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Alteration of Cell-Mediated Immunity to *Listeria monocytogenes* in Protein-Malnourished Mice Treated with Thymosin Fraction V†

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Cell-mediated immune reactivity, measured by lymphocyte responsiveness to phytohemagglutinin, was higher in both young or aged mice fed a 4% casein diet compared with age-matched controls. Treatment in vivo with bovine thymosin fraction V decreased the responsiveness to phytohemagglutinin of lymphocytes from mice fed either the control or moderately protein-deficient diets when compared with mice treated in vivo with saline. Resistance against *Listeria monocytogenes*, known to be a cell-mediated immune function, was impaired in young and aged mice which were fed the low-protein diet. Treatment with thymosin was able to significantly improve the cell-mediated immune resistance to *L. monocytogenes* of moderately protein-malnourished mice. Thymosin treatment impaired the resistance to *L. monocytogenes* of young or aged mice fed the control diet. The splenic natural killer cell cytotoxicity of protein-malnourished mice was impaired compared with that of mice fed the control diet. Treatment with thymosin did not restore the natural killer cell cytotoxic activity in protein-malnourished mice, but did enhance that activity in control mice.

Malnutrition in humans can adversely affect many aspects of thymus-derived (T) cell responses (1, 4, 26, 32). An increased incidence of infection with pathogenic (26) or even normal flora microorganisms (9) has been observed in protein-malnourished children. A similar decrease in resistance to a wide variety of pathogens has been shown in malnourished experimental animals (6, 15, 24). However, the mechanisms of altered disease resistance are often unclear, as moderate protein malnutrition in experimental animals has been shown to both enhance (2, 5, 24, 33) and decrease (18, 25, 31) immune responses.

Since the development of the cell-mediated immune (CMI) system is dependent upon the production of thymic hormones (8), it is not surprising that the retarded development of the thymus gland (34) and decreased thymic hormone activity (4) have been associated with nutritionally related immunological deficiencies in animals. Although certain investigators have shown beneficial effects due to treatment with thymic hormones of immunodeficient humans (8, 33), other investigators have found that the treatment of lymphocytes with thymosin fraction V can result in a suppression of immune responses. The latter result is presumably due to the increased proportion of suppressor lymphocytes (22, 35). Since malnourished animals have been found to be immunologically deficient with respect to resistance to infection, it is of interest if these hosts respond in a favorable manner to treatment with thymosin fraction V. The purpose of this investigation was to test the therapeutic value of thymosin fraction V in well-nourished and protein-malnourished mice. The effects of both moderate protein deficiency or thymosin fraction V treatment or both on the responsiveness of lymphocytes to phytohemagglutinin (PHA), natural killer cell activity (NKCC), and resistance against *Listeria monocytogenes* were examined.

MATERIALS AND METHODS

Animals and diets. Female BALB/c mice, obtained from Harlan Sprague-Dawley (Indianapolis, Ind.), were caged in groups of five. The mice which were 6 weeks (young) or 11 months (aged) of age were fed the experimental diets for 5 or 8 weeks, respectively. The mice were fed one of two experimental diets: (i) a control containing 20% casein, or (ii) a moderately protein-deficient (MPD) diet of 4% casein that was equal in calories to the control diet (U.S. Biochemical Corp., Cleveland, Ohio) (Table 1). While continuing on these diets, the mice were injected intraperitoneally with 0.5 ml of either endotoxin-free 100-μg bovine thymosin fraction V (courtesy of Teresa L. K. Low, George Washington University) or phosphate-buffered saline (PBS) (pH 7.2) every other day for 1 or 2 weeks, yielding a total treatment of 400 or 700 μg of thymosin fraction V for the mice on diets (i) and (ii), respective-

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TABLE 1. AIN semipurified rat-mouse diet[a]

Component	% of total diet for:	
	Control	Low protein
Casein high nitrogen	20.0	4.0
DL-Methionine	0.3	0.3
Corn starch	15.0	15.0
Sucrose	50.0	66.0
Fiber-Celufil	5.0	5.0
Corn oil	5.0	5.0
AIN mineral mix	3.5	3.5
AIN vitamin mix	1.0	1.0
Choline bitartrate	0.2	0.2

[a] U.S. Biochemical Corp. diet no. 10662.

ly. The preparation of thymosin fraction V was previously described (17). Briefly, the bovine thymus tissue was homogenized and centrifuged at 14,000 × *g*. The supernatant was heated to 80°C, precipitated with acetone, then precipitated with ammonium sulfate, and purified on a Sephadex G-25 column.

