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Thymic Hormones – A Clinical Update

Marcelo B. Sztein and Allan L. Goldstein

Departments of Medicine and Biochemistry, The George Washington University School of Medicine, Washington DC 20037, USA

Introduction

In the last two decades our understanding of the important physiologic role of the thymus within the immune system has improved dramatically. In the early sixties it was established that the thymus regulated the development of the immune system and that neonatal thymectomy resulted in a marked progressive depression of the immune system usually leading to death of the animals by overwhelming infections [4, 59, 65]. It was then found that partial or complete restoration of the immune function could occur following implantation of thymus glands [24] or cell-impermeable Millipore diffusion chambers containing normal thymus tissue [61, 72] or thymomas [90], or by cell-free thymic extracts [36, 41, 97].

As the first reports of restoration and/or modification of immune functions were reported [5, 36, 60, 98], it became clear that the thymus had an endocrine function and that it secreted a family of hormone-like polypeptides which have been collectively named thymic hormones (TH). It is now very well established that these hormones are produced by the thymic epithelial cells [48, 49, 51], and that at least some of them are released into the bloodstream [62, 67, 70]. Recent studies have shown that cells from other organs, such as brain [45] and spleen [47, 55], are also able to produce some peptides similar in sequence and properties to some TH, indicating that in addition to their influence in the thymic microenviroment, TH may act in a hormone-like fashion in other parts of the body as well. Although considerable progress has been made in this area, the mechanisms by which the thymus regulates the development of a competent immune system in health and its role in disease are only beginning to be understood at the biochemical, cellular, and molecular levels.

There are extensive reviews in the literature [7, 37, 39, 63, 81–83, 88, 91, 106] to which the reader is referred for detailed information covering the studies that have lead to the current knowledge of the physiology of the thymus gland and its

Offprint requests to: Marcelo B. Sztein, M. D., Ross Hall Rm 705, Department of Medicine, The George Washington University School of Medicine, 2300 I Street NW Washington DC 20037, USA

hormones. In this review, the main emphasis will be placed on the role of TH in aging and the possible implications of their use in the treatment of disease.

A number of different TH preparations are being tested for their therapeutic potential in clinical settings. Some of these preparations are partially purified, i. e., thymosin fraction 5 (TF5) [36, 54], thymostimulin (TS) (TP1) [28], thymic humoral factor (THF) [97], and thymic factor X (TFX) [87, 88], while others are homogeneous preparations, like thymosin α 1 [102], thymopoietin [41], and thymulin (FTS) [12, 17]. As can be seen in Fig. 1, these well characterized TH peptides are small molecules less than 50 amino acids (AA) long. In some cases, the biologically active portion has been able to be traced to certain portions of these molecules. For example, the biologic activity of thymosin α 1 with regard to in vivo stimulation of interleukin 2 (IL2) production and enhancement of helper T-cell function, appears to be restricted to the N-terminal AA 1–14 (N-14) of the 28 AA sequence [22, 31], while in thymopoietin, the biologic activity has been shown to reside in a pentapeptide, named TP5, corresponding to the residues 32–36 of the 49

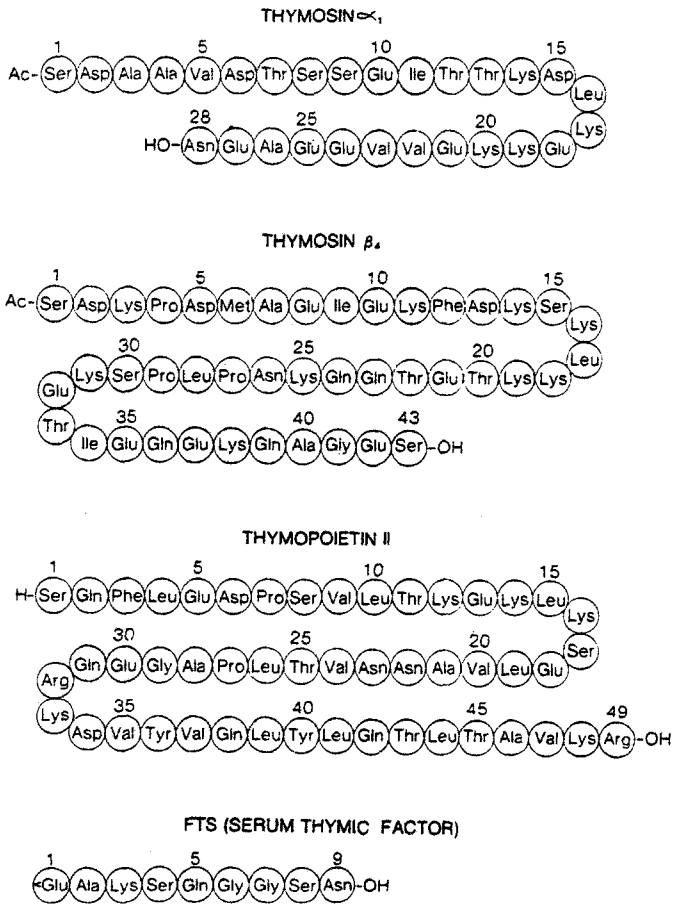

Fig. 1. Sequence analysis of well-characterized thymic hormones

AA sequence [42] (Fig. 1). Both N-14 of thymosin α 1 and TP5 have been synthesized and have been shown to be active in in vivo and in vitro studies [83].

