INFLUENCE OF THYMIC PREPARATIONS ON THE
RESULT OF EXPERIMENTAL INFECTION WITH TAENIA
CRASSICEPS (ZEDER, 1800) IN ICR MICE

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Abstract. Thymalin (Thymarin) and T-activin — thymic preparations of polypeptidic character —
were used for influencing the parasitic infection in a model system mouse — Taenia crassiceps.
A single subcutaneous application of 100 μg of Thymalin per mouse at the end of infection resulted
in a decrease (by 54.9 %) in the number of cysterci in peritoneal cavity of experimental mice com-
pared with the controls. Administration of Thymalin with T. crassiceps larval homogenate at various
intervals before and after infection resulted in a statistically significant increase of the level of
specific antibodies in the serum of infected mice, this increase, however, did not correlate with the
corresponding protective effect. Immunosuppressant azathioprine, injected subcutaneously from
7th to 3rd day preceding infection at a dose of 100 μg resulted in a significant increase in the number of
T. crassiceps larvae in the peritoneal cavity of experimental mice compared with the controls
(by 45.7 %). T-activin, injected subcutaneously to mice, immunosuppressed by azathioprine, led
to a restoration of resistance of mice to T. crassiceps infection. Subcutaneous application of T-activin
alone had a significant protective effect (decrease in cysterci number by 53.7 % in comparison
with the controls). Correlation of the level of specific antibodies in the serum of infected mice, value
of spleen index and number of T. crassiceps cysterci in peritoneal cavity of mice was not detected.

Insufficient immune response of the host organism to a helmith infection is caused by
various factors. For the parasite's part it is a low immunogenicity of most parasite
antigens, antigenic variability and antigenic mimicry; for the part of the host organism
it is the state of a certain immunodeficiency (Leid et al. 1987). At present, of particu-
lar interest is the possibility of influencing the course of infection with the modula-
tion of immunological status of the host organism (Cox 1978, Klesius 1982). Ex-
perimental infections of immunodeficient laboratory animals ("nude" — athymic mice,
animals immunosuppressed by irradiation, application of cyclostatics, etc.),
showed that in these cases the course of infection is heavier and more fatal than in
normal animals (Jacobson 1982). On the other hand, direct immunosuppressive
effects of helmiths on the host organism have been demonstrated (Good and Miller

Numerous attempts were made to influence the result of experimental infection
using various nonspecific immunomodulators, as BCG vaccine, bacterial extracts,
zymosan, levamisole, etc. (Cox 1978, Klesius 1982, Ruitenberk et al. 1988).
An appropriate model for studying the influence of these preparations on the course
of infection is asexual reproduction of developmental stages of some cestode species
in the peritoneal cavity of laboratory animals — Taenia crassiceps in mice or rats
Mesocotyloides corti in mice, etc. (Bennet et al. 1978, Thompson and Penhale 1978,

Due to the fact that T-cell part of immunity plays a decisive role in the immune
response of the host organism to the presence of helmiths (Civil and Mahmoud
1977, Pollacco et al. 1978, Lasmas et al. 1987), the thymic preparations seem to be
promising immunomodulators for the use in parasitology. These preparations influence
the process of maturation and differentiation of immature precursors of T-lymphocytes
into mature immunocompetent cells (Bach et al. 1975). At present, more than 30
various thymic preparations have been prepared (from extracts representing a complex mixture of proteins and polypeptides to individual chemical-free polypeptides) with different effects on the immune system (Bach et al. 1975, White 1980, Morozov et al. 1982, Arion 1987). Many of them are successfully used for the correction of immunodeficient states of different genesis in clinical practice (Lopukhin 1982, Byrom and Hobbs 1984, Golzand et al. 1986). Moreover, an adjuvant effect of thymic preparations during specific vaccination has been described (Zaruba et al. 1983).

In a preliminary paper, T-activin, thymic preparation, was first used in order to influence the infection in a mouse — Tenuis crassiceps model system (Hefmánek and Prokopíř 1989). In this paper, the mechanism of T-activin effects in a given model system has been verified and effects of another thymic preparation, Thymalin, have been studied.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Female ICR mice (VELA2), 6 to 8 weeks old, weighing 25—30 g, were used in all experiments. Tenuis crassiceps larvae (KBS strain) which have been passaged in the Institute of Parasitology since 1989, originated from a 3-month-passage in peritoneal cavity of ICR mice.