Resistance to infection. *L. monocytogenes* was initially cultured in Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy broth (TSB) overnight and stored at −70°C in 1-ml samples of 20% glycerol–TSB. Cells from stock suspensions were grown for 16 h in TSB at 37°C before each injection. The cells were harvested by centrifugation at 10,000 × *g* and were washed twice in sterile PBS. The concentration of washed cells was adjusted spectrophotometrically (at 550 nm) to approximately 3×10^8 cells per ml. The cells were then adjusted to the appropriate concentration, 1×10^4 per ml, needed for injection. The exact number of viable bacterial cells was determined on the basis of developing colony-forming units (CFU) on Trypticase soy agar (TSA) after incubation at 37°C for 24 h. Approximately seven mice per group were injected with *L. monocytogenes* at: (i) day 1 after receiving four injections, (ii) day 1 after seven injections, or (iii) day 7 after seven injections of thymosin fraction V or PBS. At day 7 after injection, the livers of mice were extracted and homogenized in PBS, using a Teflon-coated pestle glass homogenizer. Fifty microliters of several dilutions from an individual mouse homogenate was spread onto TSA plates in duplicate to determine the number of CFU due to infection present in mice from each group.

Preparation of lymphoid cell suspensions. The spleens of mice infected 7 days previously or spleens of uninfected mice were dispersed into a cellular suspension in sterile PBS by gently rubbing the organs through a sterile stainless steel screen. Erythrocytes were lysed by washing the suspensions in Tris-buffered 0.15 M ammonium chloride (pH 7.2). Cells were washed once in PBS and once in RPMI 1640 cell culture medium (GIBCO Laboratories, Grand Island, N.Y.) containing 10% heat-inactivated fetal calf serum (culture medium). The viability of lymphocytes was assessed by trypan blue exclusion, and cell concentrations were adjusted to 5×10^6 per ml in the culture medium.

Measurement of lymphocyte mitogenesis. Quadruplicate samples of lymphocytes from each mouse were seeded into wells of Costar Cluster 96 plates at a concentration of 2.5×10^5 cells per ml or, where indicated, at 5.0×10^5 cells per well. Mitogenesis was induced in two of the cultures with 0.25 μg of PHA (Burroughs Wellcome Co., Research Triangle Park, N.C.). The remaining two cultures, after the addition of the medium only, served as controls. The cell suspensions were incubated at 37°C in 5% CO_2–95% air for 72 h. At 24 h before the end of that incubation, 1 μCi of [*methyl*-^{3}H]thymidine (45 Ci/mmol) (Amersham Corp., Arlington Heights, Ill.) was added to each culture. The cell suspensions were harvested onto glass fiber filters and washed with absolute methanol. The radioactivity present on each filter paper was measured with a liquid scintillation spectrometer (Packard Instrument Co., Rockville, Md.).

Cytolytic assay. The NKCC was measured in young mice from each indicated dietary/injection group. Briefly, 2.5×10^4 ^{51}Cr-labeled L1210 mouse leukemia cells were incubated with 5×10^6 spleen cells in 1.5 ml of culture medium at 37°C in 5% CO_2 for 16 h. After incubation, the cell suspensions were centrifuged at 600 × *g* for 5 min, and 1 ml of the supernatant was evaluated as to radioactivity released by measurement in a gamma counter (Beckman Instruments, Inc., Fullerton, Calif.). Results were expessed as the percent lysis of target cells according to the formula, % lysis = (experimental ^{51}Cr release − spontaneous ^{51}Cr release)/(maximum ^{51}Cr release − spontaneous ^{51}Cr release).

Statistical analysis. An analysis of variance was used to determine the significance of differences or interactions among the groups of mice with respect to diet and type of treatment. To facilitate the analysis of the data from the resistance studies, dead mice were given a standard score of 8.0 $\log_{10}$ viable *L. monocytogenes* cells per liver. It was determined that moribund animals had approximately 8.0 $\log_{10}$ viable *L. monocytogenes* cells present per liver. The values reported in Fig. 1 and 2 do not contain data from dead animals; however, the number of dead animals per group was noted. Any values with $P < 0.05$ were considered significant.

RESULTS

There were no significant differences in food consumption between mice fed the 20% (2.75 g per mouse per day) or 4% (3.5 g per mouse per day) casein diet. The young mice fed the 20% casein diet weighed 19.63 ± 0.27 g, whereas the mice fed the 4% casein diet weighed significantly less (13.96 ± 0.76 g) at the end of the 5-week feeding period.

Resistance against *L. monocytogenes*. To determine the effect of moderate protein malnutrition upon CMI resistance, mice fed the control or MPD diet for 3 weeks were infected intraperitoneally with 1×10^4 *L. monocytogenes* cells. The number of viable *L. monocytogenes* cells present in infected mice was determined on days 3 and 7 after infection. The level of infection on day 3 is indicative of the native preimmune macrophage phagocytic and bactericidal capacity of the mouse (7), whereas the level of infection by day 7 is dependent upon CMI enhancement of macrophage phagocytic and bactericidal

TABLE 2. Effect of MPD diet on resistance of young mice to infection with *L. monocytogenes*

Dietary protein level (%)	Log_{10} CFU per liver and spleen at day after infection[a]		
	3[b]	7[c]	7[d]
20	5.29 ± 0.22	3.38 ± 0.47	3.38 ± 0.47
4	5.23 ± 0.19	4.11 ± 0.62	4.59 ± 0.72

[a] Six-week-old BALB/c mice were fed either a 20 or 4% casein diet for 3 weeks, then infected intraperitoneally with 4.0 log_{10} of *L. monocytogenes* cells; the CFU in the liver and spleen were evaluated on days 3 and 7 after infection.

