

Experimental toxoplasmosis in hypiodemic mice

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ABSTRACT: The hypothesis, that hypiodemia of goats causes such a compromise of the immune system, which during subsequent *Toxoplasma gondii* infections results in clinically more pronounced signs of toxoplasmosis, was verifying in laboratory mouse. Hypiodemic mice (fed by wheat and supplied by water), normiodemic mice (fed by wheat and supplied by water containing 1.25 mg KI/l) and the majority of standard mice (fed by commercial grain mixture containing 0.83 mg I/kg) were experimentally infected with *T. gondii* oocysts or tachyzoites. The susceptibility to acute *T. gondii* infection was evaluated according to mortality rate. As a criterion of cell-mediated immune function has been chosen the spleen-lymphocyte transformation test (LTT). We observed no difference in LTT between hypiodemic and normiodemic mice infected with *T. gondii* oocysts or tachyzoites and no difference in mortality of both infected groups. Four days after the exposure to 100 tachyzoites of *T. gondii* (K24 strain), all experimentally infected groups of mice showed statistically significant decrease ($P = 0.004$) in spleen cells responsiveness to stimulation by all mitogens used – as compared to non-infected standard mice group. Reduced responsiveness of cells was probably caused by *T. gondii* infection itself – the relation to iodine deficiency has not been found.

Keywords: iodine; cell-mediated immune function; *Toxoplasma gondii*; laboratory mice

Major part of the area of the Czech Republic proves to be iodine deficient. This fact results in endemic incidence of animals' iodine deficiency. Iodine deficiencies were described recently in cattle (Kursa *et al.*, 1997) in the Czech Republic. Clinically manifested toxoplasmosis of goats was documented in the Czech Republic and was confirmed by the findings of aborted foetuses, rapid seroconversion of antibodies to *Toxoplasma gondii* within the flock and, typically, pathological and anatomical findings in aborted foetuses (Šlosárková *et al.*, 1999). The pathogenic effect of *T. gondii* has been proved and the animals coming from the flock were identified as hypiodemic. Since other goat flocks in the Czech Republic showed 20% antibody prevalence to *T. gondii* without any clinical manifestations (Literák *et al.*, 1995), we assumed that in case of goat flock suffering from clinical toxoplasmosis the development of clinical toxoplasmosis was influenced by the iodine deficiency in pregnant goats (Šlosárková *et al.*, 1999). This assumption is supported also by the results of a previous study according to which iodine deficiency in

people leads to the increase of antibody prevalence to *T. gondii* within particular population (Singh *et al.*, 1994). Except these two studies (Singh *et al.*, 1994; Šlosárková *et al.*, 1999), no other literature about the relation between iodine supplementation and clinical signs of toxoplasmosis was available. We know, that iodine deficiency influences immune system negatively (Jones *et al.*, 1986; Aumont *et al.*, 1989) and that host's immune response has crucial impact on progress of toxoplasmosis (Denkers and Gazzinelli, 1998).

The aim of this work is to verify our hypothesis, that hypiodemia of goats causes such a compromise of the immune system, which during subsequent *T. gondii* infections leads to clinically more pronounced signs of toxoplasmosis than in animals with sufficient iodine reserves. The laboratory mouse was used as a model species. A spleen-lymphocyte transformation test (LTT) has been selected as a criterion of the host immune system responsiveness. This is a standard method for evaluating cell-mediated immune function, that is a predominant mechanism in the host resistance

to *T. gondii* infection (Chan *et al.*, 1986; Denkers and Gazzinelli, 1998).

MATERIAL AND METHODS

Animals

Outbred laboratory mice of CD1 stock (Anlab Charles River, Prague, Czech Republic) were divided into three groups according to served food.

Group 1. Mice fed by a commercial complete food mixture and tap water. Food and water was given *ad libitum*. Complete feed mixture was saturated with iodine (0.83 mg I/kg) and thus it covered physiological requirement of iodine. There was no iodine present in drinking water. This group was therefore marked **standard**.

Group 2. Mice fed only by wheat grains. These mice became hypiodemic after one month. Animals were considered hypiodemic because the iodine values in urine were 10-times lower than in normiodemic mice. Serving of food caused not only deficiency of iodine, but probably also in the deficiency of many other elements, that were not monitored. Mice of this group were marked **hypiodemic**.