The major clinical conditions associated with immune abnormalities in which TH may be therapeutically useful are aging, infectious diseases, primary and secondary immunodeficiences, autoimmune diseases, and cancer. We will describe the current status and the future perspectives of TH research in each of these areas.

Thymic Hormones and Their Role in Aging

The increase in the average life span of humans during the last century has been accompanied by a parallel increase of a number diseases associated with aging, including cancer, certain infectious and autoimmune diseases. The decline of immunocompetence with aging has been correlated with the increased incidence of these diseases [20, 38, 64, 101] and extensive reviews describing the changes in the immune system during the aging process have recently been published [20, 64, 101]. Although the decline of the immune system is accompanied by alterations in all its compartments, the most affected appears to be the T-cell compartment [20, 38, 64, 101]. Decline in T cell mitogen-induced proliferation, allogeneic mixed leukocyte reaction (MLR), cytotoxic T lymphocyte responses (CTL), helper T-cell activity, IL2 production, IL2 receptor expression, calcium uptake, PNP activity (PNP-purine nucleoside phosphorylase), adenylate cyclase activity, etc have been observed in T-cells from aging individuals and animals [64, 101]. These changes lead to an increased susceptibility to infections, decrease resistance to tumor growth, and an increased incidence of autoimmune diseases, probably secondary to an immunologic imbalance resulting in an increased response towards self antigens [38], characterized by a rise in the frequency of autoantibodies observed in aged individuals [64].

The thymus gland controls the maturation and differentiation of T-cells leading to normal function. The involution of the thymus, which starts at puberty, appears to precede a decline in thymic function and thymic-dependent immunity [38]. This phenomenon is accompanied by a decline in the levels of TH (i. e., thymosin, FTS, and thymopoietin) detectable in serum after puberty. However, thymosin-like activity is demonstrable in serum with advancing age, but at reduced levels, probably due to the fact that some epithelial cells of the endocrine thymus remain functional [38] and the possible contribution of extra-thymic sites of TH production (see above).

Direct evidence of the involvement of the thymus in the aging of the immune system has been provided by experiments showing that adult thymectomy accelerates the age-related decline in the immune system, and that the success of a thymic transplant in reconstituting thymectomized mice is inversely proportional to the age of the adult thymus donor [38]. Very recently, evidence was presented that sequential multiple graftings of syngeneic newborn thymus were able to partially restore immunocompetence in aging mice and extend their mean remaining life expectancy, without altering their maximal life-span [53]. Confirmation of these experiments by others may provide a model to directly study the role of the thymus gland in the aging process.

The study of the number and distribution of thymus epithelial cells at different

ages has demonstrated that there is a decrease in the number of TH-secreting cells, particularly after the third decade, which parallels the changes in thymus lymphoid volume and in blood levels of humoral factors [85]. However, these studies demonstrated a biphasic age-dependency in thymosin positive cells, with the highest positive-cell content found in the early post-natal period (2–4 months of age) and at 20–30 years, suggesting that the thymus humoral function is more complicated than suggested by the serum levels [85]. The authors hypothesized that feed-back mechanisms, the existence of which have been already demonstrated for FTS [78], may play an important role in this phenomenon. A decrease in the thymic epithelial cells with age has also been documented for cells containing thymosin α 1 [52] and FTS [79].

One of the mechanisms by which the thymus gland exerts its effects is the release of thymic factors, providing a clear rationale for the study of the in vitro and in vivo effects of TH on immune cells from aged animals and individuals. A number of studies have addressed this problem [38], and variable degrees of restoration of the immune response have been reported with several TH, including in vitro decrease in the formation of autologous rosette-forming cells (ARFC) (which are increased in the spleen of aged mice), partial reconstitution of T-cell mediated cytotoxicity and proliferative reponses, increases in T-cell helper activity and MLR, etc [16, 34, 38, 101]. In contrast, variable or no effects were observed on other immunologic parameters like Con A-induced IL2 production after in vitro preincubation with THF [43] and on the number of T-cells after in vitro incubation with TF5 [16].

