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Protective Activity of Thymosin Against Opportunistic Infections in Animal Models

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Summary. *Animal models for opportunistic infections were developed by using mice immunosuppressed by 5-FU. These mice were susceptible to various microorganisms, while normal mice had greater tolerance to such microbial infections. In these models, thymosin α_1 was found to protect mice against lethal infections with Candida albicans, Listeria monocytogenes, Pseudomonas aeruginosa, and Serratia marcescens when it was administered during 5-FU treatment prior to the infections. Thymosin α_1 was effective in some infections at 0.4–400 μg/kg/day IP, about $^1/_{100}$ of the dose required for thymosin fraction 5. Activity was also demonstrated against L. monocytogenes and Ps. aeruginosa by counting the viable bacteria in the liver after infection. The protective activity against Candida, elimination of which macrophages were essential, was abrogated by anti-thymocyte serum and/or carrageenan, indicating that thymosin α_1 serves to maintain the functions of macrophages by reducing the damage to T cells by 5-FU. On the other hand, the activity against Pseudomonas infection was not affected by anti-thymocyte serum or carrageenan. It is probable that thymosin α_1 also exerts its effect on neutrophils without participation of T cells and macrophages.*

Introduction

The thymus gland has been shown to play an important role in the development, growth, and function of lymphoid systems through a hormonal mechanisms. One of the thymic hormones, thymosin fraction 5 [10], stimulates T cell development and corrects some immunodeficiencies resulting from the lack of thymus functions [14]. Considering the importance of T cells in immunoregulatory systems, thymosin may be expected to be useful as a pharmaceutical agent for immunodeficiency diseases that are caused by or accompany the aberration of these systems. In fact, thymosin fraction 5, a partially purified material with some 40 peptides, has been shown to be effective in some primary immunodeficiency diseases [19].

It is well known that patients in the secondary immunodeficient state, either due to disease itself or as the consequence of therapies they have received, are highly susceptible to microbial infections, with serious consequences. In cancer patients, chemotherapy and radiotherapy generally decrease immunocompetence. Such defects make the patients readily susceptible to infection with common pathogens (opportunistic infection) and probably also contribute to the growth of metastatic tumors. Specifically, infections are the major cause of mortality and morbidity in cancer patients [2, 11, 16]. Therefore, it is of interest to study whether fractions 5 and its component peptide α_1 [6] are useful to remedy such defects.

For this reason, we have explored and established animal models for opportunistic infections to assess the activity of fraction 5 and thymosin α_1 given with immunosuppressive cytostatics prior to challenge with opportunistic pathogens. Thymosin fraction 5 and thymosin α_1 were found to protect mice against lethal infections with four different types of microorganisms in such models when the same cytostatic, 5-fluorouracil, was used. Furthermore, we have provided some insight into the mechanisms of action of thymosin α_1 against infection with two different types of pathogens in immunosuppressed mice using anti-thymocyte serum [12] and carrageenan [3], which depress T lymphocytes and macrophages, respectively. Two of the pathogens used were intracellular parasites, *Candida albicans* and *Listeria monocytogenes*, which are eliminated from the infected host mainly by macrophages and T cells [4]. The other is an extracellular parasite, *Pseudomonas aeruginosa*, which is eliminated mainly by neutrophils [17]. These studies suggest that thymosin α_1 might affect not only T cells but also other cell populations, such as neutrophils, whose functions are damaged by 5-FU.

In this report we describe the details of thymosin α_1 activity against opportunistic infections and its probable mode of action. A part of the study has been presented orally elsewhere [18].

Materials and Methods

Animals. Female ddY, ICR, and C57BL/6 mice (6 weeks old) used in these studies were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan.

Microorganisms. Candida albicans ATCC 10231 was purchased from ATCC and *Listeria monocytogenes* EGD was kindly provided by Prof. K. Nomoto, Kyushu University. *Pseudomonas aeruginosa* 5E81-1 (gentamicin-resistant) and *Serratia marcescens* 5A412-1 were clinical isolates. Culture media were nutrient broth (Difco) supplemented with 0.5% glucose for *Candida*, trypticase soy broth (Difco) for *Listeria*, and heart infusion broth (Difco) for *Pseudomonas* and *Serratia*. The inocula were prepared from overnight cultures

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and dilutes in saline. Mice were infected with 0.2 ml of the microorganism in each case, by the IV route and at the doses indicated in Tables 1–8 and Fig. 1.

