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MULTIPLE ORGAN DYSFUNCTION IN CONGENITAL MURINE TOXOPLASMOSIS

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Infection of gravid Nya: NYLAR (NYLAR), C57BL/6J (C57), and BALB/c mice with *Toxoplasma gondii*, on gestation day 7, resulted in fetal resorptions, abortions, and stillbirths. Fetal wastage (estimated) was 35 and 40 % in the NYLAR and C57 strains and 55 % in the BALB/c strain. Postnatally, pups were cachectic and growth-retarded, with some developing hind limb weakness, petechial lesions on ears and tail, and a blood-tinged nasal exudate. Only 13 of 97 BALB/c pups, 14 of 41 C57 pups, and 46 of 153 NYLAR pups survived the first month of life. At necropsy, swollen, blotched livers, enlarged spleens, pallid kidneys and pulmonary hemorrhages were observed. Cysts of *T. gondii* were detected in every pup, via press-smears of brain. Histologic examination revealed mineralizing cavitations, ventricular deformations, and periventricular edema in the central nervous system; extensive liver pathology marked by hepatocellular necrosis and calcification, sinusoidal dilatation, and giant cell granulomas; congestion of the spleen with blurring of red and white compartments and cavitations in the white pulp; and tubule and glomerular necrosis and calcification in the kidney. The pulmonary hemorrhages and dermal petechial lesions may reflect a bleeding diathesis due to hepatic insufficiency. The pathogenesis of congenital toxoplasmosis, in the 3 strains of mice, appears due to microvascular dysfunction characterized by dysregulation of hemostasis, perfusion failure, and multiple organ dysfunction, rather than to parasite-mediated cytopathology. We suggest that hematogenous dissemination and endothelial invasion by the parasite induced a systemic inflammatory response syndrome (i.e; toxoplasmic sepsis) leading to the microvascular dysfunction.

Key words : congenital murine toxoplasmosis, multiple organ dysfunction, wasting

INTRODUCTION

Toxoplasmosis is an infectious disease of man and animals caused by the protozoan parasite *Toxoplasma gondii*. Postnatally acquired infections, in immunologically intact hosts, are subclinical and unrecognized whereas prenatal infections can cause central nervous system (CNS) disorders and birth defects [28]. Although the pathology of congenital toxoplasmosis has been described in detail, in humans [17, 25, 36] and in animals [8, 9, 14], the pathogenesis of the congenital syndrome(s) remains poorly understood.

The present study was designed to elicit sequelae of congenital toxoplasmosis in NYLAR and C57 (susceptible) and BALB/c (resistant) strains of mice. The premise was that differences in pre and postnatal morbidity and pathology might help clarify pathogenic mechanisms. Our laboratory model of congenital toxoplasmosis is derived from a progressive and ultimately fatal wasting syndrome which develops in chronically infected NYLAR strain albino mice [32, 34]. During pilot experiments on vertical transmission of the parasite and fetal morbidity, it was found that female NYLAR mice became infertile within the first month postinfection [33]. To circumvent loss of fertility, normal female mice of the 3 strains were cohabited with male mice and then infected on the seventh day of embryogenesis. This approach proved successful, resulting in intrauterine infections marked by fetal wastage (resorptions, abortions, and stillbirths) and post-

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natal wasting. Necropsy at 1 month of age revealed a cornucopia of CNS, hepatic, and renal lesions, developmental defects, and signs of multiple organ dysfunction (MOD).

MATERIALS AND METHODS

Mice

The NYLAR, C57, and BALB/c strains of mice were obtained from a large mouse colony maintained by the Griffin Laboratory, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York. The NYLAR strain originated in 1930 from a single pair of albino mice (of unknown origin), and has since been propagated by sibling mating and systemic breeder rotation. Following parabiosis, neither rejection nor intoxication is seen, indicating that the colony is compatible at the H-2 locus [5]. The BALB/c and C57 inbred strains were originally obtained from the Jackson Labs. Female mice of the 3 strains that had previously delivered a normal litter were used. The mice, 20-22 weeks of age, were acclimated in our animal rooms for 2 weeks to screen for illness and unsuspected pregnancies. After acclimation, pairs of female mice were cohabited with proven sires (of the same strain) overnight. The following day, females with vaginal plugs were caged individually, with access to unlimited food and water. This day was designated as embryonic day 1 (E1). Gravid mice were infected on day E7 and observed daily until delivery. The morning after parturition, newborn pups of the infected dams were placed with normal, lactating BALB/c foster mothers. The exchange was to reduce infanticide, particularly by the infected NYLAR dams.