Finally, it is worth mentioning recent in vitro studies which demonstrated that supernatants from thymic adherent cells were able to augment the antigen/mitogen responses of thymocytes of 2 to 4-week-old mice if the donor of the thymic adherent cells were 2.5-month-old [77]. However, this augmenting factor was not present in the supernatant if the cells were obtained from 5-month-old donors. Additionally, it was shown that the supernatants from cells of 20-month-old donors contained an inhibitory factor [77].

With regard to the use of well characterized TH in vivo, a number of interesting experiments have been performed employing thymosin α 1. Injection of old mice with thymosin α 1 increases in vitro mitogen-induced IL2 production [31] and specifically enhances the in vitro T-cell dependent activity of their spleen cells [22]. Furthermore, these activities appear to be restricted to the N-terminal AA 1–14 (N-14) of the molecule, since the injection of N-14, but not C-14 (C-terminal AA 15–28), is as effective as the injection of the entire thymosin α 1 molecule [22, 31]. Since cells from thymosin α 1-injected old mice appear to also display an enhanced response to exogenously added IL2, it is postulated that thymosin α 1 enhancement of T-cell helper activity by spleens of injected of old mice is mediated by changes in both IL2 production and responsiveness to IL2 [31].

In another series of experiments, the effects of TF5 and thymosin α 1 on the in vitro specific antibody response by lymphoid cells from in vivo immunized young and old individuals with influenza and tetanus toxoid vaccines have been studied [25, 26]. It was demonstrated that TF5 and thymosin α 1 were able to enhance the specific antibody responses to a trivalent influenza vaccine to a greater extent in cultures established from elderly volunteers [25], while TF5 was able to increase the antibody response against tetanus toxin of 7/10 individuals from the elderly group

and of 3/12 volunteers from the younger group [26]. Additionally, it was demonstrated that thymosin α 1 injection was able to increase the specific antibody production against tetanus toxoid of inoculated young and old mice [27]. Therefore authors suggested that thymosin may be useful as an adjuvant to active immunization for the elderly.

Compatible with these observations are the results of a clinical trial designed to study the immunoprophylactic effects of TP1. In this study, 40 hospitalized aged individuals with no evidence of autoimmune, neoplastic, or acute infectious diseases were divided into two groups: one received no therapy and the other was treated with TP1 for 3 months. Individuals were followed for 180 days and several immune parameters, including Ig and complement levels, sheep rosette-forming cells, absolute lymphocyte numbers and sedimentation rate, were monitored. The incidence of new infections was also determined. Results showed a significant reduction of infections and a decreased sedimentation rate in the TP1-treated groups, while the other parameters remained unchanged [74].

Although additional research in animal models and in vitro systems is required to further determine the role of TH during the aging process, the studies completed to date point to a potentially important use of TH as therapeutic modalities in the prevention and/or treatment of diseases associated with aging and the senescence of immune responses that occurs with the aging process.

Infectious Diseases

Immunosuppressed animals have been routinely used as models to study the role of TH in the protection against infectious agents in immunocompromised patients. Studies to date are very encouraging for the possible therapeutic application in immunosuppressed patients. It has been demonstrated that in vivo administration of TF5 or thymosin α 1 enhanced immunity and improved survival of immunosuppressed mice infected with BCG [15], candida, or cryptococcus, and also enhanced the production of interferon in mice infected with Newcastle disease virus [81]. More recently, studies using nine inbred strains of mice with different degrees of susceptibility to infections with *Candida albicans*, have demonstrated that the injection of TF5 increased the resistance to *Candida albicans* by some of the susceptible strains, but decreased the resistance of the resistant strains [75]. In contrast, TF5 enhanced delayed hypersensitivity reactions to specific antigen of the resistant-sensitized mice, while no effect was observed in the susceptible strains [75]. Further experiments in this system have shown that the capacity of a particular strain to increase its resistance to the infectious agent after in vivo administration of TF5 parallels its capacity to increase the in vivo release of MIF and gamma interferon [69]. Additionally, it was demonstrated that TF5 was able to increase in vivo delayed hypersensitivity responses, MIF production, and enhance resistance to infections with *Candida albicans* in mice treated with alloxan. Alloxan induces a diabetic state characterized by hyperglycemia and a marked decrease in the cellular immune responses to *Candida* [76].

Other interesting observations reported recently involve the protection of 5-FU immunosuppressed mice against opportunistic infections (i.e., *Candida albicans*,

Listeria monocytogenes, *Pseudomonas aeruginosa*, and *Serratia marcescens*) by injection of TF5 and thymosin α 1 [58]. Finally, it has been shown that TP5 is able to greatly increase the survival rate and mean survival time of burned guinea pigs (20% of body surface) after being challenged with *P. aeruginosa* [89].

