An Immunological Approach to Aging[a]

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During senescence, the immune system undergoes profound alterations associated with progressive decline of immune responsiveness to exogenous antigens and increased incidence of autoimmune phenomena.[1] This immunologic profile is a feature of aging and reflects alterations in cell populations of the immune system.[2] Several lines of evidence suggest that the occurrence of major changes in regulatory T cell populations in aging is the result of thymic involution[3] and subsequent reduced concentration of thymic hormones involved in T cell maturation.[4]

Several extracts that promote T cell maturation have been prepared from the thymus gland.[5-9] Chemical characterization of these extracts has led to the identification of the active peptides (thymic hormones), some of which have been sequenced, synthesized, and shown to differ in amino acid sequence and biological properties.[10]

Our previous studies have demonstrated that T cell functions of immunodeficient aged mice can be improved by the administration of synthetic thymosin α_1, a 28 amino acid peptide identified in a bovine thymus extract (fraction V). T helper (Th) cell activity of spleen cell populations from old mice was found to be increased by thymosin α_1[11-13] as follows. Helper activity of spleen cells was induced *in vivo* by carrier priming and titrated *in vitro* 4 days later. Three days before carrier priming, (C57BL/10 × DBA/2)F_1, hereafter called BDF_1, mice were left uninjected or were injected intraperitoneally (ip) with 10 μg thymosin α_1, 5 μg N_{14} (N-terminal amino acid residues 1–14), or 5 μg C_{14} (C-terminal amino acid residues 15–28) synthetic fragments of the thymosin α_1 molecule (all peptides were provided by A. L. Goldstein). Mice of different ages (3, 6, 12, 18, and 24 months) were assayed in separate experiments, and the results indicated that Th cell activity is impaired by aging but is restored to a large extent by a single injection of 10 μg thymosin α_1. The injection of an equimolar amount of the N_{14} fragment is at least as effective as the entire thymosin α_1 molecule in restoring Th cell activity in 6- to 24-month-old mice, whereas the injection of the C_{14} fragment has no effect. It is noteworthy that both thymosin α_1 and its N_{14} fragment are devoid of activity when injected in 3-month-old mice, suggesting that these molecules are effective only in T cell deficient mice.

The injection of thymosin α_1 appears to stimulate also a population of nylon-wool-adherent, Lyt 2.2^+, T suppressor (Ts) cells that counteract Th cell activity.[12] Thus, thymosin α_1 injection in old mice enhances Th and Ts cell activities but, at the doses used, enhancement of helper activity is the prevailing effect.

The effect of thymosin peptide injection on IL-2 production by spleen cells from aging mice was also investigated, as previously described.[13] Spleen cells from

[a] This work was supported by a contract from ENEA-EURATOM and in part by Fondazione Pasteur-Cenci Bolognetti.

aging BDF_1 mice, uninjected or injected ip with 10 μg thymosin α_1, 5 μg N_{14}, or 5 μg C_{14} fragment 3 days before sacrifice, were stimulated in culture with ConA. After 48 hr, culture supernatants were collected and titrated on CTLL cells. It was found that IL-2 production is reduced to 46% and 15% at 6 and 12 months of age, respectively, but is fully recovered by injection of thymosin α_1 or the N_{14} fragment. No recovery was induced by injection of the C_{14} fragment. The thymosin peptides were all ineffective when injected in 3-month-old mice.

Thymosin α_1 and the N_{14} fragment, unlike the C_{14} fragment, enhance the expression of IL-2 receptors (IL-2R) in ConA-activated spleen cells from old mice.[13] These studies, performed by radioimmune assay with anti-IL-2R monoclonal antibody (7D4), were extended by use of a binding assay with radioiodinated IL-2.[14] Serial dilutions of radioiodinated IL-2 were incubated with a constant number of ConA-activated spleen cells from BDF_1 mice. Nonspecific binding was assessed by adding anti-IL-2R monoclonal antibody (PC 61). High affinity and total (low and high) affinity IL-2R binding were determined using 0.002 pM or 2 pM ligand concentration, respectively. It was found that a single ip injection of thymosin α_1 or its N_{14} fragment in 12-month-old mice 3 days before sacrifice induces an increase in number of both low and high affinity IL-2R on mitogen-activated spleen cells. The C_{14} fragment appears devoid of sizable effects.

