PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **178**, 246–249 (1985)

Hypothalamic–Adenohypophyseal Origin of Reproductive Failure in Mice following Chronic Infection with *Toxoplasma gondii* (42006)

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Abstract. Mice chronically infected with *Toxoplasma gondii* exhibited reproductive failure characterized by a constant diestrous vaginal cytology and ovarian and uterine atrophy. Chronically infected mice were treated with 20 ng of D-Leu^6-des-Gly-NH_2-Pro-ethylamide (D-Leu^6), a structural analog of luteinizing hormone-releasing hormone (LHRH), every 4 hr over a 12-hr period daily, for 3 days. Infected animals treated with D-Leu^6 had greater pituitary weight ($P < 0.01$), ovarian weight ($P < 0.01$), and uterine weight ($P < 0.025$), than did infected control mice treated with saline. In addition, a change in vaginal cytology to estrus, metestrus, and proestrus of the D-Leu^6-treated animals was observed, although a contiguity of normal estrous cycles and reproductive function was not determined. Comparable basal levels of serum luteinizing hormone (LH) were seen in infected mice and uninfected normal mice. However, the infected animals demonstrated a decreased pituitary responsiveness to D-Leu^6 when monitored at 60 ($P < 0.025$) and 120 min ($P < 0.010$) following intraperitoneal administration of a bolus of 200 ng of the analog. Thus, the observed reproductive failure involves the readily releasable pool of pituitary LH, since basal LH is similar in both groups, and appears to be due to a dysfunction of the hypothalamic–adenohypophyseal axis. © 1985 Society for Experimental Biology and Medicine.

Two phases are commonly recognized in postnatally acquired *Toxoplasma gondii* infections: (a) a brief acute phase of 2- to 3-weeks duration, during which proliferative forms (trophozoites) invade and multiply within a variety of host cells and (b) a lifelong chronic phase, characterized by the development and persistence of *T. gondii* cysts in the brain and other organs and tissues of the host (1, 2). The acute phase of the toxoplasma infection has been extensively investigated both clinically and experimentally, whereas the study of chronic toxoplasmosis has received less attention. Probably contributing to this disproportion is the widespread belief that chronic toxoplasmosis is of little or no clinical significance except in those rare individuals immunologically compromised by underlying hematological malignancies and/or receiving immunosuppressive drug therapy (3). Furthermore, chronic maternal infection is discounted as a significant factor in the etiology of human congenital toxoplasmosis (4, 5), although vertical transmission has been shown to occur in several animal species (2).

During the course of preliminary studies on chronic toxoplasmosis and its sequelae, we observed that infected female mice failed to reproduce. In a series of pilot experiments, a total of 60 female mice, infected from 1 to 4 months, were mated with proven studs. The 60 matings resulted in only one litter, whereas 38 of 45 uninfected female mice gave birth. The objective of this study was to identify the locus of reproductive failure in mice chronically infected with *T. gondii.*

Materials and Methods. *Animals.* Female albino Nya:NYLAR mice (Griffin Laboratory, Center for Laboratories and Research, New York State Dept. of Health, Albany, N.Y.) were used throughout this study. Mice were housed up to 10 per cage and allowed free access to food and water.

Parasite and infection. The nonlethal Cornell strain (CS) of *T. gondii* (obtained from Dr. A. Kimball, Cornell University School of Medicine, New York, N.Y.) was maintained in the cyst stage in the brains of Nya:NYLAR mice and passaged into normal mice every 6 months. To obtain CS cysts, infected mice were sacrificed by cervical dislocation and their brains were removed and homogenized in saline. The standard inocu-

[1] Recipient of NIA NIH-HD-16675.

0037-9727/85 $1.50

lum for this study was 12 cysts in a 0.5-ml aliquot of brain emulsion, administered by intraperitoneal (ip) injection.

Pituitary response of infected mice to D-Leu^6 treatment. Mice were infected with *T. gondii* at 8–10 weeks of age and treatment began 6 months later. Nine mice were allocated to each group D-Leu^6-treated and saline control. Treated animals received multiple injections of D-Leu^6-des Gly-$NH_2{}^{10}$-LHRH-ethylamide (D-Leu^6) (6), 20 ng, ip every 4 hr over a 12-hr period daily (8:00 AM to 8:00 PM), for 3 days. D-Leu^6 is 10 times more potent than endogenous hypothalamic luteinizing hormone-releasing hormone (LHRH) in causing the release of luteinizing hormone (LH) from the adenohypophysis (7). On the morning of the fourth day all the mice were weighed individually, then sacrificed by cervical dislocation and autopsied. The ovaries and uteri were removed and weighed on a torsion balance. Student's t test was used to determine whether a difference existed between groups. The level of significance was set at $P < 0.05$. The cytology of vaginal smears was examined daily for 12 days prior to and including the last day of treatment.