Infection of pregnant mice

The Cornell (CS) strain of *T. gondii* was originally recovered from the brain of a congenitally infected infant that died soon after birth. The parasite was obtained from Dr. A. Kimball, Cornell University School of Medicine, New York and was maintained in the cyst stage in the brains of chronically infected NYLAR mice. Cysts *T. gondii* were recovered, quantitated and inoculated as previously described [33, 34]. On day E7, the gravid mice were inoculated intraperitoneally (ip) with 0.5 ml of infected NYLAR brain emulsion containing 8 toxoplasma cysts. To monitor for viral contaminants in the infecting inoculum [19], groups of control mice received infected-mouse brain emulsion that had been repeatedly snap-frozen and thawed. This procedure has been shown to effectively destroy viable toxoplasma organisms [32], but should not affect viruses. Other groups of control mice received untreated emulsion of normal mouse brains. All inocula were identical in volume and dilution. After inoculation, the gravid mice were inspected daily, but with minimal handling to avoid undue stress.

Postnatal procedures

After parturition, the number of pups per litter was recorded. The experimental neonates, segregated by strain, were randomly assorted into groups of 6 to 8 pups and placed with normal, lactating BALB/c foster mothers. All the pups born to infected dams were used, to compensate for expected postnatal attrition. The control litters were culled of male pups and, in groups of 8, placed with normal BALB/c dams. The pups were monitored for a period of 1 month to allow further development of pathologic sequelae.

Verification of maternal infection

After removal of their litters, the postpartum BALB/c, C57, and NYLAR dams were set aside for future examination. Four months later, the dams were killed and their brains examined by squash preparations and by histologic processing as described below.

Reason for using foster mothers

During pilot experiments, it was noted that many infected dams, the NYLARS in particular, were either killing their sickly newborn pups or refusing to suckle them, with the consequent loss of entire litters. Therefore, to avert infanticide by parasitized dams, all putatively infected litters were placed with normal lactating BALB/c dams. The BALB/c foster mothers were quite tolerant and usually accepted the sickly neonates although an occasional mishap still occurred.

Necropsy and tissue processing

At 1 month of age, the surviving pups were lightly anesthetized with ether, individually weighed, then euthanized. After opening the skull to expose the brain, the mouse was decapitated and the entire head placed in 10 % buffered formalin. Next, the liver, spleen,

and kidneys were excised and also placed in formalin. After several days, the brain was removed from the skull and put into fresh formalin for further fixation and hardening. Prior to weighing, all organs were placed on coarse paper toweling to drain excess fluid, then trimmed of extraneous tissue. The larger organs were weighed on a Sartorius model 1205-MP electric balance, sensitivity +/− 1.0 mg. The smaller organs were weighed on a Sartorius model 2434 analytical scale, sensitivity + / − 0.01 mg. Relative organ weights were calculated as mg/100 g body weight. Tissues were embedded in paraffin and sectioned at 5 µm. Serial sections were cut and routinely stained either with hematoxylin and eosin (H&E) or periodic acid Schiff (PAS). Other sections were left unstained and used for immunohistochemical studies.