[b] Mean ± standard error on log_{10} CFU per liver and spleen from a minimum of eight mice.

[c] Only surviving mice.

[d] Surviving plus dead mice.

activity (18). The early resistance against *L. monocytogenes* was not significantly affected in young mice fed the low-protein diet (Table 2). By day 7, the CMI clearance of infecting *L. monocytogenes* cells was impaired in mice fed the MPD diet, when CFU from dead mice were included (control, 3.38 ± 0.47 versus malnourished mice, 4.59 ± 0.72).

Effect of thymosin treatment on CMI resistance. To test the hypothesis that the impairment in CMI resistance against *L. monocytogenes* observed in mice fed the MPD diet was at least partially the result of low thymic hormone production, we treated mice from each dietary group of both ages with a series of injections of bovine thymosin fraction V or PBS. One day after a total treatment of 400 or 700 μg of thymosin, mice were infected with 1×10^4 *L. monocytogenes* cells. In the younger mice, the CMI resistance 7 days after infection was significantly affected ($F = 9.79$, $P = 0.003$) by treatment with thymosin (Fig. 1). This effect upon CMI resistance was dependent on the diet which the mice received during the treatment ($F = 12.36$, $P < 0.0001$). Although the CMI resistance of mice fed the MPD diet was not altered by the thymosin treatment, the CMI resistance of mice fed the control diet was significantly suppressed by the thymosin treatment.

A similar pattern for the effects of thymosin treatment upon CMI resistance was observed in aged mice (Fig. 2). Again, the effect that thymosin treatment had upon the CMI resistance was significantly dependent upon the diet which the mice were fed ($F = 17.31$, $P < 0.001$). The resistance against *L. monocytogenes* of aged mice fed the MPD diet was enhanced by the thymosin treatment, being most dramatic at 7 days after treatment. The resistance of mice fed the control diet was impaired at all times after thymosin treatment. It should be noted that, for an unknown reason, there seemed to be a decrease in resistance after the first treatment point, irrespective of whether the animals were treated with thymosin or PBS or were young or aged. As a result, there was an overall significant effect due to the duration of treatment in the young ($F = 31.24$, $P \leq 0.0001$) and aged ($F = 9.83$, $P \leq 0.0001$) mice that was not dependent upon diet or type of infection.

The responsiveness of splenic lymphocytes to the PHA of mice after day 7 of infection was significantly affected by diet in both young ($F = 39.06$, $P \leq 0.001$) and aged ($F = 22.86$, $P \leq 0.001$) mice. Spleen cells from mice fed the MPD diet had significantly higher responsiveness to PHA than did spleen cells from control mice (Fig. 3 and 4). In the younger mice (Fig. 3), thymosin treatment significantly suppressed splenic lymphocyte responsiveness to PHA ($F = 12.83$, $P \leq 0.001$) compared with that of diet-matched mice treated with PBS. Unlike the young mice, the effect of thymosin treatment upon spleen cell responsiveness to the PHA

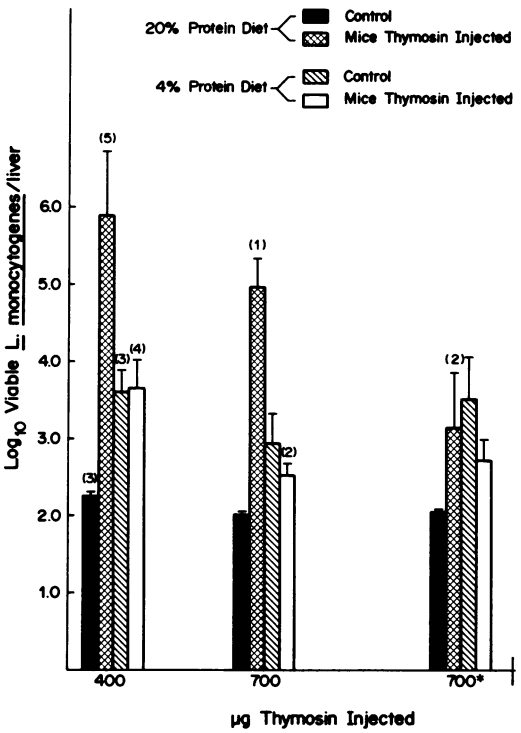

FIG. 1. CFU (standard error indicated by bars) in livers at 7 days after infection of seven mice intraperitoneally with 1×10^4 *L. monocytogenes* cells. The numbers in parentheses indicate dead mice by day 7 after infection. Young (6-week-old) mice fed either the control or MPD diet were treated with a series of injections with PBS or a total of 400 or 700 μg of thymosin. Mice were infected 2 days after treatment with 400 or 700 μg of thymosin or 1 week after treatment with 700 μg of thymosin (*).