Group 3. Mice fed with the same food as hypiodemic mice, but served water was supplemented with potassium iodide in concentration of 1.25 mg KI/l water (i.e. 1 mg iodine/l water). We created this group in order to be able to evaluate the impact of hypiodemia separately. These mice were marked **normiodemic**. These animals represented control group.

For the production of *T. gondii* oocysts, a serologically negative cat (tested with commercial ELISA kit, Immunocomb, Biogal Galed Labs., Haifa, Israel) 15 weeks old was infected.

T. gondii strains

There were used two types of *T. gondii* strains: avirulent (K 2) oocysts and virulent (K 24) tachyzoites.

The K2 strain of *T. gondii* was used during the first experiment. This strain was isolated in 1995 from a cat and, based on genotype characteristics, classified as a strain from the clonal line of the so-called avirulent strains (Literák *et al.*, 1998). Oocysts of that strain were obtained from the faeces of a cat infected *per os* by a brain tissue suspension of the CD1 mice containing *T. gondii* tissue cysts as described by Dubey and Beattie

(1988). The oocysts were kept for 3 to 7 days in 2% sulphuric acid at room temperature to sporulate, and then stored at 4°C. Before inoculation, the oocysts suspension was neutralised in 3.3% sodium hydroxide. The number of oocysts was determined by visual counting under a microscope.

In the second experiment the mice were infected with tachyzoites of K24 strain from 91st tachyzoite-tachyzoite passage of laboratory mice. This K24 strain was isolated from a cat in 1995 and classified as a representative of virulent clonal line (Literák *et al.*, 1998). First, the strain was maintained in mice by intraperitoneal (i.p.) passages of tissue cysts (mice brain tissue suspension) every 4–6 months. Mice, in which were prepared tachyzoites for infection of monitored animals, were previously treated by subcutaneous injection of hydrocortisone sodium succinate (Research Institute of Antibiotics and Biotransformation Roztoky near Prague) in dosage 40 mg/kg every second day for a period of 20 days. Within 8–10 days after *T. gondii* inoculation of tissue cysts we obtained tachyzoites from mice peritoneal exudate. These tachyzoites were used for the subsequent passages in mice every 3–4 days.

In the LTT, the commercial antigen of *T. gondii* was used (Sevac, Prague, Czech Republic).

Iodine values determination

The level of iodine in organism of mice has been determined by testing of the iodine presence in their urine. The concentration of iodine in urine was assessed after alkaline digestion according to the method by Sandel-Kolthoff (Bednář *et al.*, 1964). We collected the samples of 10 mice in order to obtain sufficient amount of urine. Mice were placed into plastic container with two bottoms – the upper bottom was made from wire grid while the lower fixed bottom was made from a plastic. Mice were placed on wire grid and exposed to 8°C temperature for 1 hour. After their return to breeding facility we drew 1–2 ml urine in the syringe and poured it into the test tube. The test tube was closed and frozen at –18°C temperature. At this temperature we stored mouse urine until the examination.

Lymphocyte transformation test (LTT)

We randomly selected and euthanised three mice from each experimental group. Then we prepared a

pooled cell suspension from spleens of these three mice. The suspension, used intentionally without any further purification steps, was cultivated in the final concentration of 2×10^6 living cells/ml in medium RPMI 1640–HEPES modification (Sigma-Aldrich, St. Louis, USA), supplemented with 2 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, 100 µg/ml streptomycin, and 5% foetal calf serum (Sigma). The cells were cultivated either without any stimulant or they were stimulated by mitogens or antigen *T. gondii* in several concentrations. The most suitable appeared to be phytohaemagglutinin (PHA HA 15, Murex Bio-tech, Dordrecht, England) in the final concentrations 12.5 and 25 µg per ml, concanavalin A (Con A, Pharmacia, Uppsala, Sweden) in the final concentrations 1 and 2.5 µg/ml, lipopolysaccharid *Escherichia coli* O55:B5 (LPS, Sigma) in the final concentration 5 µg/ml, and antigen *T. gondii* (Sevac, Prague, Czech Republic) in the final concentration 10 µg/ml.