Immunohistochemistry

Tissue sections were deparaffinized in a xylene-ethanol gradient. Endogenous peroxidase was quenched with 0.3 % hydrogen peroxide in methanol (30 min), and nonspecific binding blocked by 1 % normal rabbit serum in phosphate-buffered saline (PBS, pH 7.4, 30 min). The sections were then incubated overnight at 4 °C with polyclonal rabbit antibodies to glial fibrillary acidic protein (GFAP, 1:1,000) and to toxoplasma antigen (1:50). Next, a biotinylated goat anti-rabbit IgG (1:50) was applied for 1 hour at room temperature. Between each step, tissue sections were washed 3 times in PBS. Bound antibodies were visualized by the avidin-biotin-peroxidase complex (ABC) assay using the appropriate Vectastain ABC kit (Vector Laboratories, Burlingame, California) for 1 hour at room temperature. The brown reaction product was developed by the application of 3',3-diaminobenzidine tetrachloride (DAB), 10 mg in 20 ml of 0.05 M TRIS buffer, pH 7.41, with 0.033 ml of hydrogen peroxide added immediately before use. The sections were counterstained with hematoxylin, dehydrated, and mounted. As controls, the primary antibodies were either omitted or replaced with non-immune serum. The control sections did not develop positive reactions at any time.

RESULTS

Maternal infection and fetal wastage

Gravid dams of the 3 strains of mice, inoculated with untreated infective brain emulsion, experienced fetal resorptions and the passage of bloody clots during the last week of gestation. In some BALB/c dams, pregnancy was aborted with the loss of all embryos. In the NYLAR and C57 mice, embryogenesis was affected to a lesser degree in terms of fetal resorptions, expulsion of macerated embryos, and stillbirths. Parturition typically occurred at night, and pups found dead in the morning were considered stillborn. Extrapolating from the number of pups born to normal dams, fetal wastage was estimated at 35 and 40 % in the NYLAR and C57 strains and 55 % in the BALB/c mice. Postnatal attrition also was very high although the use of foster mothers curtailed the problem of infanticide. Nevertheless, 30 and 34 % of the NYLAR and C57 pups, respectively, and 87 % of the BALB/c pups did not survive the first month of postnatal life (Table 1).

Postnatal development and attrition

Pups born to infected dams were less active than their normal counterparts. During early postnatal development, other differences emerged. In addition to smaller size, the affected pups developed a shriveled, hunched posture, ataxia, dragging of the hind limbs, and huddling with little movement. Some BALB/c pups developed patches of alopecia on the head and snout, while others developed swollen paws on their hind legs. During the latter half of the 1 month period of postnatal observation, petechiae appeared on the ears and tail of some NYLAR and C57 pups. Several terminally ill BALB/c pups exhibited a slight, blood-tinged bubbling exudate from the nares suggesting intrapulmonary hemorrhaging. The nasal bleeding was rarely seen in the NYLAR and C57 pups. Many pups of the 3 strains of mice died during the 1 month period of observation. A small portion of the brain of pups found dead was pressed between 2 glass slides and scanned for toxoplasma cysts. In the BALB/c squash preparations, from 0-3 cysts per smear were detected, occasionally necessitating the examination of 2 or 3 press smears to detect parasites. In the NYLAR and C57 squash preparations, 10-20 cysts per smear were usually seen. Although not every macerated fetus or partially eaten pup could be examined, our survey indicates

Table 1 Estimated fetal and postnatal wastage in 3 strains of mice infected with *Toxoplasma gondii* during gestation.

MOUSE STRAIN	GROUPS	GRAVID* DAMS n	LITTERS n	PUPS/ LITTER n	LIVE BORN PUPS n	ESTIMATED** FETAL WASTAGE	POSTNATAL*** WASTAGE
BALB/c	CONTROLS	5	5	11	57	—	—
	INFECTED	18	16	5	97	55 %	84/97 (87 %)
NYLAR	CONTROLS	9	9	11	98	—	—
	INFECTED	22	22	7	153	35 %	46/153 (30 %)
C57	CONTROLS	3	3	10	29	—	—
	INFECTED	7	7	6	41	40 %	14/41 (34 %)

*Gravid dams inoculated on embryonic day 7 with mouse brain emulsion containing cysts of *T. gondii*

**Fetal wastage was estimated by comparing number of pups born to infected dams vs. control dams and calculating percentage reduction.

***The percentage of pups surviving 1 month of postnatal life is misleadingly high because all of the 13 BALB/c pups and several of the NYLAR and C57 pups were comatose when killed.