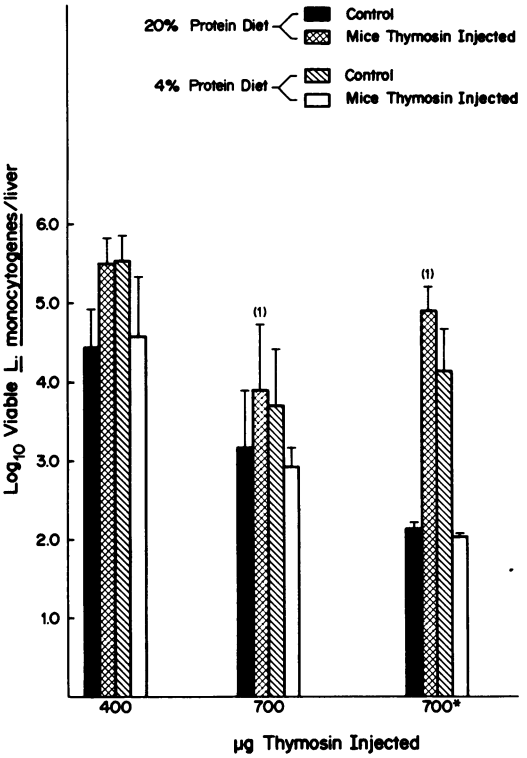

FIG. 2. CFU (standard error indicated by bars) in livers at 7 days after infection of seven mice intraperitoneally with 1×10^4 *L. monocytogenes* cells. Numbers in parentheses indicate dead mice by day 7 after infection. Old (11-month) mice fed either the control or MPD diet were treated with a series of injections with PBS or a total of 400 or 700 μg of thymosin. Mice were infected 2 days after treatment with 400 or 700 μg of thymosin or 1 week after treatment with 700 μg of thymosin (*).

from the older mice was not significant, irrespective of the level of dietary protein.

Splenic lymphocyte responsiveness to PHA. A separate group of 6-week-old mice was fed either the control or MPD diet for 5 weeks, to determine whether a thymosin-induced suppression of spleen lymphocyte responsiveness to PHA was present in uninfected mice. Mice were then treated every other day for 2 weeks with a total of 700 μg of thymosin or PBS. The spleen cell numbers and responsiveness to PHA were evaluated 1 day after the last thymosin treatment. The number of splenic lymphocytes was reduced significantly by the low dietary protein levels (Table 3). Treatment with thymosin did not significantly enhance the number of lymphocytes in mice fed the MPD diet. However, thymosin treatment significantly increased the lymphocyte number in mice fed the control diet. The responsiveness of lymphocytes to PHA was significantly enhanced ($F = 9.53$, $P = 0.004$) in mice fed the MPD diet. As in the previous experiment, the thymosin treatment significantly lowered the lymphocyte responsiveness to PHA, especially at the higher concentration ($F = 4.66$, $P = 0.038$). Again, the suppressive effect of the thymosin treatment upon splenic lymphocyte responsiveness to PHA was not dependent ($F = 0.161$, $P = 0.691$) upon the level of dietary protein fed to the mice. The thymosin treatment only affected the T lymphocytes; no significant effect in the mitogenic responsiveness of lymphocytes to lipopolysaccharide was seen (Table 4) (4,693 ± 572 and 3,908 ± 429 cpm in mice fed a 20% casein diet in PBS or thymosin-treated mice, respectively; 2,979 ± 542 and 3,641 ± 653 cpm in mice fed a 4% casein diet in PBS or thymosin-treated mice, respectively).

NKCC. The θ antigen-bearing lymphocytes,

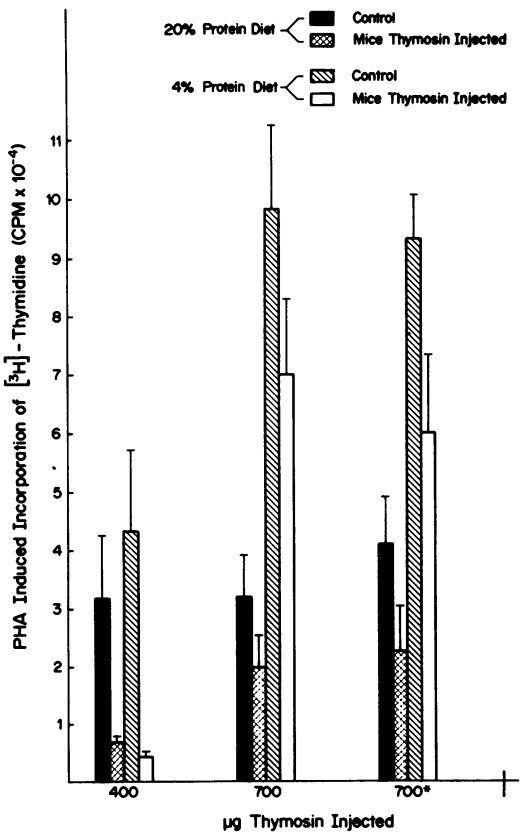

FIG. 3. Counts per minute (standard error indicated by bars) of [^{3}H]thymidine in 2.5×10^5 PHA-stimulated spleen cells from mice infected intraperitoneally 7 days earlier with 1×10^4 *L. monocytogenes* cells. Young (6-week-old) mice fed either the control (20% protein) or MPD (4% protein) diet were treated with a series of injections with PBS or a total of 400 or 700 μg of thymosin. Mice were infected 1 day after treatment with 400 or 700 μg of thymosin or 1 week after treatment with 700 μg of thymosin (*).