2×10^5 cells in the volume of 0.2 ml per a well (4 parallel wells) of flat-bottomed Microwell plates (Nunc, Roskilde, Denmark) were cultivated in humid atmosphere of 5% CO₂ in air at 37°C for 72 hours. After the last 6 hours of cultivation we added 1 µCi [6-³H] thymidine (LACOMED s.r.o., Řež near Prague, Czech Republic) per a well. The cells were harvested by means of an automatic cell harvester Mash II (Dynatech, Zug, Switzerland); the radioactivity was measured with spectrophotometer Betaszint BF 5000 (Berthold, Germany). The response of the cells was expressed as an arithmetic mean of counts per minute (CPM) of four parallel wells with appropriate standard deviation.

Experimental design

Experiment No. 1 (mice infected with *T. gondii* oocysts).

Standard ($n = 36$), hypoidemic ($n = 33$) and normoidemic ($n = 32$) mice were fed by relevant diet for 3 months. The level of iodine in mice organism was determined by monthly urine examination.

After 3 months, three mice from each group were randomly selected and tested in the LTT. All remaining mice with the exception of three mice from standard group, were infected *per os*, using a micropipette, with oocysts suspension (50 µl inoculum) in phosphate buffered saline (PBS; 7.2 pH) in a dose of 10^3 oocysts of the K2 *T. gondii* strain. After a period of 35 days, during which we noticed mortality in the mice, three survived animals out of each group were euthanized and their spleen cells were tested in the LTT. The three non-infected standard mice were also tested in the LTT.

Experiment No. 2 (mice infected with *T. gondii* tachyzoites).

Standard ($n = 35$), hypoidemic ($n = 22$) and normoidemic ($n = 29$) mice were fed by relevant diet for 6 months. The level of iodine in mice was determined by monthly urine examination. After 2 months, three mice from each group were selected randomly and tested in the LTT.

After 4 months three mice from each group were selected randomly and were infected i.p. with 10 tachyzoites of virulent strain K24 in 1 ml PBS. After 4 days, the mice were euthanized and tested in the LTT. Other three randomly chosen non-infected standard mice were tested in the LTT as a non-infected control.

In respect to the results achieved up to this time were intentionally after 6 months three randomly selected mice from each group infected i.p. by more infectious dose – 100 tachyzoites of virulent strain K24 in 1 ml PBS. After 4 days, the mice were euthanized and tested in the LTT. Other three randomly chosen non-infected standard mice were tested in the LTT as a non-infected control again.

After the end of the main experiment remained 20 standard, 13 hypoidemic and 20 normoidemic mice. These mice were i.p. infected with 100 tachyzoites of K24 strain in 1 ml PBS with the aim to find out possible differences in the duration of their survival. Their survival was recorded and statistically evaluated.

Statistical methods

The method of one-way analysis of variance (ANOVA, Sokal and Rohlf, 1995) was used to compare iodine concentrations in the urine of mice of each group.

The main hypothesis was verifying by data obtained in the LTT in both experiments. Due to the limited amount of data we compared the groups by two-way analysis of variance without replication (with the following factors: experimental group and stimulant) where the variability of interaction effect of both factors is used for estimation of error variability (Mittenecker, 1963; Sokal and Rohlf, 1995). We used logarithmic CPM values for calculation because they better meet the model assumptions. In cases where the differences in mean CPM were generally proven, the different groups were identified by analysing preselected pairs of groups by means of so-called linear contrasts (partial *t*-test).

The difference in proportion of mice that died within 35 days after the infection with *T. gondii* oocysts

was evaluated by Fisher's exact test for fourfold table (Sokal and Rohlf, 1995).

Mice mortality after the infection with *T. gondii* tachyzoites was evaluated by the survival analysis. The equality of survival function in particular groups was tested by the log-rank test (Collett, 1997).

All statistical tests were performed at the 5% significance level. We used statistical program SPSS (Norušis, 1994) for the calculations.

RESULTS

Experiment No. 1 – The reaction of hypoidemic mice infected with *T. gondii* oocysts.