PREPARATION OF T. CRASSICEPS LARVAE HOMOGENATE

T. crassiceps larvae washed out from the peritoneal cavity of ICR mice were washed in PBS several times and homogenised in an Ultraturrax homogenizer for 2 x 2 min in a minimal volume of PBS. The homogenate was lyophilized and dissolved in a sterile PBS (10 μg homogenate in 1 ml) prior to application.

PREPARATION OF T-ACTIVIN

T-activin was prepared from calf thymus using a standard method of the Laboratory of Molecular Immunology, Research Institute of Physical-Chemical Medicine, Moscow (Arion 1982). This method is described in detail in the paper by Hefmánek and Prokopíř (1989).

Thymalin preparation (drug form of the preparation Thymalin) was kindly provided by Dr. Arion, Laboratory of Molecular Immunology, Research Institute of Physical-Chemical Medicine, Moscow. Method of the preparation of Thymalin is described in the literature (Morozov et al. 1977).

APPLICATION OF IMMUNOMODULATORS

Experiment 1: Eighteen ICR mice were injected s.c. with 100 μg of Thymalin in 0.5 ml sterile PBS per mouse at definite time intervals before and after T. crassiceps infection (Table 1). Six control mice were injected with 0.5 ml of sterile PBS. On day 9°, each mouse was injected i.p. with 3 cysticerci of T. crassiceps in 1 ml of PBS (transparent, movable cysticerci of medium size, with buccal). After 6 weeks, the mice were exsanguinated and dissected and the number of developed T. crassiceps cysticerci in their peritoneal cavity was detected.

Experiment 2: Twenty mice of four groups (L, M, N, O — Table 2) were injected i.p. with 10 μg of lyophilized T. crassiceps homogenate in 1 ml of PBS 14 days before infection. Mice of M, N and O groups were injected i.c. with 100 μg Thymalin in 0.5 ml of sterile PBS at definite time intervals before and after infection. Five control mice were injected s.c. with sterile PBS. T. crassiceps infection was performed in the same way as in Experiment 1.

Experiment 3: Thirty-four ICR mice were divided into 4 experimental groups (Table 3). Mice of groups K2 and X were injected s.c. with 100 μg of antrichrine (Aranthin, Medica Helsinki) in 0.5 ml of sterile PBS on days 7, 6, 5, and 4 before infection. Mice of groups K3 and X were injected s.c. with 100 μg of T-activin in 0.5 ml of sterile PBS on days 2 and 1 before infection and on day of infection. Eight control mice of K1 group were injected s.c. with 0.5 ml of sterile PBS on days 7—1 before infection. Mice of all groups were infected with larvae of T. crassiceps in the same way.
### Table 2. The influence of subcutaneous application of Thymalin on the effectiveness of vaccination with the homogenate of *T. cruziiceps* (Experiment 2)

| Exp. group (No. of mice) | Treatment | Parameters of infection | Spleen index | ELISA (Abs. cm)
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<tr>
<td></td>
<td>Homogenate <em>T. cruziiceps</em> (10 mg. intraperitoneally)</td>
<td>Thymalin (100 μg s.c.)</td>
<td>No. of larvae <em>T. cruziiceps</em></td>
<td>Protection (± %)</td>
</tr>
<tr>
<td>K (5)</td>
<td>—</td>
<td>—</td>
<td>49-296 179 ± 113</td>
<td>—</td>
</tr>
<tr>
<td>L (5)</td>
<td>—-14th</td>
<td>—</td>
<td>19-241 160 ± 95</td>
<td>+10.9</td>
</tr>
<tr>
<td>M (5)</td>
<td>—-14th</td>
<td>—14th</td>
<td>3-348 153 ± 107</td>
<td>+14.9</td>
</tr>
<tr>
<td>N (5)</td>
<td>—-14th</td>
<td>0</td>
<td>3-358 271 ± 270</td>
<td>-50.9</td>
</tr>
<tr>
<td>0 (5)</td>
<td>—-14th</td>
<td>+7th</td>
<td>5-856 331 ± 320</td>
<td>-84.5</td>
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1) — days before infection, + days post infection, 0 day of infection
2) N. S. not significant