The pituitary response to a 10-fold higher dose of D-Leu^6 was then examined. Following the 3 days of D-Leu^6 treatment, all animals were lightly anesthetized and approximately 200 μl of blood were withdrawn from the retro-orbital plexus. All the mice then were given a bolus ip injection of 200 ng of D-Leu^6. Sixty minutes later, the mice again were anesthetized and sacrificed by exsanguination. The pituitary, ovaries, and uterus of each animal were removed and examined as described above. Serum was separated by centrifugation and stored at −20°C until assayed for LH (8). Purified ovine LH (LER-1056-C2) was used for iodination and NIH-LH-S17 was used as a reference standard (9).

Time course of pituitary response to D-Leu^6. The secretory pattern of LH release in response to a single bolus ip injection of 200 ng of D-Leu^6 was examined in 9 uninfected and 10 infected mice. Animals were bled prior to D-Leu^6 injection (basal LH) and at 30, 60, and 120 min postinjection. Serum was collected and assayed as described above.

Results. *Uterine response of mature T. gondii-infected mice to D-Leu^6.* Vaginal cytology for 8 days preceding treatment exhibited a constant diestrous cycle phase, indicating that all infected mice were anovulatory. The intensity of infection for 16 mice evaluated at this stage of infection was approximately 4650 ± 3980 cysts/brain. Mice at this stage are sick. However, atrophy of endocrine sensitive organs occurs before sickness, usually apparent at 1 month. Following treatment with D-Leu^6, vaginal cytology began to change on Day 3 of treatment for over 50% of the mice. Vaginal cytology on Day 4 indicated a range of estrous, metestrous, and proestrous cycle phases for all mice treated with D-Leu^6. All untreated infected mice remained in the diestrous cycle phase.

Infected animals treated with D-Leu^6 exhibited increases in uterine and ovarian weight with no change in body weight (Table I). Uterine weight for D-Leu^6-treated mice was about three times greater than that of controls. Although the ovarian weight gain was only slightly higher for analog-treated mice, the difference was significant.

TABLE I. OVARIAN AND UTERINE WEIGHT RESPONSE OF *T. gondii*-INFECTED MICE TO D-Leu^6

Group	n	Body wt (g)	Ovarian weight mg	Ovarian weight mg%	Uterine weight mg	Uterine weight mg%
Infected saline	9	20.1 ± 3.1	6.0 ± 1.1	30.1 ± 3.6	31.2 ± 12.3	152.4 ± 39.5
Infected D-Leu^6	9	20.4 ± 2.4	7.5 ± 1.7	36.3 ± 4.2	87.9 ± 24.3	430.6 ± 97.7
P-Level		NS	$P < 0.05$	$P < 0.01$	$P < 0.001$	$P < 0.001$

Note. Effect of D-Leu^6 treatment on body, ovarian, and uterine weight of Nya:NYLAR mice infected with *T. gondii.* D-Leu^6: four injections of 20 ng ip at 4-hr intervals (8:00 AM to 8:00 PM) daily for three days. Differences between treatments were examined by Student's t test. All animals were infected at 8–10 weeks of age and treated 6 months later. NS = no significant difference. Data are expressed as the means ± the standard deviation.

Pituitary response of T. gondii infected mice to D-Leu⁶. Serum LH basal levels were comparable in chronically infected mice following 3 days of treatment with either D-Leu^6 or saline (Table II). The analog-pretreated mice had less of a readily releasable pool of LH than the mice treated with saline since their LH levels were lower than the saline-treated mice 60 min after the injection of the 200-ng bolus of D-Leu^6 ($P < 0.01$). Despite an apparently greater pituitary reserve of LH, the saline-treated mice remained acyclic, whereas the D-Leu^6-pretreated mice began to cycle suggesting that a lack of pituitary responsiveness was not the etiology of their acyclicity. Pituitary weights in both groups were comparable.

Time course of pituitary response of T. gondii-infected mice to D-Leu^6. Significant differences in serum LH levels did not exist between infected and uninfected mice until 60 min after D-Leu^6 administration (Table III). Therefore, the elapsed response time was similar for both groups over the first 30 min postinjection. Significant differences in serum LH levels between groups became apparent at 60 ($P < 0.05$) and 120 ($P < 0.02$) min following D-Leu^6 administration. Thus, there appeared to be less of a readily releasable pool of pituitary LH in the chronically infected mice.