Iodine supplementation. In the group of normoiodemic mice in comparison to the group of standard mice, we found in urine significantly higher iodine concentrations in second and third month of experiment duration, however, differences in iodine concentration of these groups (Table 1) in the 4th day before

and in the 27th day after the infection were already not significant. Throughout the experiment, iodine supplementation was significantly lower in hypoidemic mice in comparison with normoiodemic mice (Table 1).

Mortality. Only 1 mouse from the standard group (3%, $n = 36$) died before 35th day after experimental infection with *T. gondii* oocysts; 7 mice from hypoidemic group (21%, $n = 33$); and 3 mice from normoiodemic group (9%, $n = 32$). The difference in the proportion of dead mice between hypoidemic and normoiodemic group was not significant (Fisher's one-sided test, $P = 0.164$).

LTT results. The reactivity of spleen cultures of three monitored mice groups examined after 3 months of special diet is stated in Table 2. The spleen culture reactivity of 4 mice groups is stated in Table 3. Three of them were infected with *T. gondii* oocysts 35 days ago and 4th group was a non-infected control. There was no statistically significant difference in average CPM values between the three mice groups from Table

Table 1. Iodine concentration in urine ($\mu\text{g/l}$) of mice experimentally infected with *T. gondii* oocysts (geometric means, 95% confidence intervals are in brackets)

Mice group	n	1 month	2 months	3 months	3.5 months (4 days before infection)	4.5 months (27th day after infection ³)
Standard	36	870 (593; 1 276)	575 (485; 683)	481 (375; 617)	857 (626; 1 173)	484 (277; 848)
Hypoidemic ¹	33	Nt	49 (30; 79)***	97 (52; 181)***	41 (18; 94)***	11 (7; 16)***
Normoiodemic ²	32	Nt	1 282 (1 153; 1 621)***	913 (839; 995)*	1 034 (441; 2 423) ^{NS}	444 (277; 713) ^{NS}

¹the significance of differences between hypoidemic and normoiodemic mice groups

²the significance of differences between standard and normoiodemic mice groups

³ $n = 32, 25$ and 27 in standard, hypoidemic, and normoiodemic mice groups, respectively

Nt = not tested

* $P \leq 0.05$; *** $P \leq 0.001$; ^{NS} not significant

Table 2. Responsiveness of spleen cells of mice ($n = 3$, mixed sample) in the lymphocyte transformation test. Mice were feeding by special diet for 3 months and were preparing for experimentally infection with *T. gondii* oocysts (average counts per minute \pm standard deviations)

	Standard mice	Hypoidemic mice	Normoiodemic mice
No stimulant	2 825 \pm 317	849 \pm 81	1 990 \pm 528
PHA 12.5 $\mu\text{g/ml}$	21 244 \pm 1 258	21 060 \pm 438	24 413 \pm 1 247
PHA 25 $\mu\text{g/ml}$	27 307 \pm 1 757	38 606 \pm 3 424	28 089 \pm 1 509
Con A 1 $\mu\text{g/ml}$	32 425 \pm 2 821	23 306 \pm 2 486	25 605 \pm 1 291
Con A 2.5 $\mu\text{g/ml}$	76 446 \pm 4 213	61 377 \pm 3 956	101 700 \pm 9 895
LPS 5 $\mu\text{g/ml}$	21 819 \pm 1 301	25 598 \pm 1 630	25 052 \pm 2 650
<i>T. gondii</i> antigen 10 $\mu\text{g/ml}$	3 725 \pm 255	1 194 \pm 180	2 545 \pm 461

Table 3. Responsiveness of spleen cells of mice ($n = 3$, mixed sample) in the lymphocyte transformation test, 35 days after the infection with 10^3 oocysts of *T. gondii* (average counts per minute \pm standard deviations)