Anti-Thymocyte Serum (ATS). Anti-thymocyte serum was prepared by the method described by Levey and Medawar [12]. Three rabbits each received 1 ml IV of a suspension of thymocytes (10^9 cells) from C57BL/6 mice, and booster injections of the same doses of thymocytes on two occasions at intervals of 2 weeks. Serum was obtained from the rabbits at 1 week after the last treatment. The sera (50 ml) were treated at 56° C for 30 min, and absorbed at 4° C for 60 min with liver sediments prepared by homogenization of 50 g liver from C57BL/6 mice and then washing 10 times with phosphate-buffered saline. The neutralization titer of ATS thus obtained was determined to be 1 : 320 ~ 1 : 640 by cytotoxicity against thymocytes from C57BL/6 mice with guinea pig complement. Inhibition of the mitogenic response of spleen cells by injection of the ATS was determined by measuring incorporation of ^{3}H-thymidine [7].

Treatment with 5-Fluorouracil and Thymosin. Strain ddY mice were pretreated daily for 8 or 10 days with 0.2 ml each of thymosin α_1 (0.4 ~ 400 μg/kg/day) or thymosin fraction 5 (4 mg/kg/day) and 5-FU (25 mg/kg/day) in saline by the IP route. At 24 h after the last treatment the mice were infected with microorganisms. Thymosin fraction 5 and α_1 peptide were dissolved in saline, divided into one-dose portions, and kept at −20° C. They were used immediately after thawing.

In vivo Growth. Each group of three ddY mice which had been treated daily for 7 days with saline, 5-FU (25 mg/kg/day), or thymosin α_1 (40 μg/kg/day) by the IP route was infected by *L. monocytogenes* or *Ps. aeruginosa* at 24 h after the last treatment. The mice were then sacrificed by excessive bleeding after mild anestesia. The liver was aseptically removed, rinsed in saline, and homogenized. The homogenate, diluted with saline, was spread on heart infusion agar plates. After 24 h incubation, the number of viable bacteria was determined. The data are presented as the mean of the value obtained in three mice.

Compounds. Thymosin fraction 5, prepared from calf thymus, and chemically synthesized α_1 peptide were kindly supplied by Drs A. Ramel and J. Meienhofer, Hoffmann-La Roche Inc., Nutley, NJ, USA, respectively.

Statistical Analyis. Analyses of mortality differences were compared by the Mann-Whitney *U*-test (Rank sum test). Differences were considered to be significant when probability (*P*) values < 0.05 were obtained.

Results

Suppression by Treatment with 5-FU of Host Defense System Against Microbial Infection

Some microbes (bacteria, fungi, and viruses) that are not pathogenic in healthy individuals with normal immunocompetence often cause infections in immunosuppressed individuals, with serious consequences (opportunistic infections). Similar phenomena were demonstrated in mice immunosuppressed by a cytostatic, 5-FU, prior to infection.

When mice were treated daily 8–10 times with 5-FU (25 mg/kg/day) by the IP route, various immune parameters

Table 1. LD_{50} of various microorganisms in normal and 5-FU-treated mice

Microorganism	LD_{50} (No. of microorganisms)[a]	
	Normal mice	5-FU-treated mice
Listeria monocytogenes EGD	2×10^4	5×10^2
Candida albicans ATCC 10231	2×10^6	1×10^5
Pseudomonas aeruginosa 5E81-1	2×10^7	1×10^4
Serratia marcescens 5A412-1	8.6×10^7	4×10^4

[a] ddY mice pretreated daily for 8 days with saline or 5-FU (25 mg/kg/day, IP) were infected with various doses of microorganisms by the IV route. LD_{50} was determined by the method of Reed and Muench [15]

Table 2. Effect of pretreatment with thymosin α_1 on lethal infection with *L. monocytogenes* or *C. albicans* in 5-FU-treated mice

Pretreatment[a]			Infection[b] at day 0	Survival/tested at day			*P* value[c] (*U*-test)
Thymosin (IP)		5-FU (IP)		4	8	15	
Expt 1.							
L. monocytogenes							
Control (saline)		+	−	10/10	10/10	10/10	
		−	+	10/10	10/10	9/10	
		+	+	9/10	0/10	0/10	
Thymosin α_1	4 μg/kg/day	+	+	10/10	7/10	6/10	< 0.001
	40 μg/kg/day	+	+	10/10	6/10	4/10	< 0.001
Expt 2.							
C. albicans							
Control (saline)		+	−	7/7	7/7	7/7	
		−	+	7/7	7/7	7/7	
		+	+	2/7	2/7	1/7	
Thymosin Fr. 5	4 mg/kg/day	+	+	6/7	5/7	4/7	< 0.006
Thymosin α_1	4 μg/kg/day	+	+	5/7	5/7	3/7	< 0.02
	40 μg/kg/day	+	+	7/7	6/7	5/7	< 0.005

