research is needed to identify the relative contribution of cytokine signaling through thymocytes or thymic epithelial cells in the role of stress-induced thymic atrophy. Regardless, these data and other observations support a new paradigm, in which cytokines act as thymosuppressors in settings of stress-induced acute thymic atrophy.

In contrast, we have identified leptin as a novel thymostimulatory agent that can protect against LPS-induced acute thymic atrophy. Recent work done with bone marrow chimeras generated between leptin receptor-deficient db/db mice and healthy mice has ruled out the possibility of a cellular defect between thymocytes or thymic stromal cells in the absence of leptin in a normal, metabolic environment. These observations indicated that chronic thymic atrophy seen in db/db mice may be a result of the abnormal corticosterone levels or other metabolic abnormalities found in db/db mice. Taken together, these observations discussed herein support a model in which leptin does not play a major role in a young, healthy mouse but rather, is important in settings of stress-induced thymic atrophy such as LPS or in settings of leptin deficiency. Leptin has also shown
promise as a thymus stimulant in aged mice [88], thus suggesting that leptin may be beneficial as a therapeutic in other thymic atrophy models and in settings of T cell immune reconstitution.

In summary, much has been done to define the role of cytokines, steroids, and hormones in acute thymus atrophy; however, there are still many unanswered questions. If we are to develop effective therapeutics to prevent atrophy or accelerate thymic recovery, then there must be a focused effort about defining the underlying mechanisms that drive acute thymic involution associated with stress events.

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