	Standard mice		Hypoidemic mice	Normoidemic
	non-infected	infected	infected	mice infected
No stimulant	1 620 \pm 145	3 887 \pm 519	7 431 \pm 2 065	2 908 \pm 290
PHA 12.5 μ g/ml	11 216 \pm 1 568	50 947 \pm 8 220	102 483 \pm 7 566	33 931 \pm 1 165
PHA 25 μ g/ml	76 675 \pm 4 067	111 456 \pm 7 053	104 673 \pm 9 108	108 732 \pm 8 782
Con A 1 μ g/ml	6 068 \pm 2 372	20 313 \pm 2 293	77 414 \pm 2 923	44 234 \pm 6 443
Con A 2.5 μ g/ml	65 380 \pm 14 970	128 263 \pm 1 921	161 697 \pm 7 299	157 871 \pm 10 445
LPS 5 μ g/ml	138 564 \pm 6 212	21 168 \pm 2 951	16 736 \pm 2 957	17 741 \pm 1 644
<i>T. gondii</i> antigen 10 μ g/ml	1 733 \pm 260	14 183 \pm 3 112	21 066 \pm 4 252	30 288 \pm 5 270

Table 4. Iodine concentration in urine (μ g/l) of mice experimentally infected with tachyzoites of *T. gondii* (sample mixed from randomly selected 10 mice)

Mice group	1 month	2 months	3 months	4 months (day of infection with 10 tachyzoites)	5 months	6 months (day of infection with 100 tachyzoites)
Standard	3 553	550	852	333	371	1 028
Hypoidemic	21	37	34	33	0 ¹	2
Normoidemic	1 130	2 384	581	501	1 080	456

¹out the limit of determination

2 (ANOVA, $P = 0.272$), or between the three infected groups from Table 3 (ANOVA, $P = 0.088$).

There were some differences between the values characterising mitogenic responses in individual experimental groups, but these differences have no connection with iodine availability in the diet. The response to LPS was in all three infected groups markedly lower than in non-infected control (Table 3). On the other hand, the response to PHA and Con A was significantly higher in infected groups, irrespective of whether we tested the significance of the difference from the control group in all three infected groups together (ANOVA, $P = 0.009$) or in preselected group pairs. We compared the following pairs of groups: standard non-infected mice vs. standard infected mice (partial t -test, $P = 0.019$), standard non-infected mice vs. normoidemic infected mice ($P = 0.010$) and standard non-infected mice vs. hypoidemic infected mice ($P = 0.001$). The availability of iodine had no effect on intensity or dynamics of the above-mentioned reactivity changes.

Experiment No. 2 – The reaction of hypoidemic mice infected with *T. gondii* tachyzoites.

Iodine supplementation. The differences in iodine supplementation of three groups of mice kept under special diet for 6 months are stated in Table 4. Iodine supple-

mentation of hypoidemic mice was noticeably lower in comparison with normoidemic and standard mice.

Mortality. After i.p. infection with 100 tachyzoites of virulent strain K24, average time of surviving was 8.0 days in the case of standard mice, 7.4 days in hypoidemic and 7.7 days in normoidemic mice (Table 5). There was no statistically significant difference in average time of surviving between these three groups (ANOVA, $P = 0.3$). Equally, there was no statistically significant difference in the distribution of

Table 5. Mice mortality after the infection with 100 tachyzoites of virulent *T. gondii* strain K24

Days after the infection	Number of dead mice		
	standard ($n = 20$)	hypoidemic ($n = 13$)	normoidemic ($n = 20$)
1–5	0	0	0
6	2	0	1
7	6	10	14
8	5	2	1
9	5	0	1
10	2	1	1
11	0	0	2
Mean survival time (days)	8.0	7.4	7.7

dying (i.e., the shape of survival function) between these three monitored groups (log-rank test, $P_{1-2} = 0.53$, $P_{2-3} = 0.83$, $P_{1-3} = 0.17$).

LTT results. There is no statistically significant difference in average CPM values between the three groups of mice (ANOVA, $P = 0.141$) examined after 2.5 months of special diet (Table 6). The result is analogous to the result of experiment No. 1 (Table 2) where either no statistically significant difference between the groups was found. The reactivity of spleen

cells of 4 groups of mice (in all cases there were 3 groups infected with *T. gondii* and one non-infected) examined after 4 and 6 months of special diet is stated in Tables 7 and 8. There was no statistically significant difference in average CPM values between the four groups of mice (ANOVA, $P = 0.776$) examined after 4 months of special diet and after the infection with 10 *T. gondii* tachyzoites (Table 7).