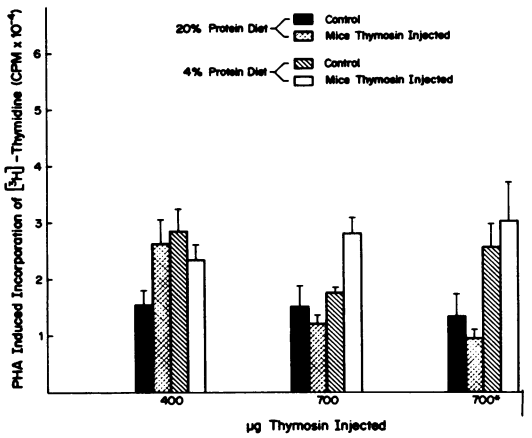

FIG. 4. Counts per minute (standard error indicated by bars) of [^{3}H]thymidine in 2.5 × 10^5 PHA-stimulated spleen cells from mice infected intraperitoneally 7 days earlier with 1 × 10^4 *L. monocytogenes* cells. Old (11-month) mice fed either the control (20% protein) or MPD (4% protein) diet were treated with a series of injections with PBS or a total of 400 or 700 μg of thymosin. Mice were infected 1 day after treatment with 400 or 700 μg of thymosin or 1 week after treatment with 700 μg of thymosin (*).

which are responsive to PHA (12), have functions within the CMI system distinct from the θ-antigen-negative lymphocytes responsible for NKCC against tumor cells (10). The effects of thymosin treatment upon the spleen cell population responsible for NKCC activity was determined in young mice fed either the control or MPD diet injected every other day for 2 weeks with a total of 700 μg of thymosin or PBS. The splenic NKCC activity against L1210 mouse leukemia cells was measured 1 to 7 days after the last thymosin or PBS injection (Table 5). Unlike spleen cell responsiveness to PHA, NKCC activity was significantly reduced in mice fed the MPD diet ($F = 19.42$, $P < 0.001$). No significant effect upon NKCC activity was observed as a result of thymosin treatment in mice fed the MPD diet. However, NKCC activity did increase 7 days after the last thymosin treatment in mice fed the control diet.

DISCUSSION

Animal model studies of the effects of malnutrition upon immunity and resistance to infection have yielded results that sometimes differ from clinical observations in humans (32). The results presented in this report further demonstrate this dichotomy, in that moderate protein malnutrition in mice resulted in higher responsiveness of spleen cells to PHA, but lower CMI resistance against *L. monocytogenes*. The major acquired resistance mechanism of mice against *L. monocytogenes* is the CMI system (16), whereas the preimmune susceptibility is governed by monocyte phagocytic and bactericidal activity (21). Defective CMI resistance against *L. monocytogenes*, as in mice fed the MPD diet, may be the result of one or more "lesions" in the cascade of events that are part of CMI resistance, which leads to the killing of bacteria. For instance, one such lesion may be in the production of lymphokines by immune T cells, which activate monocytes and macrophages to increase phagocytic and bactericidal activity (18). Even though T cell activity in mice fed the MPD diet was enhanced on a per cell basis, as the concentration of cells for the assay was adjusted to a fixed level, the total lymphocyte activity in the whole host may be less, resulting in lower CMI resistance. Indeed, splenic lymphocyte concentrations were significantly impaired in mice fed the MPD diet. Another such lesion may be the macrophage level, the cell responsible for killing the infecting bacterial cells. However, Cooper et al. (5) have shown that native peritoneal macrophages of moderately protein-malnourished mice actually phagocytize *Listeria* cells better than macrophages from well-fed mice. Watson et al. (33) have also shown that macrophages from protein-malnourished animals are larger and have higher levels of superoxide dismutase. Peritoneal macrophages from protein calorie-malnourished rats had intact bactericidal activity against both gram-positive and gram-negative bacterial cells (13), but an impairment in non-CMI resistance factors, such as chemotactic factors and the serum opsonins of these protein calorie-malnourished animals. These two events would impair the in vivo function of macrophages. Complement, a non-CMI resistance factor, has been shown to play a role in the decreasing susceptibility of mice to *L. monocytogenes* (23). Indeed, certain complement components have been shown to be impaired by protein malnutrition in rats (32).