In humans, TH are beneficial in enhancing the in vitro response to influenza and tetanus toxoid vaccines in old individuals (see above). In vivo, clinical studies using various TH preparations, including THF, TFX, and TS, have suggested that they can be of benefit to patients with viral infections (i. e., *Herpes zoster*, *Herpes simplex*, adenovirus, hepatitis, and cytomegalovirus), since these TH were able to shorten the course of viral infections and accelerate the restoration of T-cell immunity in such patients [81]. Unfortunately, these studies did not include randomized clinical trials and therefore they can not be interpreted as conclusive evidence for the efficacy of TH in viral infections. However, more recently two randomized clinical trials involving control groups of patients have been reported. In one, TS treatment successfully decreased the number of recurrences of *Herpes simplex labialis* infection and improved several immunologic parameters in immunosuppressed patients with recurrent *Herpes simplex labialis* infection as compared to a placebo group [3]. In the second study, TS was found to improve the score for respiratory infections and distribution of T-cell subsets in children with recurrent respiratory infections as compared to a control group of untreated patients [19].

Thus, it appears that TH may be of therapeutic relevance in preventing or attenuating infectious diseases in immunocompromised hosts, although additional, well controlled clinicals trials and the development of additional animals models are required.

Immunodeficiencies

The observations that TH are able to stimulate immune responses in immunocompromised animal models, provided the rationale for the initiation of clinical trials with thymic preparations in patients with primary and secondary immunodeficiencies.

Primary Immunodeficiencies

Primary immunodeficiencies include a number of syndromes related to congenital defects of the immune system. They may involve B, T or both lymphocyte populations. The in vitro effects of TH on PBL from these patients have been documented for TF5, THF, thymopoietin, TP5, and thymulin [81, 83, 103]. The most frequently observed change was an in vitro increase, although not a complete normalization, in the percentage and numbers of E-rosette-forming cells after incubation with TH. In addition, TF5 was found to enhance the MLR responses of lymphoid cells from a subgroup of primary immunodeficiency patients [103].

Several of the TH preparations, including TF5, TS, TP5, thymulin, THF, and TFX, have already entered clinical trials [2, 7, 18, 81, 83, 88, 103]. The administration of these preparations to children with primary immunodeficiencies is based on the effects observed in animals and on patient's PBL in vitro and is an

attempt to replace the activity of the endocrine thymus. One of the problems involved in analyzing these trials has been the low incidence of these diseases which precludes randomized trials with placebo groups. However, immunoreconstitution has been achieved in a significant number of cases reported. The most consistent results were observed in children with DiGeorge syndrome [83].

Improvements in the immune cell function of these patients have been reported with TF5 [81, 103], TP1 and TP5 [2], and thymulin [7]. Additionally, improvements have been observed in patients with ataxia-telangiectasia syndrome (A-T) treated with thymulin [7] and in patients with Wiskott-Aldrich syndrome treated with TF5 [81] and occasionally in some combined immunodeficiency disease (CID) patients treated with TF5 [103], and A-T syndrome patients treated with THF [46]. From the reported studies it appears that the patients most likely to benefit from the TH therapy are those with the mildest immune defects. In contrast to the above mentioned trials, no beneficial effects have been observed in severe CID or most patients with chronic mucocutaneous candidiasis [103]. It has been suggested that the lack of response in SCID patients is based on the fact that the defect appears to be at the stem cell level, and therefore these patients lack a population of TH-responsive pre-T cells.

In summary, clinical trials on the effects of TH in primary immunodeficient patients have suggested that TH are effective in reconstituting cellular immunity and improving the clinical status in these patients. Further trials involving an increased number of patients are warranted to conclusively establish the therapeutic value of TH in these syndromes.

Secondary Immunodeficiencies

Secondary immunodeficiencies are usually associated with a number of clinical situations like severe burns, viral infections, chronic renal failure, autoimmune diseases, cancer, etc. TH appear to be effective in improving the immune status in animals affected by several of these diseases. In vitro it has been demonstrated that TH are able to improve the percentage of E-rosette forming cells from patients with severe burns, viral infections, uremia, tuberculosis, etc [83]. Furthermore, it has recently been reported that thymosin $\beta 4$ was able to increase in vitro to almost normal levels the percentage of helper T cells (OKT4+ cells) and all peripheral T cells (OKT3+ cells) without changing the percentage of suppressor T cells (OKT8+ cells) in patients with chronic renal failure [1]. It is known that OKT3+ and OKT4+ cells, but not OKT8+ lymphocytes, are decreased in these patients. No effect was observed in normal individuals [1].