[a] Pretreated daily for 8 days (expt 1) or 10 days (expt 2)
[b] Infected with *L. monocytogenes* (1×10^3, IV, expt 1) or *C. albicans* (2×10^5, IV, expt 2) at day 0
[c] Compared with the control mice pretreated with 5-FU and then infected

were decreased. In such mice, the infections with microorganisms used were lethal at doses at least 20 times lower than those in normal mice (Table 1). Decrease in the host defense systems was particularly obvious against *Pseudomonas* and *Serratia* infections. The daily dose of 5-FU used in the experiments, 25 mg/kg IP, was found to be approximately in the optimal range for the treatment of animal tumors in our system, as reported by other investigators [9].

Protective Activity of Thymosin Against Infection with Intracellular Parasites

Since thymosin α_1 is known to affect the population of T cells, the effect of this agent was first examined against *L. monocytogenes* and *C. albicans*, for which T cells are known to participate in the elimination from infected hosts.

As shown in Table 2 (expt 1), mice immunosuppressed by 5-FU were susceptible to *Listeria*, while normal mice had quite high tolerance. On the other hand, when thymosin α_1 was administered with 5-FU prior to the infection, the mice largely survived. The activity of thymosin α_1 was further demonstrated by measuring the number of the viable bacteria in the liver of mice pretreated with 5-FU after infection at a relatively high dose of bacteria (Table 3). The number of bacteria was higher 5 h after infection in mice pretreated with 5-FU than that in normal mice. On the other hand, pretreatment with thymosin α_1 (40 μg/kg/day, IP) together with 5-FU reduced the number to a lower level than that in normal mice. However, when the clearance rate of bacteria from blood was measured during the first 30 min after infection there was no significant difference between these groups (data not shown). The treatment regimen for thymosin α_1 was investigated as shown in Table 4. Long-term treatment with thymosin α_1 seemed to be required for protective activity against *Listeria*, although a few treatments at high doses before the infection also showed a significant activity.

Thymosin was next examined for its activity against *Candida* infection in the immunosuppressed mice. As shown in Table 2 (expt. 2), thymosin fraction 5 and thymosin α_1 protected mice from the lethal infection to a similar extent at doses of 4 mg/kg/day and 40 μg/kg/day, respectively. Although not studied in detail, thymosin α_1 appeared to be about 100 times more active than thymosin fraction 5 on a per weight basis. The activity of thymosin α_1 was also observed in a different strain (ICR) of mice at similar dose ranges (data not shown).

Protective Mechanisms against Candida albicans

Since macrophages and T cells are known to be essential for elimination of *C. albicans* [4], treatment with thymosin α_1 appears to reduce the damage caused to the populations and/or functions of such cells by 5-FU. In an attempt to examine the above possibility, the effect of thymosin α_1 was studied in C57BL/6 mice treated with ATS. As shown in Table 5 (expt. 1), treatment with ATS during the thymosin treatment abolished the protective activity against *Candida* infection. When normal mice (C57BL/6) were infected once IV with 0.2 ml anti-thymocyte serum, the mitogenic response measured by ^{3}H-thymidine incorporation of spleen cells to Con A (3 μg/ml) was reduced to 4.5%, while that to LPS (60 μg/ml) was reduced to 54.4% compared with the response of spleen cells from control mice. These data suggest that T cells participate in the protective activity of thymosin α_1.

Treatment with carrageenan 1 day before the infection increased susceptibility in all groups, including normal mice, and consequently the protective activity of thymosin α_1 was abrogated (Table 5, expt. 2). The presence of functional macrophages is quite important in preventing the *Candida* infection. On the other hand, when carrageenan was given prior to the treatment with thymosin α_1 and/or 5-FU, the protective activity of thymosin α_1 was retained (Table 5, expt. 3). Thymosin α_1 may serve to maintain the function of macrophages, which is damaged by carrageenan and 5-FU, probably through reducing the damage to the T cells by 5-FU.