TABLE 3. Effect of thymosin fraction V (Thy) on splenic lymphocyte number in control or moderately protein-malnourished BALB/c mice[a]

Level of dietary protein (%)	Treatment type	Splenic lymphocytes (10^7)
20	PBS	9.69 ± 0.50[b]
20	Thy	11.23 ± 0.84
4	PBS	5.07 ± 0.81
4	Thy	5.53 ± 0.71

[a] Mice from each dietary group were injected intraperitoneally with 0.5 ml of either 100-μg thymosin fraction V or PBS, on every other day for 2 weeks for a total of 700 μg of thymosin.

[b] Mean ± standard error.

TABLE 4. Effect of thymosin fraction V (Thy) on responsiveness of spleen cells from control or moderately protein-malnourished BALB/c mice to PHA[a]

Level of dietary protein (%)	Treatment type	cpm of [^{3}H]thymidine incorporated[b]			
		2.5×10^5 cells		5.0×10^5 cells	
		With PHA	Without PHA	With PHA	Without PHA
20	PBS	29,228 ± 2,916	1,691 ± 364	84,789 ± 5,705	2,014 ± 313
20	Thy	27,804 ± 4,901	634 ± 148	67,144 ± 8,052	1,775 ± 328
4	PBS	51,255 ± 9,208	816 ± 204	100,601 ± 6,548	2,319 ± 330
4	Thy	38,514 ± 7,947	888 ± 139	85,572 ± 1,160	1,233 ± 120

[a] Mice from each dietary group were injected intraperitoneally with 0.5 ml of either 100-μg thymosin fraction V or PBS, on every other day for 2 weeks, for a total of 700 μg of thymosin. Cells stimulated with lipopolysaccharide had 4,693 ± 572, 3,908 ± 429, 2,979 ± 542, 3,641 ± 653 cpm in PBS-treated 20%, Thy-treated 20%, PBS-treated 4%, Thy-treated 4% mice, respectively.
[b] Mean ± standard error.

Since mice fed the MPD diet were immunosuppressed with respect to splenic lymphocyte numbers and CMI resistance against *L. monocytogenes*, while exhibiting an enhanced responsiveness to PHA, attempts were made in this study to restore that resistance with bovine thymosin fraction V. Thymosin fraction V contains at least 30 different polypeptides and has been used to help restore CMI responses in humans with immunodeficiency diseases (8, 33) and increase resistance to tumor cells in well-fed and malnourished mice (24a). In the present study, treatment with thymosin had a suppressive effect upon lymphocyte responsiveness to PHA in animals fed either the control or MPD diet. We have shown, for the first time, a suppressive effect due to thymosin treatment upon the CMI resistance against *L. monocytogenes* in well-nourished mice. However, thymosin treatment enhanced the CMI resistance against *L. monocytogenes* in mice fed the MPD diet compared with saline-treated mice fed the MPD diet. These results were observed in both young adult and middle-aged mice. It is possible that the thymosin treatment of mice induced an increase in the suppressor lymphocyte population, therefore decreasing the responsiveness of the total lymphocyte population to PHA. Thymosin α_7, one of the polypeptides within fraction V, has been shown to induce suppressor T cells (17). Also, splenic lymphocytes from the thymosin-treated nude mice were able to actively suppress the in vitro generation of cytotoxic T cells (22). Other studies in immunocompetent mice have shown that thymosin treatment has no effect or a slightly suppressive effect upon spleen cell responsiveness to PHA (26). Wolf (35) has indicated that in vitro pretreatment of immunocompetent human lymphocyte populations with thymosin can suppress responsiveness to PHA and pokeweed mitogen. Interestingly, thymosin has been able to successfully reconstitute concanavalin A responsiveness in spleen cells from nude mice. This mitogen is capable of inducing suppressor T cells (28). Previous studies have indicated that the increased responsiveness of lymphocyte to PHA or sheep erythrocytes in protein-malnourished mice is related to a decrease in suppressor lymphocytes (14, 20). The data from the present report have shown that thymosin treatment is able to insignificantly decrease the responsiveness of lymphocytes from malnourished mice to PHA. This would indicate a partial restoration of the suppressor lymphocyte population in these mice as a result of the thymosin treatment.

Other polypeptides from the thymosin preparation have been shown to have other specific

TABLE 5. Effect of thymosin fraction V (Thy) on NKCC against L1210 leukemia cells in control of moderately protein-malnourished BALB/c mice[a]

Level of dietary protein (%)	Treatment type	% Cytotoxicity on day after last treatment:	
		1	7
20	PBS	15.84 ± 1.49[b]	18.46 ± 2.08
20	Thy	15.16 ± 1.19	24.22 ± 1.60
4	PBS	11.40 ± 1.31	13.33 ± 1.71
4	Thy	12.72 ± 2.68	12.15 ± 2.76

[a] Mice from each dietary group were injected intraperitoneally with 0.5 ml of either 100-μg thymosin fraction V or PBS, on every other day for 2 weeks; the lymphocyte/target cell ratio was 200:1.
[b] Mean ± standard error.

functions. Thymosin B_4 induces the expression of terminal deoxynucleotidyl transferase, stimulates the secretion of luteinizing hormone-releasing factor, and is involved in the early stages of T lymphocyte differentiation (17). Thymosin α_1 is involved in the generation of helper T lymphocytes (17), whereas thymosin B_1 to date has no known immunological activity.