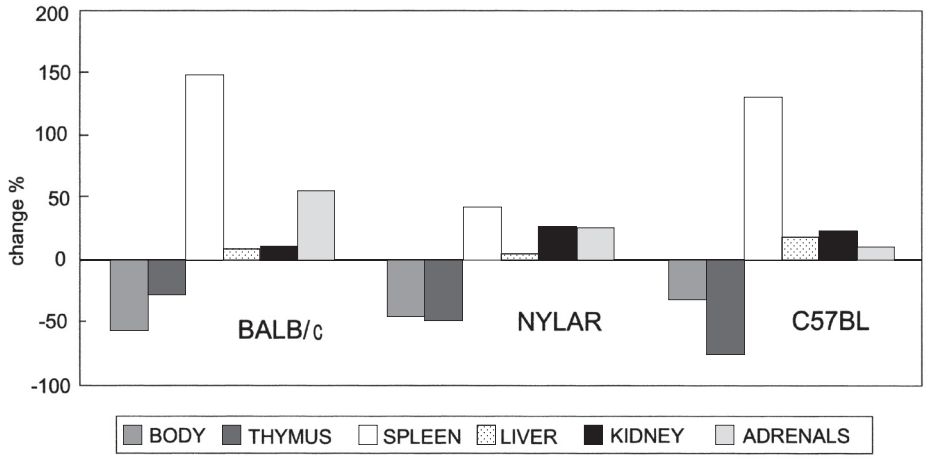


Fig. 1 Percentage changes in body and relative organ weights of 1 month-old mouse pups congenitally infected with *Toxoplasma gondii*. Relative organ weight: wet weight/body weight × 100. Percentage change in organ weights of infected pups were expressed as differences compared with control values set at 100 %.

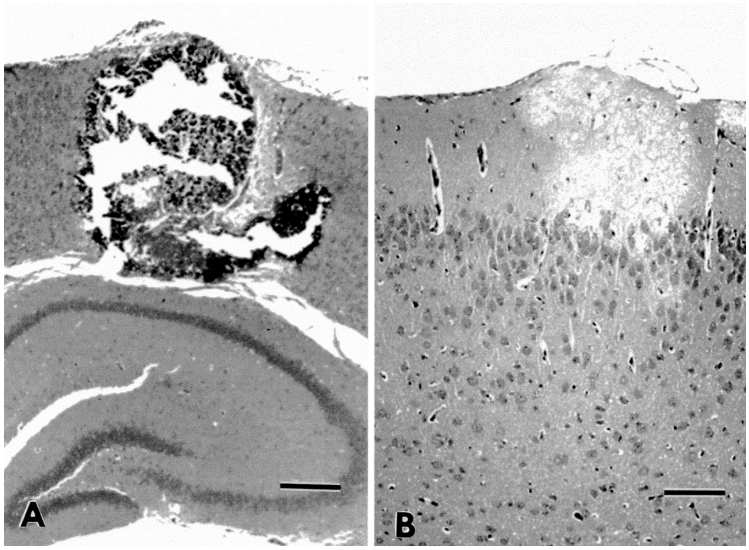
an apparent congenital infection rate of 100 % in the NYLAR, C57 and BALB/c pups.

Body and organ weights of pups at 1 month of age

The percentage change in relative weights of 5 selected organs of the 3 strains of mice are presented in Fig. 1. Among the 3 strains, the BALB/c pups lost the most body weight. In terms of percentage change in organ weights, the BALB/c pups also showed the greatest degree of splenic and adrenal hypertrophy and the least amount of thymic atrophy.