Although some clinical trials are reportedly under way, like the case of patients with severe burns [89], most of them have involved immunosuppressed patients with infectious diseases. We will focus now on the acquired immunodeficiency syndrome (AIDS). This syndrome, which appears to be caused by a retrovirus termed HTLV III, is characterized by an increased number of opportunistic infections, a progressive crippling of the thymus-dependent immune system, the presence of Kaposi sarcoma and other tumors in about 40% of the cases, and is usually fatal within 2 years [23, 29]. It affects predominantly young homosexual males, but has also been observed in increased numbers in other high-risk groups,

including hemophiliacs and i. v. drug users [23, 29]. It now appears that AIDS has been present in central Africa for about 10 years, and may have originated from that area [80]. A number of immunologic abnormalities have been described [23, 29, 50], including an inversion of the T4/T8 ratio due to a deficiency in helper T cells, abnormally elevated levels of thymosin α 1 (in both adult and pediatric AIDS patients), decreased response to mitogens and antigens, decreased MLR, decreased IL2 production, etc [9, 23, 29, 40, 50, 68]. We [68] and others [44] have attemped to immunologically reconstitute these patients with TH. A pilot clinical trial to evaluate the in vivo and in vitro potential of TF5 to immunorestore homosexuals and hemophiliacs at high risk of contracting AIDS, who already exibited a depressed T4/T8 ratio, has just been completed [68, R. S. Schulof, M. B. Sztein, A. L. Goldstein et al. submitted for publication]. Results indicate that TF5 is able to increase MLR and mitogen-induced IL2 production in vitro (G.L. Simon, R.S. Schulof, M. B. Sztein, A. L. Goldstein et al. submitted for publication). Additionally, treatment of these patients for 10 weeks have also lead to an improvement in allogeneic MLR and mitogen-induced IL2 production by PBL obtained from these patients at several time points. Positive immunomodulation was restricted to a group receiving 60 mg TF5/m^2: 30 and 120 mg TF5/m^2 were not effective. No responses were observed in other immunologic parameters, including NK, T4/T8 ratios, absolute T cell numbers, etc [83, R. S. Schulof, G. L. Simon, M. B. Sztein, A. L. Goldstein et al. submitted for publication]. It is interesting to note that, as was the case for the treatment of primary immunodeficiencies, the patients that appear to benefit the most are those with the least compromised immune system. A clinical trial administering thymosin α 1 to a similar group of patients at high risk of developing AIDS has just been started to confirm and extend the preliminary findings using TF5 (R. S. Schulof, M. B. Sztein, A. L. Goldstein et al. (in preparation)).

Autoimmune Diseases

It is currently accepted that the emergence of autoimmunity is associated with an immunoregulatory T-cell imbalance, probably due to a decrease in T-lymphocyte suppressor cell activity, which leads to an uncontrolled antibody production by B-lymphocytes and T-cell dyscrasias. Several animal models have been established as correlates of human autoimmune disorders. One that has been used extensively as a model of human systemic lupus erythematosus (SLE) is the NZB mouse. Evidence obtained in the last few years supports the hypothesis that an abnormality of the endocrine thymus may be involved in the development of autoimmunity in NZB mice [81]. A decrease in FTS-like activity in the sera of these mice precedes the onset of the disease and grafting of a newborn thymus to an adult NZB mice can correct some of the defects associated with the disease (i. e., autoimmune hemolytic anemia) [81]. Additionally, a number of the immune abnormalities associated with this disease can be partially corrected by the administration of several thymic preparations, including TF5, FTS, THF, TP5, and thymopoietin. For example, TF5 after short-term administration has been demonstrated to restore the capacity of lymph node lymphocytes to respond to PHA and Con A and increase MLR

responses [32] and induce some reduction in the production of antinucleic acid antibodies [94]. Long-term administration of TF5 does not appear to have any effect on the overall survival rate or immune parameters [33]. More recently it has been shown that FTS is able to decrease the high NK activity observed in the NZB mice [8], apparently through the activation of cells that suppress NK activity. The treatment of young female B/W ([NZB × NZW] F_1) mice with FTS retards the appearance of Sjögren syndrome (which is usually associated with the autoimmune disease in NZB mice) but appears to lead to an enhancement of anti-DNA autoantibody production and glomerulonephritis [7]. Conversely, FTS treatment of aging B/W mice decreased anti-DNA antibody formation and improved glomerulonephritis [7].