The reason that thymosin treatment had contradictory effects upon malnourished mice as opposed to the mice fed the control diet with respect to resistance against *L. monocytogenes* is unclear. Based upon the ability of lymphocytes to be stimulated by PHA from the two populations of mice, it seems likely that the thymosin treatment induced a supranormal level of suppressor T lymphocytes in control mice but failed to restore a normal suppressor T lymphocyte activity in malnourished mice. The CMI resistance against *L. monocytogenes* has been shown to be accompanied by a rapid increase in splenic Thy 1^+ cells from days 5 to 8 after infection (21). In this report the thymosin treatment resulted in decreased spleen lymphocyte responsiveness to PHA in mice 7 days after infection with *L. monocytogenes*, irrespective of the diet which the animals were fed, a response mediated by Thy 1^+ cells.

Whereas thymosin treatment had a suppressive effect upon CMI resistance against bacterial infection, it had an enhancing effect upon NKCC activity in mice fed the control diet. These differences in the effect of thymosin could be caused by separate populations of T cells which are responsible for each response. Spleen cells responsive to PHA have surface θ antigen (12), whereas cells responsible for NKCC have very little, if any, surface θ antigen (10). Preliminary results from our laboratory indicate that mice fed the MPD diet have a significant increase in the percentage of θ plus splenic lymphocytes (61.93 ± 4.63) compared with mice fed the control diet (48.53 ± 9.39), whereas the total number of θ plus splenic lymphocytes remained the same.

In conclusion, in vivo treatment with bovine thymosin fraction V had a suppressive effect upon both spleen cell responsiveness to PHA and resistance to infection in mice fed a diet adequate in protein. Decreased CMI resistance to infection in mice fed a MPD diet was partially restored by thymosin treatment, which suggests that there was low thymic hormone activity in malnourished mice.

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LITERATURE CITED

1. **Beatty, D. W., and E. B. Dowdle.** 1978. The effects of kwashiorker serum on lymphocyte transformation in vitro. Clin Exp. Immunol. **32**:134–143.
2. **Bounous, G., and P. A. L. Kongshavn.** 1978. The effect of dietary amino acids on immune reactivity. Immunology **35**:257–266.
3. **Chandra, R. K.** 1979. Serum thymic hormone activity in protein-energy malnutrition. Clin. Exp. Immunol. **38**:228–230.
4. **Chandra, R. K.** 1980. Cell-mediated immunity in nutritional imbalance. Fed. Proc. **39**:3088–3092.
5. **Cooper, W. C., R. A. Good, and T. Mariani.** 1974. Effects of protein insufficiency on immune responsiveness. Am. J. Clin. Nutr. **27**:647–664.
6. **Dubos, R. J., and R. W. Schaedler.** 1958. Effect of dietary proteins and amino acids on the susceptibility of mice to bacterial infections. J. Exp. Med. **108**:69–81.
7. **Emmerling, P., H. Finger, and H. Hof.** 1977. Cell-mediated resistance to infection with *Listeria monocytogenes* in nuded mice. Infect. Immun. **15**:382–385.
8. **Goldstein, A. L., D. W. Wara, A. J. Amman, H. Sakai, N. S. Harris, G. B. Thurman, J. A. Hooper, G. H. Cohen, A. S. Goldman, J. J. Costanzi, and M. C. McDaniel.** 1975. First clinical trial with thymosin: reconstitution of T cells in patients with cellular immunodeficiency diseases. Transplant. Proc. **7**:681–686.
9. **Gracey, M. O., S. Suharjono, and D. E. Stone.** 1973. Microbial contamination of the gut: another factor of malnutrition. Am. J. Clin. Nutr. **26**:1170–1174.
10. **Herberman, R. B., and H. T. Holden.** 1978. Natural cell mediated immunity. Adv. Cancer Res. **27**:305–377.
11. **Jackson, R. B., and H. T. Holden.** 1978. Natural cell mediated immunity. Adv. Cancer Res. **27**:305–377.
12. **Janossy, G., and M. F. Greaves.** 1972. Lymphocyte activation. II. Discriminating stimulation of lymphocyte subpopulations by phytomitogens and heterologous antilymphocytesera. Clin. Exp. Immunol. **10**:525–536.
13. **Keusch, G. T., S. D. Douglas, G. Hammer, and K. Braden.** 1978. Antibacterial functions of macrophages in experimental protein calorie malnutrition: II. Cellular and humoral factors for chemotaxis, phagocytosis, and intracellular bactericidal activity. J. Infect. Dis. **138**:134–142.
14. **Khorshidi, M., and N. Mohagheghpour.** 1979. Effect of protein deficiency upon suppressor cells. Infect. Immun. **24**:770–773.
15. **Koerner, T. A., H. R. Getz, and E. R. Long.** 1949. Experimental studies on nutrition in tuberculosis. The role of protein in resistance to tuberculosis. Proc. Soc. Exp. Biol. Med. **71**:154–158.
16. **Lane, F. C., and E. R. Unanue.** 1972. Requirement of thymus (T) lymphocytes for resistance to listeriosis. J. Exp. Med. **135**:1104–1112.
17. **Low, T. L. K., G. B. Thurman, C. Chincarim, J. E. McClure, G. D. Marshall, S. Hu, and A. L. Goldstein.** 1979. Current status of thymosin research: evidence for the existence of a family of thymic factors that control T-cell maturation. Ann. N.Y. Acad. Sci. **332**:33–48.
18. **Mackaness, G. B.** 1970. The mechanism of macrophage activation, p. 61–75. *In* S. Mudd (ed.), infectious agents and host reactions. The W. B. Saunders Co., Philadelphia, Pa.
19. **Malave, I., and M. Layrisse.** 1976. Immune response in malnutrition: differential effect of dietary protein restriction on the IgM and IgG response to all θ antigens. Cell Immunol. **21**:337–343.
20. **Malave, I., and M. Pocino.** 1980. Abnormal regulatory control of the antibody response to heterologous erythrocytes in protein-calorie malnourished mice. Clin. Immunol. Immunopathol. **16**:19–20.
21. **Mandel, T. E., and C. Cheers.** 1980. Resistance and susceptibility of mice to bacterial infection: histopathology of listeriosis in resistant and susceptible strains. Infect. Immun. **30**:851–861.