Confirmation of murine strain differences in susceptibility to *T. gondii*

The experimental dams, inoculated with infective (i.e., untreated) brain emulsion on day E7 of pregnancy, were sequestered after parturition. Four months later, the detection of toxoplasma cysts in the brain verified infection. The chronically infected NYLAR and C57 dams underwent progressively severe wasting compounded by hemorrhagic necrosis of the ears and tail, corneal opacities, and hind limb paralysis. Histologic examination of the maternal NYLAR and C57 brains disclosed meningeal infiltration,



Figs. 2A, B Photomicrographs of coronal sections of the cerebrum of 1-month-old BALB/c mouse pups congenitally infected with *Toxoplasma gondii*. **A** Large cavitation in parietal cortex, containing necrotic and calcifying debris. Note absence of inflammation. **B** Clearly demarcated infarction in cerebral cortex. Hematoxylin and eosin. Scale bars 50 μm (A) and 100 μm (B)

perivascular cuffing, ventricular enlargement, and numerous parasitic cysts, findings typical of chronic toxoplasma infection in these 2 susceptible strains of mice [32]. In sharp contrast to the severe disease syndrome in the NYLAR and C57 dams, the chronically infected BALB/c dams were vigorous, healthy in appearance, and symptom-free throughout the 4 months of observation. In the BALB/c brains, despite the presence of scattered parasitic cysts, pathologic tissue changes were not found, confirming the strong resistance of the BALB/c adult mice to the adverse effects of *T. gondii* [14, 29].

Control dams, inoculated either with frozen-thawed infected brain emulsion or with normal brain emulsion, did not exhibit any fetal wastage or signs of illness before or after parturition nor were parasites or pathologic changes found in the CNS 4 months later. The absence of parasites confirmed that repeated freeze-thawing kills toxoplasma, and the lack of overt neuropathology alleviated concern over the possible presence of viral contaminants in the brain emulsion [19].

Histopathologic findings in congenitally infected pups

CNS: Marked pathologic changes were found in the cerebrum of every pup. The most conspicuous anomalies were numerous cystic cavitations and large necrotic and mineralizing lesions scattered throughout the gray and white matter of the cerebrum. The basal ganglia and periventricular regions were invariably affected. Some necrotic masses, with diameters exceeding 1 mm, were found in the cortical parenchyma, extending from the roof of a lateral ventricle to the meninges and grossly disrupting the architecture of the intervening hippocampus and corpus callosum (Fig. 2A). The numerous areas of infarction (Fig. 2B) likely are the forerunners of the many cystic cavitations riddling the CNS of the pups. The smaller cavitations revealed a tenuous meshwork of collagenous trabeculae and threadlike glial processes but otherwise were largely free of necrotic debris. Many cavitations contained sheaves of filariform spicules undergoing dystrophic calcification. Many of the cystic cavitations were encircled by reactive (GFAP-positive) astrocytes and large macrophages. Typically, neither parasites nor inflammatory cells were detected in or around these cystic cavities and necrotic masses. In the NYLAR and C57 cerebrums, an occasional small glial nodule containing flakes of amorphous debris was occasionally encountered. Some of the flakes of debris stained weakly for toxoplasma antigen. Less