Another animal model of autoimmune diseases in which TF5 demonstrated its immunomodulatory capacity is experimental autoimmune thyroiditis (EAT), which is characterized by a lymphoid infiltration of the thyroid gland and the presence of anti-thyroglobulin antibodies. In this system TF5 was able to suppress the EAT development in a strain of guinea pigs which is a high responder to thyroglobulin immunization, while no effect was observed in a low responder strain [96]. In some animal models TH have been shown to exert different effects. For example, it has recently been shown that TF5 has no suppressive effect on the incidence and severity of experimental allergic encephalomyelitis (EAE), an animal autoimmune disease used as a model for multiple sclerosis (MS) [105]. In contrast, thymulin has been shown to reduce EAE development [7].

The studies of the effects of TH on PBL isolated from patients with autoimmune diseases have shown that TF5, THF, thymulin, and thymostimulin were able to increase in vitro one or more immune parameters, such as E-rosette forming cells (E-RFC), autologous-MLR (A-MLR), production of lymphokines such as leukocyte migration inhibition factor (LMIF), graft versus host responses, etc [81].

Particularly important are the observations showing that TF5 [56, 107] and FTS [66] are able to modulate suppressor cell activity in autoimmune disorders, such as systemic lupus erythematosus (SLE) [56], rheumatoid arthritis (RA) [107], chronic active hepatitis (CAH) [66], and autoimmune hemolytic anemia (AHA) [57], all associated with abnormal suppressor cell activity. Additionally, FTS has been found to increase the percentage of autologous rosette-forming cells (Tar cells) in PBL of patients with SLE [73]. Tar cells, considered to be post-thymic T-cell precursors, are OKT3, 4, 8 + Dr- PBL which are able to generate cytotoxic effector cells against allogeneic target cells and TNP-labeled self-target cells provided they are activated by IL2 or FTS [92].

Taken together, these observations indicate that TH exert an homeostatic role in diseases associated with an imbalanced immune system, and have provided a rational basis to use TH therapeutically in autoimmune diseases. Clinical trials are in progress in several autoimmune disorders, including SLE, RA, CHA, AHA, multiple sclerosis (MS), aplastic anemia, and sarcoidosis. However, there are few published reports concerning the therapeutic efficacy of TH in patients with these disorders.

Preliminary evidence indicates that some improvement in SLE and RA was obtained with TF5 [81], although there was no effect on the levels of anti-nuclear antibodies. TFX was found to induce clinical improvements accompanied by a

decrease in rheumatoid factor levels and normalization of the hypergammaglobulinemia in 16 out of 20 RA patients [88]. These studies have also shown an improvement in clinical and laboratory findings in 60–80% of CAH patients, while no promising results were observed in MS patients [88]. Thymulin is another TH which has shown promising preliminary results in the treatment of RA patients, since a clear improvement of the clinical signs, particularly those related to inflammation, has been observed [7]. Additionally, TP5 has also been demonstrated to significantly improve the clinical status of some, but not all, RA patients [100]. A recent report on two children with AHA suggested that TF5 may be of therapeutic value in the treatment of the T-suppressor-cell deficiency associated with this disease [57]. Finally, hematologic improvements have been reported in aplastic anemia patients treated with TS [35]. Although the clinical trials to date are encouraging for a possible therapeutic use of TH in the treatment of autoimmune disorders, such as RA, larger, placebo-controlled randomized studies will be necessary to provide definitive answers.

Cancers

Since it is well established that a nonspecific deterioration of the immune system occurs in tumor-bearing hosts, this appears to be one of the potentially more important areas in which TH immunotherapy may exert a beneficial effect. Several animal tumor models have been established in an effort to mimic the clinical status of cancer patients. Early experiments in mice have clearly established that neonatal thymectomy increased the susceptibility to tumor transplantation and carcinogenesis, and thymic grafts in diffusion chambers could restore normal anti-tumor responses [81]. Later, it was shown that TF5, FTS, and THF were able to accelerate the rejection of syngeneic tumors in several immunosuppressed murine models [81]. Additionally TH have been shown to modulate natural killer cell activity [11, 21, 30]. However, these experiments are difficult to apply to clinical situations in which chemotherapy, radiotherapy, surgery, and other procedures that depress cellular immunity are used. Therefore, recent studies have attempted to employ TH along with concurrent radiotherapy or chemotherapy as the primary anti-tumor treatment similar to that received by patients. For example, it has been demonstrated that in vivo administration of TF5 or thymosin α 1, in conjunction with cyclophosphamide (CY), can prevent reappearance of the MOPC-315 plasmacytoma resulting in increased survival compared to the animals treated with CY alone [106]. In a different study, the combination of TF5 and bischloroethylnitrosourea (BCNU) was found to increase survival of mice with lymphocytic leukemia as compared to mice treated with BCNU alone [13].