Table 3. Effect of thymosin α_1 on the growth of *L. monocytogenes* in 5-FU treated mice

Treatment[a]	No. of bacteria in liver[b]
Saline (control)	$1.7 \pm 0.1 \times 10^6$
5-FU	$> 5 \times 10^7$
5-FU + thymosin α_1	
4 μg/kg/day	$4.8 \pm 0.7 \times 10^5$
40 μg/kg/day	$4.7 \pm 0.4 \times 10^5$

[a] Daily treatment (days $-7 \sim -1$) prior to infection with *L. monocytogenes* (5×10^6, IV)

[b] The number of the viable bacteria in liver at 5 h after the infection

Table 4. Effect of treatment schedule for thymosin α_1 on lethal infection by *Listeria* in mice treated with 5-FU[a]

Treatment with thymosin α_1 (μg/kg/day) (schedule)	Treatment with 5-FU (day $-8 \sim -1$)	Survival/tested at day 15	*P* value (*U*-test)
Saline	−	10/10	
Saline	+	2/10	
4×1 (day -1)	+	2/10	
40×1	+	3/10	
400×1	+	6/10	< 0.02
4×3 (day $-3 \sim -1$)	+	4/10	
40×3	+	6/10	< 0.02
400×3	+	7/10	< 0.02
4×5 (day $-5 \sim -1$)	+	6/10	< 0.02
40×5	+	9/10	< 0.005
40×8 (day $-8 \sim -1$)	+	9/10	< 0.005

[a] Mice treated with 5-FU and thymosin α_1 at day indicated were infected with *L. monocytogenes* (1×10^3, IV) at day 0

Table 5. Effect of anti-thymocyte serum or carrageenan on the protective activity of thymosin α_1 against *C. albicans* in 5-FU-treated mice

Pretreatment[a]			Infection[d] with *Candida*	Survival/tested at day			*P* value (*U*-test)[e]
				4	8	15	
Expt 1							
Saline			+	7/7	7/7	7/7	
Saline	5-FU		−	7/7	7/7	7/7	
Saline	5-FU		+	2/7	1/7	1/7	
Saline	5-FU	ATS[b]	+	4/7	2/7	1/7	
Thymosin α_1			+	6/7	6/7	6/7	< 0.001
Thymosin α_1	5-FU	ATS	+	4/7	3/7	3/7	NS
Expt 2							
Saline			+	8/8	8/8	8/8	
Saline	5-FU		−	8/8	8/8	8/8	
Saline	5-FU		+	6/7	3/7	2/7	
Saline		Ca[c]	+	3/8	1/8	1/8	
Saline	5-FU	Ca	+	3/7	1/7	1/7	
Thymosin α_1	5-FU		+	8/8	8/8	8/8	< 0.006
Thymosin α_1	5-FU	Ca	+	3/8	3/8	3/8	NS
Expt 3							
Saline			+	6/6	6/6	6/6	
Saline	5-FU		−	7/7	7/7	7/7	
Saline	5-FU		+	6/7	4/7	2/7	
Saline		Ca[c]	+	7/7	5/7	3/7	
Saline	5-FU	Ca	+	4/6	2/6	1/6	
Thymosin α_1	5-FU		+	6/6	6/6	6/6	< 0.006
Thymosin α_1	5-FU	Ca	+	6/6	4/6	4/6	< 0.005

[a] C57BL/6 mice (expt 1) or ddY mice (expts 2 and 3) were pretreated with 5-FU (25 mg/kg) or thymosin α_1 (40 μg/kg) daily for 10 days from days −10 to −1

[b] ATS (0.2 ml, IV) was injected at days −11, −8, −5, −3, and −1

[c] Carrageenan (Ca, 200 mg/kg, IP) was injected at day −1 (expt 2) or −11 (expt 3)

[d] Infected with *C. albicans* (expt 1, 2.5×10^5; expts 2 and 3, 4×10^5) by the IP route at day 0

[e] Compared with the control mice pretreated with 5-FU, or 5-FU plus Ca or ATS, and then infected
NS, not significant

Table 6. Effect of pretreatment with thymosin α_1 on lethal infection with *Ps. aeruginosa* or *S. marcescens* in 5-FU treated mice

Pretreated (day −10 ~ −1)[a]			Infection at day 0[b]	Survival/tested at day			*P* value (*U*-test)[c]
Thymosin α_1		5 FU		4	8	15	
Expt 1.							
Ps. aeruginosa							
Control (saline)		−	+	10/10	10/10	10/10	
		+	−	10/10	10/10	10/10	
		+	+	0/10	0/10	0/10	
Thymosin α_1	4 μg/kg/day	+	+	4/10	4/10	4/10	< 0.0001
	40 μg/kg/day	+	+	8/10	8/10	8/10	< 0.0001
Expt 2.							
S. marcescens							
Control (saline)		−	+	10/10	10/10	10/10	
		+	−	10/10	10/10	10/10	
		+	+	1/10	1/10	1/10	
Thymosin α_1	0.4 μg/kg/day	+	+	6/10	5/10	4/10	< 0.085
	4 μg/kg/day	+	+	6/10	6/10	6/10	< 0.016
	40 μg/kg/day	+	+	7/10	7/10	7/10	< 0.013