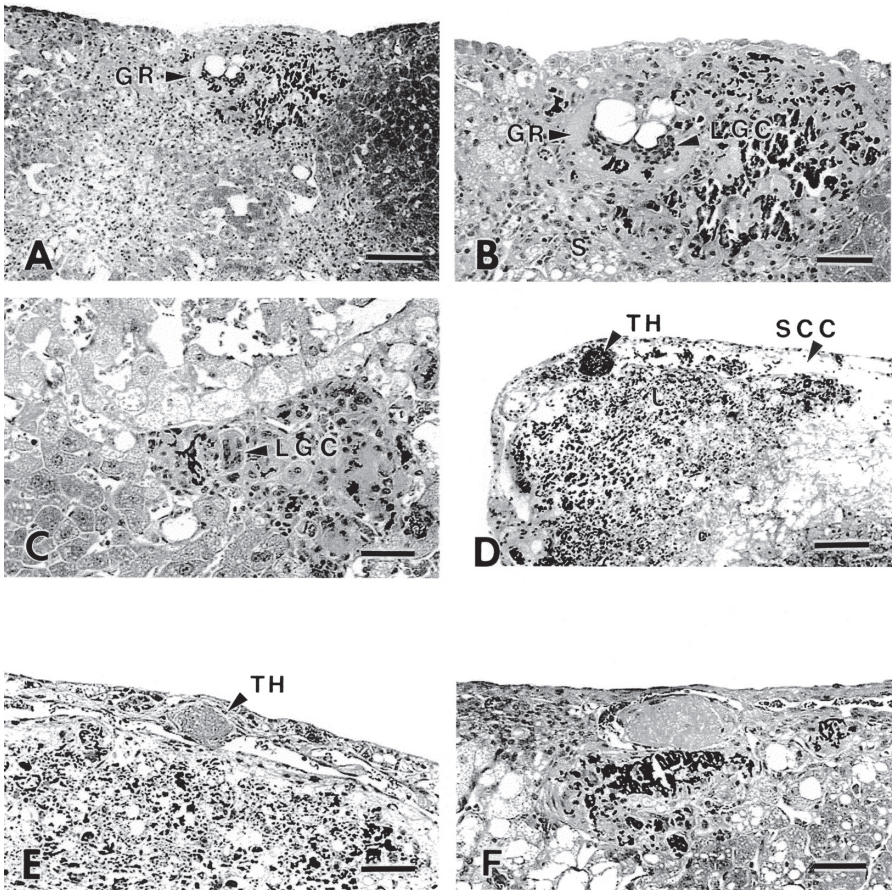
commonly, aggregates of tachyzoite-infected cells, poorly visualized by light microscopy but recognized after immunohistochemical staining, were observed adjacent to or within the glial nodules. These aggregates of parasites were not found in the BALB/c brains. In the susceptible strains, a mononuclear cell infiltration of the meninges and mild perivascular cuffing of some blood vessels in the hippocampus were seen. Among the most destructive and bizarre lesions in the cerebrum were periventricuIar cavitations rupturing into the lateral ventricles. Extensive segments of the ventricular walls were affected, especially around the angles of the ventricles. The subependymal astrocyte precursor cells normally bordering the dorsolateral angle and lateral wall of the lateral ventricle were either absent or greatly reduced in number. With the loss of ventricular integrity, marked edematous changes in the dorsal aspects of the ventricles were seen, greatly affecting the myelinated fibers of the corpus callosum. It is of interest to note that the most pronounced and disruptive ventricular lesions occurred in the BALB/c pups. In all the pups, small clumps of subependymal cells were occasionally detected floating free within the ventricles or closely approximating the ventricular wall. Some clumps had flattened into a layer of cells over areas exhibiting ependymal disruption. In the cerebral cortex, cytoarchitectural disarray and reductions in pyramidal cell numbers were observed in the hippocampus. The third ventricle also was the site of unusual changes, particularly in the BALB/c pups. Ventricular enlargement, periventricular edema, intraluminal clumps of cells, lengthy breaches in the ependymal layer, and bilateral fluid- filled appendage-like cavities were seen.

The cerebral vasculature in the 3 strains of mice exhibited mural thickening, calcinosis, and endothelial cell hypertrophy and discontinuity. Many blood vessels throughout the cerebrum, including some of the larger vessels penetrating the cortex, were swollen and congested with "foamy" macrophages (lipophages), mononuclear cells, erythrocytes and, occasionally, strands and clumps of fibrin. In blood vessels in the basal ganglia, margination of mononuclear cells was seen in several mice. Within the edematous perivascular spaces, numerous foamy macrophages and swollen astrocytic foot processes were observed. The cerebellar cortex was marked by the persistence of remnants of the external germinal layer, ectopic granule cells, swollen and fragmented astrocytic foot processes, and focal edematous gaps in the Purkinje layer. Granule cell necrosis was noted within some cerebellar folia. These anomalies were present in the 3 strains of mice.