TABLE II. PITUITARY WEIGHT AND SERUM LH CHANGES OF *T. gondii*-INFECTED MICE AFTER ADMINISTRATION OF D-Leu^6

	Pituitary weight		LH (ng/ml)	
Group	mg	mg%	0 min	60 min
Saline	3.0 ± 0.5 (9)	15.1 ± 1.8 (9)	0.8 ± 0.4 (9)	9.8 ± 5.2 (7)
D-Leu^6	3.2 ± 0.2 (9)	15.8 ± 0.9 (9)	0.6 ± 0.1 (10)	3.1 ± 3.0 (10)
P-Level	NS	NS	NS	$P < 0.01$

Note. Pituitary weights and LH response of chronically infected mice to a bolus of D-Leu^6 (200 ng, ip) after 3 days of pretreatment with either D-Leu^6 or saline. Pretreatment was accomplished by ip injection of 20 ng of D-Leu^6 four times daily, from 8:00 AM to 8:00 PM, for 3 days. Serum LH levels were determined on the morning of Day 4. Data are expressed as means ± the standard deviation. The values in parentheses indicate the number of animals per group.

TABLE III. TIME COURSE OF PITUITARY RESPONSIVENESS OF *T. gondii*-INFECTED MICE TO D-Leu^6

	Time after treatment with D-Leu^6 (min)			
Group	0	30	60	120
	Serum LH (ng/ml)			
Uninfected	0.4 ± 0.1 (8)	2.5 ± 0.3 (8)	12.7 ± 3.0 (8)	25.6 ± 16.8 (9)
Infected	0.4 ± 0.2 (7)	2.1 ± 0.2 (8)	5.6 ± 2.7 (8)	9.2 ± 9.5 (10)
P-Level	NS	NS	$P < 0.05$	$P < 0.02$

Note. Time course of pituitary responsiveness of *T. gondii*-infected and normal, uninfected female mice to a single ip injection of 200 ng of D-Leu^6. The zero time values represent the basal serum LH levels of the two groups. The numbers in parentheses represent the number of animals which contribute to each treatment mean and standard deviation. Student's *t* test was used to determine whether differences existed between the means obtained at each time period. Animals were infected with *T. gondii* at 8–10 weeks of age and used 6 months later. Uninfected control animals were the same age.

Discussion. Toxoplasmosis has long been implicated in abortion, fetal wastage, perinatal mortality, and congenital malformations in humans and in laboratory and domestic animals (2). To these areas of reproductive failure we now believe that we can add infertility.

In our laboratory model of murine toxoplasmosis, we observed that chronically infected Nya:NYLAR mice became acyclic and remained in permanent diestrus. Other constant findings were ovarian and uterine atrophy and apparent cessation of ovulation. Clearly, the functional integrity of the hypothalamic–adenohypophyseal–ovarian axis had been compromised. For normal cycling and ovulation to occur, hypothalamic secretion of LHRH is required. LHRH stimulates the secretion of pituitary gonadotropin (luteinizing hormone and follicle-stimulating hormone) which in turn promotes maturation of ovarian follicles, ovulation, and corpus luteum function.

In the present study, we employed D-Leu^6 as a physiological probe to test pituitary responsiveness. If we could succeed in reversing the hypogonadism, we could then point to the hypothalamus as the site of the primary endocrinologic lesion.

The experimental data support our contention that the locus of reproductive failure is a malfunction of the hypothalamic control of the adenohypophysis. This conclusion is based upon the observation that administration of D-Leu^6 restored the uterus and ovaries to a stimulated state, demonstrating that the atrophied ovary of the infected mouse was capable of responding to gonadotropin. It, in turn, stimulated uterine hypertrophy through steroid production.

The hypothalamus is further implicated because infected and uninfected animals do not differ in basal levels of serum LH. One possible mechanism that might account for a difference in the readily releasable pool of LH is that there is a lack of hypothalamic priming of the pituitary in infected animals. The priming effect of LHRH on the pituitary is much greater at proestrus than at diestrus (12, 13), which is the stage at which cycling was arrested in the infected animals. The lack of estrogen from the ovary may lessen the priming effect, thus complicating further the lack of pituitary responsiveness, although estrogen may not be the primary modulator of pituitary responsiveness (14). The lack of a tonic stimulation of the pituitary would lead to acyclicity and reproductive failure.

If pituitary responsiveness had been defined as the LH response between 0 and 30 min, we would have concluded that there was no difference between infected and uninfected animals. However, careful observation of LH response over a protracted time period clearly illustrated a fundamental difference between the normal and infected animal response to D-Leu^6. Basal levels of serum LH were also poor indicators of gonadotropin sufficiency, since infected and uninfected animals did not differ in the basal levels of LH and yet were clearly different in reproductive capacity. Thus, the presence of LH is necessary but not sufficient to ensure normal reproductive function. Whether chronic toxoplasmosis compromises reproductive capacity in other laboratory animals, domestic animals, or humans remains to be determined. This study demonstrates that, in the mouse, reproductive failure was caused by hypothalamic dysfunction resulting from long-term chronic infection with *T. gondii.*

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Received May 14, 1984. P.S.E.B.M. 1985, Vol. 178.
Accepted October 18, 1984.