Recently, the effect of thymosin α 1 has been examined in mice immunosuppressed by cytostatics or X-ray irradiation [99]. These treatments caused the mice to die within a few days after challenge with P388 or L1210 leukemic cells. It was shown that thymosin α 1 given with cytostatics (5-FU or BCNU) or after irradiation prevented the decrease in resistance to tumors caused by those agents [99]. These studies also demonstrated that thymosin α 1 was able to restore NK activity in the spleens of mice treated with 5-FU or irradiation [99]. In another study, thymosin α

1 was shown to restore 5-FU-induced bone marrow cytotoxicity in mice, as measured by immune reconstitution of colony formation and lymphokine production [71]. Additionally, restoration of T-cell mediated immune responses and induction of specific anti-tumor responses were obtained with TF5 in a fibrosarcoma model in spontaneously hypertensive rats with congenital T-cell depression [93]. In other studies, TF5 was able to consistently stimulate T-cell responses against several tumors in vitro, as measured by enhanced MLR and the development of cytotoxic effector cells in mixed lymphocyte tumor response cell-mediated cytotoxicity assay (MLTC-CMC) [95]. Additionally, these studies also demonstrated that TF5 can act as an immunoadjuvant in vivo for the development of specific effector cells capable of rejecting a tumor challenge, as well as exhibit some therapeutic effects against pre-existing metastatic tumor burden [95].

Immunomodulatory effects of TH have also been observed in vitro in PBL obtained from cancer patients. For example, increased E-RFC formation has been described after incubation of lymphocytes from cancer patients with TF5, THF, TS, and TFX [83]. As described before, positive effects were seen if pre-incubation E-RFC percentages were below normal levels. Additionally, enhanced LMIF production by TF5, increased proliferative responses by TF5 and TS, an increase in the percentage of Tar cells by thymosin α 1, increased proliferative responses to allogeneic tumor cells by TP1, and a decrease in the suppressor cell activity of cells from cancer patients with evidence of abnormally elevated suppressor activity have all been reported [83, 86].

These studies, together with the results obtained in cancer, infectious diseases, and immunodeficiency animal models, demonstrate the ability of TH to modulate the immune system and have provided an extensive rationale for the use of these preparations in cancer patients. Since cancer is usually associated with depressed immune responses, patients usually have a higher incidence of viral, fungal, and other opportunistic infections. It is hoped that TH can be employed as adjuncts to chemotherapy or radiotherapy to ameliorate the immunosuppressive side-effects associated with these treatments.

Several hundred patients have already entered clinical trials, constituting the largest group of patients in which TH have been studied therapeutically. Reviews have recently been published describing in detail the therapeutic effects of TH in cancer patients [39, 81, 83, 88]. We will only briefly describe the results obtained and report the latest clinical trials.

To date, the TH preparations which have been reportedly used in cancer patients include TF5, synthetic thymosin α 1, TS, THF, and TFX. However, extensive clinical trials have only been reported with TF5, thymosin α 1, and TS. Phase I studies have demonstrated that TF5 can be administered without toxicity other than occasional allergic reactions noted at high and repetitive doses. In phase II non-randomized trial improvements in the percentage of E-RFC, proliferative responses to PHA and LMIF activity were observed in patients with Hodgkin disease following treatment with TS [83]. Phase II randomized trials were performed with TF5 on unresectable head and neck cancer patients concurrently with radiotherapy, but no significant clinical responses were observed. Furthermore, efforts to accelerate immunologic reconstitution and reduce chronic graft versus host disease after bone marrow grafting in 10 patients with leukemia

and four patients with aplastic anemia by TF5 administration have so far proven unsuccessful. Although modulation of some immunologic parameters were observed, TF5 did not alter the incidence of chronic graft versus host disease or leukemic relapse in these patients [104]. In another phase II randomized trial it was demonstrated that TS was able to increase the circulating T-cell population and DTH responses in a group of advanced metastatic gastrointestinal cancer patients who received TS plus chemotherapy, as compared to the group receiving chemotherapy alone [83].

More recently, the first randomized double blind phase II trial using a synthetic TH in the treatment of lung cancer has been reported with encouraging results [84]. This study involved the administration of thymosin α 1 to patients with localized, unresectable, non-small cell lung cancer, starting 1 week after the completion of radiotherapy to the primary tumor and mediastinum. Two different schedules of administration were used: loading [daily × 14 + twice a week (BIW) maintainance] or BIW. Serial immune monitoring revealed that only the patients who received thymosin α 1 by the loading dose schedule had a normalization of the immune function in MLR [84]. It is important to mention that although this study was not designed as a therapeutic trial, the follow-up of the patients until relapse or death showed that patients treated with either schedule exibited a significant improvement in relapse-free and overall survival compared to those treated with placebo [84] (Fig. 2). Several additional confirmatory trials of thymosin α 1 in lung cancer sponsored by the National Cancer Institute are currently under way.