LIVER: Hepatic pathology in the congenitally infected pups ranged from tiny foci of calcification in 4 of 41 C57 pups to marked destruction of hepatocytes, lobular disarray, and large calcifying lesions in 13 of 13 BALB/c mice. The NYLAR pups exhibited lesions similar to those seen in the BALB/c mice, but reduced in magnitude and restricted to the subcapsular margins and to the narrow angles of the liver lobes and their wedges of hepatic tissue. The pathologic changes seen in the BALB/c pups were characterized by extensive areas of necrosis and calcification, microvesicular steatosis, sinusoidal dilatation with influx of eosinophils, and numerous granulomas, many with giant cells (Figs. 3A-C). Hepatocyte necrosis and calcifying lesions were marked along the margins of the liver, with broad extensions of necrosis penetrating deep into the parenchyma. Scattered islands of monocytes and lymphocytes, rimmed by neutrophils, were frequently seen in and around the portal tracts and around the central vein. In the subcapsular sinuses, especially in the acute angles of the liver, amorphous clumps of varying sizes were found either floating free or blocking the subcapsular channels (Figs. 3D-F).

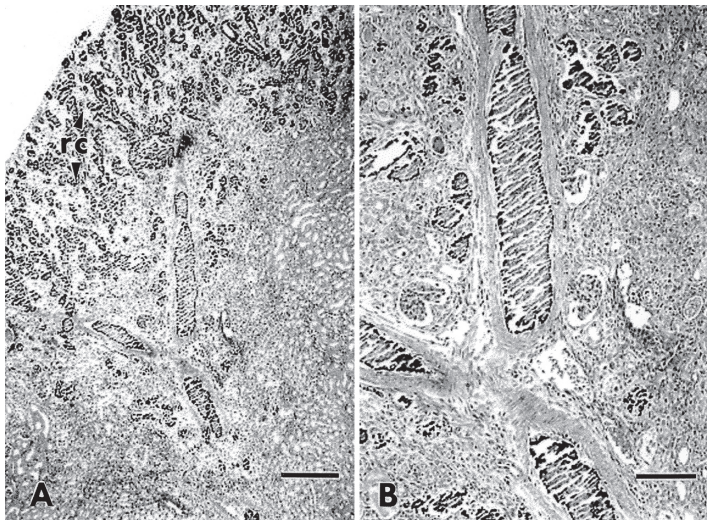
KIDNEY: Renal changes ranged from: miniscule and rare in the C57 strain: scattered foci of calcification in the NYLAR strain: to extensive areas of glomerular and tubular necrosis and calcification in the BALB/c strain. In some BALB/c pups, there were areas of massive necrosis in the renal cortex, with calcification of degenerating tubules and glomeruli (Figs. 4A, B). Calcifying foci were also present in the renal medulla as well. In none of the pups, regardless of strain, were parasites or parasitic antigens detected in the kidney.

SPLEEN: Splenic changes in the congenitally infected pups consisted of depletion of lymphoid cells in the white pulp and expansion of the red pulp, resulting in slight