Results from limiting dilution analysis of T cell precursors in BDF_1 mice[15] indicate that ip injection of thymosin α_1 or its N_{14} fragment increases the frequency of ConA-responsive T lymphocytes in old, but not in young, mice whereas injection of the C_{14} fragment is devoid of any effect in both young and old mice. Thus, active thymosin peptides amplify the pool of the T cell precursors in immunodeficient old mice.

Recently, we have performed a long-term experiment in which BDF_1 male mice were chronically treated with thymosin α_1 and its fragments, and examined for possible effects on life span and tumor incidence. Starting at the age of 3 months, 120 mice/group were left untreated or were weekly injected ip with saline, 1 μg thymosin α_1, 0.5 μg N_{14}, or 0.5 μg C_{14} for 52 consecutive weeks. Eighty mice/group were inspected daily until spontaneous death, and survivors weighed every 3 months until the age of 24 months. Mice were autopsied within 24 h from death, and organs and tissues fixed and stained for histologic examination. From the remaining 40 mice/group at the age of 3 months, 4 mice were sacrificed every 3 months until the age of 18 months, and their spleen cells were pooled, stimulated with ConA, and tested for IL-2 production, as previously described.[13] From the curves in FIGURE 1, it appears that the survival was not affected by treatment with thymosin α_1 or its fragments, the mean survival time being about 700 days in all groups. Also the body weight was unaffected by the treatment (FIGURE 2). Tumor incidence at death was not statistically different in mice injected with thymosin α_1 as compared to untreated controls (TABLE 1). The major contribution of tumors in soft tissues to the total number of solid tumors could have resulted from the trauma induced by the repeated ip injections. Indeed, macroscopic examination of the abdominal region of all mice in each of the other groups revealed the presence of solid tumors at the injection sites as follows: 3 (saline), 3 (N_{14}), 4 (C_{14}). Taken together, these results clearly indicate that repeated injections of thymosin peptides had no effects, either beneficial or detrimental, on life span, body weight, and tumor incidence. Similar results have previously been obtained[16] in mice chronically injected for life with 10 μg thymosin fraction V, thus ruling out toxic effects of this treatment.

Whether the repeated injections of thymosin α_1, N_{14}, or C_{14} fragments were effective on the immune system was monitored every 3 months by assessment of

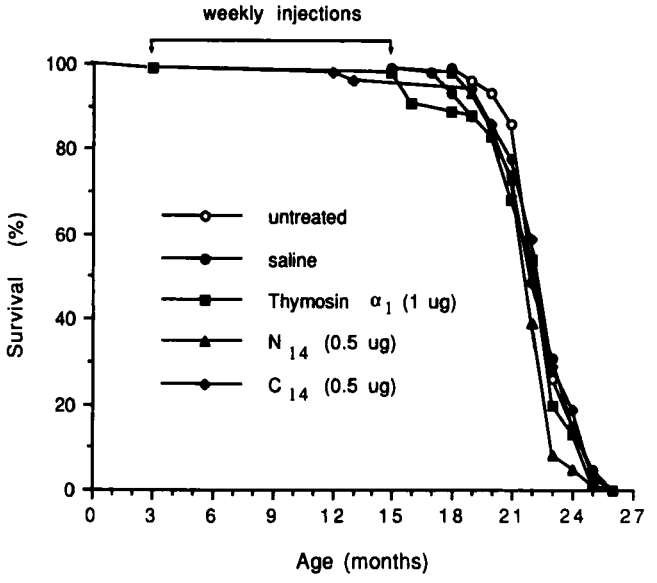

FIGURE 1. Life span of mice injected with thymosin peptides for 52 consecutive weeks.