### Table 3. The influence of subcutaneous application of azathioprine and T-activin on the proliferation of *T. cruziiceps* cysticerci in the peritoneal cavity of ICR mice (Experiment 3)

| Exp. group (No. of mice) | Treatment | Parameters of infection | Spleen index | ELISA (Abs. cm)
<table>
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<tr>
<td></td>
<td>Azathioprine (100 μg)</td>
<td>T-activin (100 μg)</td>
<td>No. of larvae <em>T. cruziiceps</em></td>
<td>Protection (± %)</td>
</tr>
<tr>
<td>K1 (8)</td>
<td>—</td>
<td>—</td>
<td>49-1,104 311 ± 334</td>
<td>—</td>
</tr>
<tr>
<td>K2 (9)</td>
<td>—-7, -6, -5, -4, -3</td>
<td>—</td>
<td>93-1,204 462 ± 441</td>
<td>-48.7</td>
</tr>
<tr>
<td>K3 (8)</td>
<td>—</td>
<td>—</td>
<td>6-204 144 ± 76</td>
<td>53.7</td>
</tr>
<tr>
<td>X (9)</td>
<td>—-7, -6, -5, -4, -3</td>
<td>—</td>
<td>125-422 233 ± 98</td>
<td>25.9</td>
</tr>
</tbody>
</table>

1) — days before infection, + days post infection, 0 day of infection
2) N. S. not significant
as in Experiments 1 and 2. In all the three experiments, the number of cysticerci developed in peritoneal cavity of infected mice was detected and spleen index was calculated. The antibody level in the serum was detected by ELISA. The protective effect of the preparations was calculated after the formula \( R = \frac{K - X}{K} \times 100 \% \), where \( K \) and \( X \) are arithmetical means of the numbers of \( T. \) cresseps larvae developed in the peritoneal cavities of mice of the control and experimental groups. Statistical evaluation of experimental data was made by a non-parametric Student’s \( t \)-test using Apple Ile computer - BISTAT 3 programme.

**ELISA**

The level of specific antibodies in the serum of infected mice was detected by indirect ELISA method, described in detail in the paper by Vančo and Lukaš (1987). \( T. \) cresseps (full homogenate (10 \( \mu \)g of protein in 1 ml) was used as antigen, conjugate SWAM/Px (SEVAC, Praha). The sera were diluted with PBS-Tween-20 in the ratio of 1:90. The amount of specific antibodies in the serum was expressed in units of optical absorbance of the substrate at the wavelength of 492 nm.

**RESULTS**

**Effect of Thymalin**

The results obtained in Experiment 1 (Table 1) show that the proliferation of \( T. \) cresseps cysticeri in peritoneal cavity of experimental mice is affected by subcutaneous application of Thymalin. A single injection of Thymalin at various intervals before and after infection resulted in a different effect. As a matter of fact, the application of the preparation 7 days prior to infection did not stimulate any response (decrease in cysticeri number by 4.8\% in comparison with the controls). Thymalin applied at the day of infection resulted in a significant decrease in the number of \( T. \) cresseps larvae (by 54.9\%, \( P = 0.09 \)). On the other hand, the application of the preparation 7 days after infection resulted in an increase in the number of cysticeri in the peritoneal cavity of experimental mice in comparison with the controls (by 44.3\%). Mean values of the spleen index of individual experimental groups showed a high degree of correlation with the number of cysticeri in the peritoneal cavity of mice (correlation coefficient \( r = +0.975 \)), the differences among individual groups were not statistically significant. The level of specific antibodies in the serum of infected mice detected by an indirect ELISA method was in all experimental groups higher than in the control group. However, the level of antibodies in the serum did not correlate with the intensity of infection (\( r = +0.583 \)).

The possibility of influencing Thymalin with a crude homogenate of \( T. \) cresseps larvae (applied 14 days prior to infection) was verified in Experiment 2 using a single application of Thymalin (Table 2). The applied scheme of vaccination led to a statistically significant increase in the level of specific antibodies in the serum of experimental mice of all groups compared with the controls (\( P < 0.001 \)). The application of Thymalin — groups M, N, O; \( P = 0.001 \), application of homogenate alone). The differences between individual groups were not statistically significant. This increase did not correlate with the corresponding protective effect. The intraperitoneal application of the homogenate alone 14 days prior to infection led to a reduction in the number of cysticeri (by 10.9\%). The subcutaneous application of Thymalin at the same time had an increased protective effect (by 4\%) — statistically the insignificant results, whereas the application of Thymalin (groups N and 0 at the day of infection or 7 days post infection resulted in an inverse effect: the number of cysticeri in the peritoneal cavity of experimental mice was higher than in the controls (by 80.9\% and 84.5\%, respectively). The value of the spleen index was significantly lower in the groups with the largest numbers of cysticeri (groups N and 0; \( P = 0.02 \) and \( P = 0.05 \), respectively), but the correlation between these parameters was not found (\( r = -0.641 \)).