There was a statistically significant difference in spleen cultures reactivity between 4 groups of mice

Table 6. Responsiveness of spleen cells of mice ($n = 3$, mixed sample) in the lymphocyte transformation test. Mice were feeding by special diet for 2.5 months and were preparing for experimental infection with tachyzoites of *T. gondii* (average counts per minute \pm standard deviations)

	Standard mice	Hypoidemic mice	Normoidemic mice
No stimulant	13 057 \pm 2 099	5 736 \pm 927	7 518 \pm 1 501
PHA 12.5 μ g/ml	150 669 \pm 2 270	165 749 \pm 7 531	178 757 \pm 9 535
PHA 25 μ g/ml	126 311 \pm 6 609	122 853 \pm 844	127 975 \pm 5 323
Con A 1 μ g/ml	213 154 \pm 11 561	Not tested	222 051 \pm 35 162
Con A 2.5 μ g/ml	132 584 \pm 1 929	172 924 \pm 10 309	230 114 \pm 12 940
LPS 5 μ g/ml	61 819 \pm 7 839	66 137 \pm 4 497	82 008 \pm 11 709

Table 7. Responsiveness of spleen cells of mice ($n = 3$, mixed sample), that were feeding by special diet for 4 months, 4 days after the infection with 10 tachyzoites of *T. gondii* in the lymphocyte transformation test (average counts per minute \pm standard deviations)

	Standard mice		Hypoidemic mice	Normoidemic mice
	non-infected	infected	infected	infected
No stimulant	1 371 \pm 210	3 652 \pm 573	4 050 \pm 1 026	4 721 \pm 406
PHA 12.5 μ g/ml	55 861 \pm 1 723	38 103 \pm 5 000	60 054 \pm 2 849	29 719 \pm 4 540
PHA 25 μ g/ml	133 941 \pm 6 457	135 530 \pm 4 872	159 290 \pm 7 666	133 199 \pm 7 530
Con A 1 μ g/ml	72 846 \pm 1 944	36 302 \pm 3 374	53 837 \pm 1 847	39 138 \pm 7 084
Con A 2.5 μ g/ml	154 763 \pm 7 546	106 767 \pm 1 424	118 961 \pm 4 962	100 908 \pm 11 134
LPS 5 μ g/ml	81 472 \pm 14 334	60 588 \pm 3 322	51 797 \pm 3 945	48 413 \pm 5 753

Table 8. Responsiveness of spleen cells of mice ($n = 3$, mixed sample), that were feeding by special diet for 6 months, 4 days after the infection with 100 tachyzoites of *T. gondii* in the lymphocyte transformation test (average counts per minute \pm standard deviations)

	Standard mice		Hypoidemic mice	Normoidemic mice
	non-infected	infected	infected	infected
No stimulant	2 567 \pm 571	2 229 \pm 354	1 926 \pm 297	2 683 \pm 396
PHA 12.5 μ g/ml	89 791 \pm 2 430	17 556 \pm 2 248	7 906 \pm 1 725	20 723 \pm 4 319
PHA 25 μ g/ml	187 812 \pm 4 534	65 719 \pm 5 466	60 015 \pm 7 107	109 249 \pm 13 356
Con A 1 μ g/ml	30 827 \pm 3 224	5 227 \pm 759	7 917 \pm 1 767	7 403 \pm 1 194
Con A 2.5 μ g/ml	134 251 \pm 11 783	25 326 \pm 4 992	68 052 \pm 6 155	62 922 \pm 3 316
LPS 5 μ g/ml	69 480 \pm 6 702	38 016 \pm 3 153	38 203 \pm 4 743	36 051 \pm 2 731

infected with 100 *T. gondii* tachyzoites (ANOVA, $P = 0.004$) that were examined after 6 months of special diet (Table 8). Similarly to experiment No. 1, when comparing preselected group pairs, the difference was only found between standard infected group and standard non-infected group (partial t -test, $P = 0.001$). However, there was no difference between standard infected and normoiodemic infected group (partial t -test, $P = 0.230$), nor between hypoiodemic infected and normoiodemic infected group (partial t -test, $P = 0.270$). There were markedly differences in spleen cultures reactivity between 3 infected groups, that were examined after 4 months and those, that were examined after 6 months of special diet (Table 7, 8).