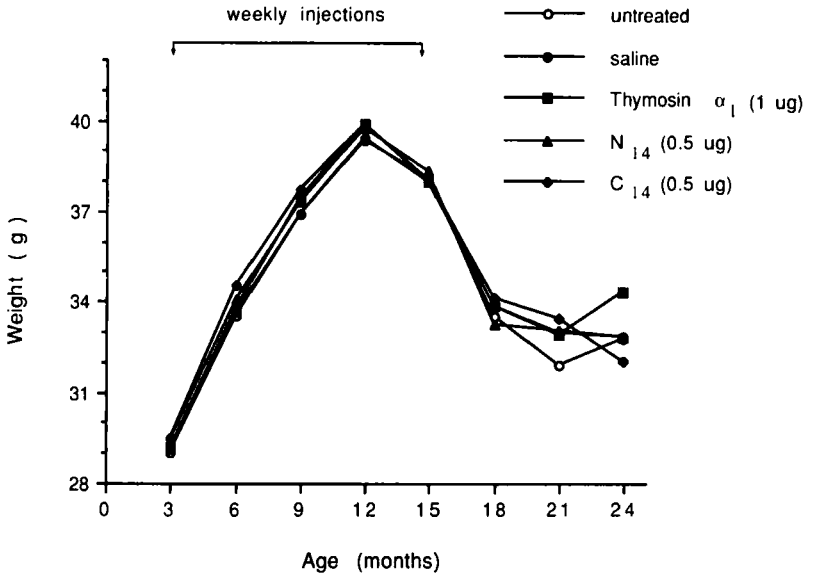

FIGURE 2. Body weight of mice injected with thymosin peptides for 52 consecutive weeks.

TABLE 1. Tumor Incidence at Death[a]

Treatment	Number of Mice Autopsied	Malignant Lymphomas	Solid Tumors				
			Lung	Liver	Soft Tissues	Kidney	Total
Untreated	76	12	3	—	—	—	3
Thymosin α_1	76	14	1	2	5	—	8
Effect of treatment							
χ^2		0.19					2.45
p		0.67					0.12

[a] Mice were injected weekly with 1 μg thymosin α_1 for 52 consecutive weeks from 3 to 15 months of age.

IL-2 production by mitogen-activated spleen cells. Results in FIGURE 3 show that IL-2 production was increased by thymosin α_1 or N_{14}, but not C_{14}, fragment at 3 and 6 months after the beginning of the treatment and declined as in controls during the subsequent 6 months. It is conceivable that excessive stimulation of IL-2-producing T cells by prolonged administration of thymosin peptides may have exhausted the mature T cell pool. This interpretation is supported by *in vivo* and *in vitro* studies suggesting that thymic hormones stimulate postthymic T cells.[17]

In conclusion, the results of our studies demonstrate that thymosin α_1 is a potent modulator of T cell function in aging mice, since it is able to recover Th cell activity up to the level displayed by spleen cells from young mice. This effect

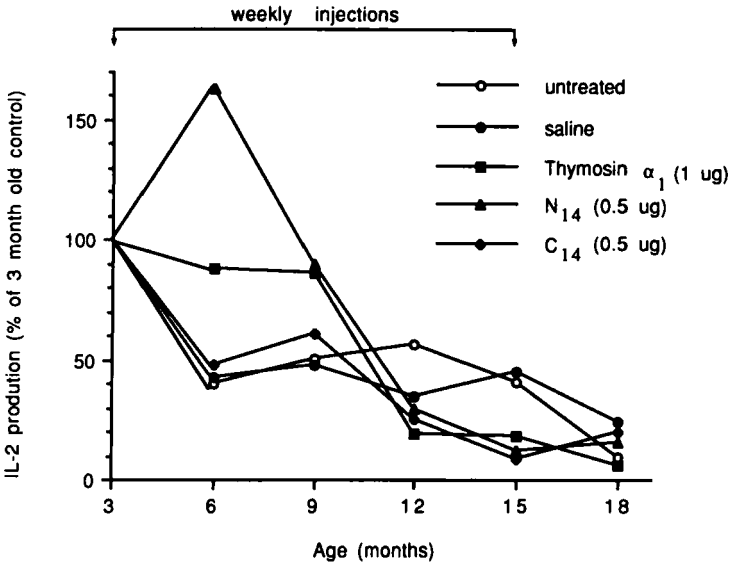

FIGURE 3. IL-2 production by spleen cells from mice injected with thymosin peptides for 52 consecutive weeks.

of thymosin α_1 is restricted to the N-terminal half of the molecule and may depend not only on the increase in the precursor cell frequency of mitogen-responsive T cells but also on the increased number of IL-2R and on the enhanced production of IL-2 that, in turn, favors the expression of IL-2R. Chronic injections of thymosin peptides for 12 months enhance T cell functions during the first 6 months of treatment but, although devoid of demonstrable toxicity, do not prolong the life span or reduce the incidence of lymphomas and solid tumors at death.

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