22. **Marshall, G. D., G. B. Thurman, J. L. Rossio, and A. L. Goldstein.** 1981. In vivo generation of suppressor T cells by thymosin in congenitally athymic nude mice. J. Immunol. **126**:741–744.
23. **Petit, J.** 1980. Resistance of listeriosis in mice that are deficient in the fifth component of complement. Infect. Immun. **27**:61–67.
24. **Petro, T. M., and J. K. Bhattacharjee.** 1981. Effect of dietary essential amino acid limitations upon the susceptibility of mice to infection with *Salmonella typhimurium*. Infect. Immun. **32**:251–259.
24a. **Petro, T. M., and R. R. Watson.** 1982. Resistance to L1210 mouse leukemia cells in moderately protein-malnourished BALB/c mice treated in vivo with thymosin fraction V. Cancer Res. **42**:2139–2145.
25. **Purkayastha, S., B. M. L. Kapoor, and M. G. Deo.** 1975. Influence of protein deficiency on homograft rejection and histocompatibility antigens in rats. Ind. J. Med. Res. **63**:1150–1155.
26. **Schopfer, K., and S. D. Douglas.** 1976. In vitro studies of lymphocytes from children with kwashiorkor. Clin. Immunol. Immunopathol. **51**:21–30.
27. **Scrimshaw, N. S., C. E. Taylor, and J. E. Gordon.** 1968. WHO monograph no. 57, p. 3–329. World Health Organization, New York, N.Y.
28. **Shou, L., J. L. Schwarts, and R. A. Good.** 1976. Suppressor cell activity after concanavalin A treatment of lymphocytes from normal donors. J. Exp. Med. **143**:1100–1110.
29. **Thurman, G. B., A. Ahmed, D. M. Strong, M. E. Gershwin, A. D. Steinberg, and A. L. Goldstein.** 1975. Thymosin induced increase in mitogenic responsiveness of lymphocytes of C57B1/6J, NAB/Ward nude mice. Transplant Proc. **7**:299–309.
30. **Wara, D., A. L. Goldstein, N. E. Doyle, and A. J. Ammann.** 1975. Thymosin activity in patients with cellular immunodeficiency. N. Engl. J. Med. **292**:70–74.
31. **Watson, R. R., and K. Haffer.** 1980. Modifications of cell-mediated immune responses by moderate dietary protein stress in immunologically immature and mature BALB/c mice. Mech. Aging Dev. **12**:269–278.
32. **Watson, R. R., and D. N. McMurray.** 1979. The effects of malnutrition on the secretory and cellular immune processes. Crit. Rev. Food Sci. Nutr. **12**:113–159.
33. **Watson, R. R., M. Rister, and R. L. Baehner.** 1976. Superoxide dismutase activity in polymorphonuclear leukocytes and alveolar macrophages of protein malnourished rats and guinea pigs. J. Nutr. **106**:1801–1808.
34. **Weindruch, R. H., and S. C. Suffin.** 1980. Quantitative histological effects on mouse thymus of controlled dietary restriction. J. Gerontol. **35**:525–531.
35. **Wolf, R. E.** 1979. Thymosin-induced suppression of proliferative response of human lymphocytes to mitogens. J. Clin. Invest. **63**:577–683.