DISCUSSION

This work was motivated by recent findings of clinically manifested toxoplasmosis in hypoiodemic goats (Šlosárková *et al.*, 1999). The purpose of the work was to verify, that hypoiodemia caused such enfeeblement of the immune system, which will induce more significant manifestation of toxoplasmosis in consequential infection. As a laboratory model we used outbred laboratory mice that were kept under special diet with respect to iodine supplementation. The hypoiodemia of mice was determined by testing of urine-excreted iodine concentration. As hypoiodemic were considered such mice, in which their urine iodine level was less than 100 µg/l. This level is known as insufficient in both people and animals (Bourdoux, 1993). The reactivity of mouse immune system was examined by LTT because the resistance to *T. gondii* infection is mediated primarily by mechanisms of cell-mediated immunity and LTT is a standard method for evaluating cell-mediated immunity. In addition to this parameter of cellular immunity we also evaluated post-infection mortality of mice.

Though it is known, that the stimulants used in LTT (PHA, Con A, LPS, specific antigen) have an effect on different subsets of immune system cells, and the functions of these cells may be influenced by iodine deficiency differently, so by simplifying the matter and evaluating the data statistically (due to the limited numbers of data obtained from the LTT), we presumed, that the response to particular stimulants is proportional – i.e., that special diet influences the response to particular stimulants also proportionally. Regarding this limitation, we were not successful in the verification of decrease in the responsiveness of spleen cells in LTT, whether we tested non-infected mice or

infected mice in various time intervals after their infection with *T. gondii* oocysts or tachyzoites. And again, the mice exposed to long-term hypoiodemia did not show stronger susceptibility to lethal progress of *T. gondii* infection too.

Differences in mitogenic response in spleen cells to LPS versus PHA and ConA in infected group in experiment No.1 are in correspondence with our experiences and with published information's (Candolfi *et al.*, 1994, 1995). We founded before, that suppression induced by *T. gondii* infection of mitogenic response to LPS is long-term remaining against the suppression to T mitogens, which is short-term remaining and after the suppression the activity of the cells goes quickly to the normal values.

Prominent differences in spleen cultures reactivity in LTT between 4th and 6th month of hypoiodemia can be explained in all three infected groups by different infectious dose. Four days after injection, the mice infected with 100 *T. gondii* tachyzoites of K24 strain showed statistically significant decrease in the responsiveness of spleen cells to stimulation by all mitogens being used. However, such decrease was observed in all mice, both normoiodemic and hypoiodemic. We suppose that the cause of this immunosuppression is the infection with *T. gondii* tachyzoites itself that is often manifested like this in the early infection stages (Strickland *et al.*, 1975; Strickland and Sayles, 1977; Charde *et al.*, 1993). The host organism responds to the *T. gondii* infection by activating its immune system, the aim of which is to stop the replication of tachyzoites before the onset of T-cell mediated immunity, and to provide for an adequate T-cell response (Denkers and Gazzinelli, 1998). In this process, macrophages start producing cytokines, particularly interferon gamma, and reactive nitrogen intermediates. An uncontrolled production of anti-inflammatory cytokines may, on the other hand, lead to the suppression of mitogen-stimulated LTT in the acute phase of the *T. gondii* infection (Chan *et al.*, 1986; Candolfi *et al.*, 1994, 1995).

No significant depression of cell response in LTT occurred in mice infected with 10 tachyzoites. The “parasite burden” probably was not hard enough to induce significant suppressive reaction within the range of stimulants concentrations being tested.

We could not prove the statistically significant decrease of spleen cells responsiveness to the mitogens used in hypoiodemic mice infected with *T. gondii* oocysts. Equally, there was no statistically significant difference between the responsiveness of spleen lymphocytes to any mitogens used in hypoiodemic and normoiodemic mice infected with *T. gondii* tachyzoites.

Hypiodemia for six months inferred from urine iodine levels caused the decrease in spleen lymphocytes responsiveness to the mitogens being used. Similarly, animals exposed to long-term hypiodemia were not responsive any stronger to acute *T. gondii* infection as compared to normiodemic mice. We can say, that our starting hypothesis was not validated on the mice model under the above-mentioned conditions. In order to verify the hypothesis, it will be necessary to further study the relation between hypiodemia, parameters of the immune system and toxoplasmosis.

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