**The influence of azathioprine and T-activin on the result of infection**

A possible mechanism of the effect of thymic preparations during the experimental infection with \( T. \) cresseps in mice was verified in Experiment 3. A daily subcutaneous application of immunosuppressant azathioprine on days 7—3 preceding infection resulted in a marked increase in the number of cysticeri in the peritoneal cavity of experimental mice — by 48.7\% compared with the controls (Table 3), as well as in a significant increase in the level of specific antibodies in the serum (\( P < 0.001 \)). The subcutaneous application of T-activin in the three successive days before infection had a significant protective effect: the number of \( T. \) cresseps cysticeri in the peritoneal cavity of experimental mice of group K3 decreased by 53.7\% in comparison with the controls. However, the level of specific antibodies in the serum was not significantly different.

The application of T-activin to a group of experimental animals immunosuppressed by azathioprine led to a restoration of resistance of mice to \( T. \) cresseps infection. The number of cysticeri in the peritoneal cavity of mice of group K was by 49.5\%, lower (\( P = 0.1 \)) than in group K2, in which azathioprine was applied and even by 25.0\% lower compared with the controls. The values of the spleen index and the level of specific antibodies in the serum of experimental mice did not correlate with the intensity of \( T. \) cresseps infection (correlation coefficients \( r = -0.100 \) and \( r = +0.546 \), respectively).

**DISCUSSION**

Although the latest technologies of vaccine preparation against helminths are used (in vitro cultivation, hybridome technology, methods of genetic engineering), their efficacy does not reach a desirable level (Kudrna and Prokopić 1986, Gamble and Murrell 1987, Lukaš 1987). Of particular interest is the use of immunomodulators in parasitology — partly as non-specific immunopotentiators increasing the resistance of the host organism against parasitic infection and partly as effective and harmless adjuvants during specific immunostimulation — vaccination (Cox 1978, Jolles 1985, Gamble and Murrell 1987).

In this paper, thymic preparation Thymalin has been used in a model system mouse — \( T. \) cresseps in both above mentioned ways. A single subcutaneous application of Thymalin at the day of infection with \( T. \) cresseps larvae resulted in a significant decrease (by 54.9\%) in the number of cysticeri in the peritoneal cavity of experimental mice. The application of the preparation 7 days prior to infection did not induce any response. By contrast, the application 7 days after infection resulted in an increase in the number of cysticeri (by 44.3\%). These results are significantly different from the effect of another thymic preparation — T-activin, which, applied at the same dose (7 days post infection) caused a maximum decrease in the number of cysticeri (by 93.6\%). T-activin had no increasing effects at any intervals (Hofmánek and Prokopić 1986). These facts confirm the importance of the time factor when the immunomodulators are used during experimental helminthiasis — for each preparation it is necessary to find an optimum scheme of application (Thompson and Penhale 1978, Toye and Jenkin 1982, Lukaš 1988).

In Experiment 2, Thymalin was used to increase the effect of vaccination with \( T. \) cresseps homogenate. Our investigation was based on the papers describing a significant adjuvant effect of Levanosol (Lukaš 1988) and of T-activin (Hofmánek).
role in the usage of thymic preparations (Sundal and Bolla 1987, Heftmänek and Prokopíř 1989).

Acknowledgements. The authors thank Mrs. V. Totoženková and H. Šimonová for their excellent technical assistance.

ВЛИЯНИЕ ПРЕПАРАТОВ ТИМУСА НА РЕЗУЛТАТ ЗАРАЖЕНИЯ МЫШЬ ИСКУССТВЕННЫМИ КУРИТУРМИ T. C. CASSIDERS (ZEDER, 1939)

П. Германик и Н. Прокошина

Решение. В работе исследовано влияние тимусовых препаратов на инъекционную тягу T. C. C. Cassiders. Препараты были введены подкожно в дозе 100 мг в течение 30 дней. Результаты инъекционной тяги были оценены по сравнению с контролем. Однако, в отличие от других исследований, тимусовые препараты не оказали влияния на инъекционную тягу трутовиков. Заключение: тимусовые препараты не оказывают влияния на инъекционную тягу трутовиков, что может быть связано с индивидуальными отличиями у различных животных.

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