[a] Pretreated daily for 10 days by the IP route

[b] Infected with *Ps. aeruginosa* (5×10^4, IV) or *S. marcescens* (5×10^5, IV) at day 0

[c] Compared with the control mice pretreated with 5-FU, and then infected

Table 7. Effect of ATS or carrageenan on the protective activity of thymosin α_1 against *Ps. aeruginosa*

Pretreatment[a]			Infection[b] with *Pseudomonas*	Survival/tested at day			*P* value (*U*-test)[c]
				4	8	15	
Expt 1							
Saline	–		+	8/8	8/8	8/8	
Saline	5-FU		–	8/8	8/8	8/8	
Saline	5-FU		+	1/8	1/8	1/8	
Saline	5-FU	ATS	+	3/8	1/8	1/8	
Thymosin α_1	5-FU		+	7/8	7/8	7/8	< 0.025
Thymosin α_1	5-FU	ATS	+	8/8	8/8	8/8	< 0.0001
Expt 2							
Saline	–		+	7/7	7/7	7/7	
Saline	5-FU		–	7/7	7/7	7/7	
Saline		Ca	+	6/7	5/7	5/7	
Saline	5-FU		+	2/7	0/7	0/7	
Saline	5-FU	Ca	+	0/8	0/8	0/8	
Thymosin α_1	5-FU		+	5/7	5/7	5/7	< 0.001
Thymosin α_1	5-FU	Ca	+	5/7	5/7	5/7	< 0.0001

[a] C57BL/6 mice (expt 1) or ddY mice (expt 2) were pretreated with 5-FU or thymosin α_1 (40 μg/kg/day) daily for 10 days from days −10 to −1. ATS (0.2 ml, IV) was injected at days −11, −8, −5, −3, and −1. Carrageenan (Ca, 200 mg/kg, IP) was injected at day −1
[b] Infected with *Ps. aeruginosa* (5×10^4 for expt 1, or 2×10^4 for expt 2) by the IV route at day 0
[c] Compared with control mice pretreated with 5-FU, or 5-FU plus ATS or Ca; then infected

Protective Activity Against Infections with Extracellular Parasites

In an attempt to evaluate whether the activity of thymosin α_1 could be useful in other types of opportunistic infections, thymosin α_1 was examined for its activity against infections with extracellular parasites such as *Ps. aeruginosa* and *S. marcescens*. For elimination of these extracellular parasites from infected hosts, neutrophils are known to be essential [17]. As shown in Table 6, thymosin α_1 protected mice from lethal infection with these bacteria, as observed against *Listeria* and *Candida* infections. Furthermore, the activity of thymosin α_1 against *Pseudomonas* was also demonstrated when the number of viable bacteria in liver was compared (Fig. 1). In mice pretreated with 5-FU the viable cells increased, while in mice pretreated with thymosin α_1 and 5-FU it decreased, as observed in normal mice.

Protective Mechanism Against Ps. aeruginosa

The mode of action of thymosin α_1 against *Ps. aeruginosa* infection in the immunosuppressed mice was also investigated in a similar manner to that used in the case of *Candida* infection. While the treatment with ATS or carrageenan abrogated the thymosin activity and increased susceptibility to *Candida* infection, such treatments did not affect the thymosin activity against lethal infection with *Ps. aeruginosa* in the immunosuppressed mice (Table 7). Treatment with thymosin α_1 may exert a direct effect on neutrophils or their precursors, and maintain or recover their immunological function.