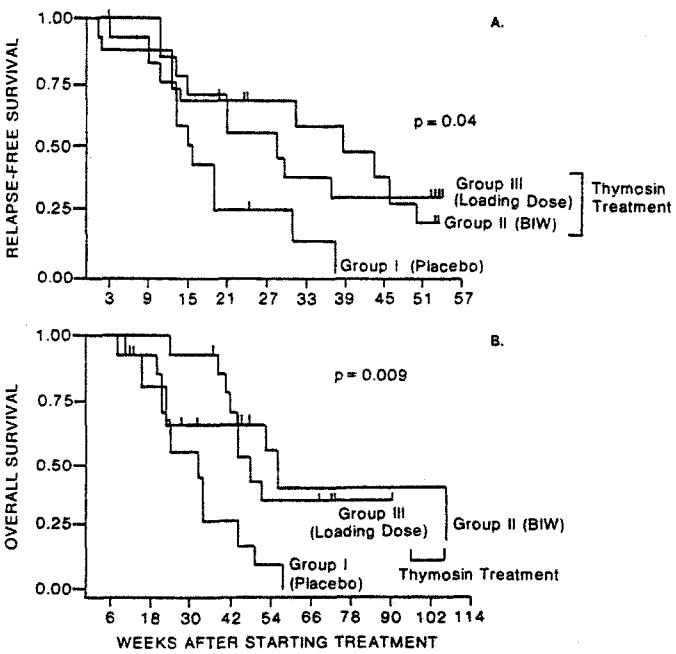

Fig. 2. Phase II randomized trial of thymosin α 1 in patients with non-oat lung cancer following radiotherapy. A Relapse-free survival, B overall survival (from Schulof et al. [84])

In phase III trials, TF5 has been shown to significantly prolong survival in patients with small cell bronchogenic carcinoma as an adjunct to intensive remission-induction chemotherapy [14]. In this study, the increased survival was restricted to patients who previously exhibited depressed T-cell function that had a good response to chemotherapy. However, these results have not been confirmed in trials in advanced oat and non-oat cell lung cancer patients, using different chemotherapy and radiotherapy protocols [39, 83]. Other phase II studies with TF5 in head and neck cancer and malignant melanoma have shown only minimal or borderline results, particularly in patients who are immunoincompetent [83].

TS trials in patients with excised stage I melanoma and low circulating T cells have shown that patients receiving TS exhibited a significant improvement in the metastasis-free interval as compared to patients who received chemotherapy (DTIC) or surgery alone [6, 10]. Confirmatory trials of these results are currently under way. In contrast, TS appear to have no effect on non-small cell lung cancer patients treated with chemotherapy [83].

Although the effects of TH as adjuvants to chemotherapy and/or radiotherapy in selected cancer patients are encouraging, a number of major problems exist with the interpretation and planning of clinical trials in cancer patients. One is based on the heterogeneity of patients which even in the same staging exhibit differences in their immune status. Thus, the planning of a trial should attempt to select groups as homogeneous with regard to their immune status as possible. In addition, the dose and schedule of administration appear to be critical. Finally, it is anticipated that definitive answers regarding the efficacy of TH will only be provided by large-scale, multicenter phase III trials, similar to the studies initiated with thymosin α 1 in non-small cell lung cancer and with TS in malignant melanoma.

Future Perspectives

In spite of the rapid progress that is being made in the field of thymic hormones, the current understanding of thymic physiology is far from complete. Although several thymic peptides have been isolated, sequenced, and synthesized, no specific biologic assays to measure them have yet been developed. It is anticipated that the development of such assays will greatly contribute to a better understanding of the role of TH as they relate not only to the development and maturation of the T-cell dependent immune system, but also to other components of the immune system as well as the autonomic, neuroendocrine, and endocrine systems. Another very intriguing area which deserves increased attention is the study of the role of the thymus and its endocrine products in the pathogenesis of disease.

Through the knowledge gained in the last few years, it is clear that TH may be of therapeutic relevance in aging and in a number of disease states, including infectious and autoimmune diseases, primary and secondary immunodeficiencies, and cancer. However, the doses, schedules, and routes of administration employed, the eligibility criteria for patients to be admitted into clinical trials, the inclusion of placebo control groups that are derived through double-blind randomization, etc is far from optimal. Future trials should address these questions very carefully in order to attempt to achieve optimum immunomodulatory effects. Finally, the develop-

ment of additional and better animal models to be used to explore the efficacy of the administration of TH alone or in combination with other standard therapies (radiation, chemotherapy, surgery) and products, such as lymphokines, where synergistic immunomodulatory influences are possible, may prove of importance in the design of future clinical trials.

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