Effect of Thymosin α_1 on Infection in Normal Mice

Treatment with thymosin α_1 prevented the bacterial growth to some extent, as seen when the growth of *Listeria* in liver was measured after the infection at a relatively low dose of the bacteria (Table 8). Both control and treated groups survived with such dosages. However, for lethal infection of normal

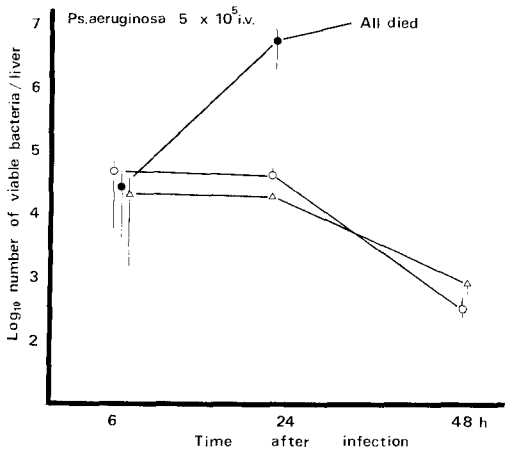

Fig. 1. Effect of thymosin α_1 on the growth of *Ps. aeruginosa* in 5-FU treated mice. ddY Mice were treated daily for 7 days with saline, 5-FU, or 5-FU and thymosin α_1 (40 μg/kg) prior to infection with *Ps. aeruginosa* (5×10^5, IV) (○) saline; (●) saline, 5-FU; (△) thymosin α_1, 5-FU

Table 8. Effect of thymosin α_1 on growth of *L. monocytogenes* in normal mice

Treatment[a]	No. of bacteria/liver		
	Days after infection[b]		
	1	2	3
Control (saline)	2.2×10^6	1.3×10^6	7.7×10^4
Thymosin α_1 4 μg/kg/day	1.1×10^5	1.0×10^6	1.9×10^5
40 μg/kg/day	7.3×10^3	4.7×10^4	2.5×10^3

[a] Daily treatment with saline or thymosin α_1 from days −7 to −1
[b] Infected with *L. monocytogenes* (2×10^3, IV) at day 0

mice with much higher doses of *Candida* and *Listeria*, thymosin α_1 showed essentially no activity (data not shown).

Discussion

The present study deals with the effect of thymosin α_1 against opportunistic infections in mice made immunodeficient by administration of a cytostatic, 5-FU. In these models, co-administration of thymosin α_1 with 5-FU was found to protect mice from lethal infection with all opportunistic pathogens tested so far, i.e., *C. albicans, L. monocytogenes, Ps. aeruginosa,* and *S. marcescens*. The activity of thymosin α_1 was reproducible and the minimum dose showing the activity was quite low (about 4–40 μg/kg/day), which was about $1/100$ of the dose required for fraction 5. Both this dose range and the difference between thymosin α_1 and fraction 5 were similar to those observed by Ohta (unpublished work), who assessed the thymosin activity by measuring the restoration of DTH response in mice immunosuppressed by 5-FU. Some activity was also observed in normal mice, but the activity was much clearer in immunosuppressed mice.

Since thymosin α_1 has been reported to affect mainly T cells, it is reasonable that thymosin α_1 could protect mice from the lethal infection with *Candida* and *Listeria,* since T cells participate either directly or indirectly in their elimination from infected hosts [4]. The participation of T cells in the thymosin activity against *Candida* was proved by the fact that the activity was abrogated by treatment with ATS. In addition, treatment with carrageenan increases the susceptibility of normal mice to *Candida,* and consequently abrogated the thymosin activity, indicating that macrophages are crucially important for elimination of the fungi. On the other hand, when carrageenan was given prior to the treatment with thymosin α_1 the activity remained. Thymosin α_1 may serve to maintain the function of macrophages, which is damaged by carrageenan and/or 5-FU, through an effect on T cells. Since it is known that macrophages are activated by sensitized T cells or their products, lymphokines, and that thymosin α_1 affects the differentiation of T cells [1, 5] and enhances the production of lymphokines such as MIF [6] and interferon [8], this is likely.

Thymosin α_1 also showed activity against different types of bacteria, *Pseudomonas* and *Serratia.* For elimination of such bacteria, neutrophils are known to be essential [17]. In contrast to infection with *Candida,* the treatment with ATS or carrageenan could not abrogate the activity of thymosin α_1 against *Pseudomonas* infection. These results indicate that thymosin α_1 might exert some effect on neutrophils or their progenitor cells, either directly or indirectly. This possibility was supported by Ohta and Yagi, who showed that treatment with thymosin α_1 restored the granulocyte-macrophage colony-forming capacity of bone marrow cells in 5-FU-treated mice [13]. Further studies are required to establish this interesting possibility.

In another series of experiments, thymosin α_1 was shown also to be effective in preventing the rapid death otherwise caused by the inoculation of leukemic cells to immunosuppressed mice (Y. Umeda et al. 1982, unpublished work). Both these results indicate the usefulness of this agent in cancer as an adjuvant to conventional therapies.

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