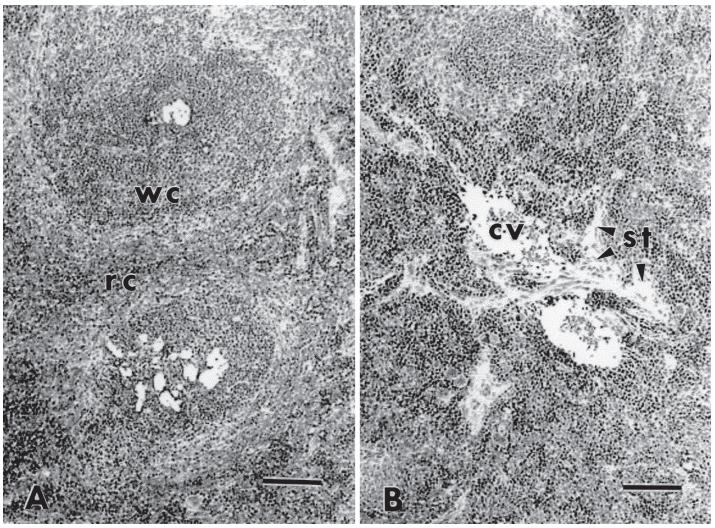


Figs. 3A-F Photomicrographs of sections of liver of 1-month-old mouse pups congenitally infected with *Toxoplasma gondii*. **A** BALB/c mouse: subcapsular granuloma (GR) and focal calcification. Extensive cellular destruction at left, normal-appearing hepatocytes at right. Within the granuloma are 2 vacuolated Langhan's giant cells, each with multiple nuclei. **B** Higher magnification of the granuloma (GR) and Langhan's giant cells (LGC) shown in (A). Hepatocyte degeneration does not appear due to direct infection by *T. gondii*. **C** BALB/c mouse: Langhan's giant cell (LGC) in hepatic granuloma. The multiple nuclei are arranged in the shape of a horseshoe. Hepatocyte degeneration is seen above the granuloma. **D** NYLAR mouse: Thrombus (TH) in subcapsular channel (SCC) of liver. Large area of necrosis and calcification of hepatocytes in wedge of liver. **E** NYLAR mouse: Thrombus (TH) blocking subcapsular channel. Extensive hepatocyte necrosis and calcification in subcapsular parenchyma has obliterated hepatic cytoarchitecture. **F** BALB/c mouse: Large thrombus situated above a calcifying granuloma. Note numerous cavitations in nearby parenchyma. Hematoxylin and eosin. Scale bars 100 μm (A, D, E) and 50 μm (B, C, F)

blurring of the white and red compartments. Numerous multi-lobulated cavitations were observed, many within lymphoid follicles (Fig. 5A). In the NYLAR and BALB/c mice, lymphoid thinning resulted in the emergence of splenic trabeculae from beneath the covering mantle of cells (Fig. 5B). In the BALB/c and NYLAR spleens, vaguely defined granulomatous swirls of large, pale cells were found, some enclosing multi-nucleated giant cells. Parasites were found in 3 of 31 NYLAR spleens. In 1 pup, a small cyst was detected abutting the splenic capsule; a few scattered infected cells were seen in the others. The few infected cells were visualized only in sections stained with PAS. Neither cysts nor infected cells were found in spleen sections of the BALB/c or C57 pups. Histopathologically, the spleens in the C57 pups seemed the least affected; the changes



Figs. 4A, B Photomicrographs of section of kidney of 1-month-old BALB/c mouse pup congenitally infected with *Toxoplasma gondii*. **A** Wide area of tubuloglomerular necrosis and calcification in renal cortex (RC) with distortion of renal architecture. **B** Higher magnification of (A). Extensive tubular calcification and obliteration of glomeruli. Infiltration of inflammatory cells not seen. Hematoxylin and eosin. Scale bars 100 μm (A) and 50 μm (B)



Figs. 5A, B Photomicrographs of cross sections of spleen of 1-month-old BALB/c mouse pups congenitally infected with *Toxoplasma gondii*. **A** Distinction between red (RC) and white (WC) compartments is blurred. Cavitations have developed in white compartment. **B** Marked depletion of lymphocytes has led to emergence of splenic trabeculae (ST) from beneath covering mantle of white cells. There is marked blurring between red and white compartments; note the 2 large cavitations (CV). Hematoxylin and eosin. Scale bars 100 μm (A, B)

in the NYLAR pups the most pronounced.

DISCUSSION

Based on pilot experiments with NYLAR mice, it was anticipated that congenitally acquired toxoplasmosis in the susceptible NYLAR and C57 strains would result in fetal wastage and progressive postnatal morbidity. What was *not* anticipated was that the "resistant" BALB/c mice would experience greater pre and postnatal morbidity and the most devastating pathologic changes. The gross disruption of cerebral architecture by calcifying cystic cavitations, ventricular malformations, periventricular edema, microvascular pathology, and calcifying granulomatous hepatic lesions clearly were most pronounced and destructive in the BALB/c pups. Hepatic pathology of the nature and magnitude seen in the BALB/c pups has not been reported previously in the literature on congenital toxoplasmosis, with 1 notable exception. Cowen and Wolf [9], in a detailed study of congenital murine toxoplasmosis, alluded to "unusual and conspicuous changes in the livers of at least 75 % of young mice". Unusual changes were also found in the kidneys of some mice. Their detailed description of the hepatic and renal changes could, in large measure, be used to describe the pathologic changes observed in the BALB/c pups in the present study. It is also noteworthy that Cowen and Wolf conjectured that the changes were related to the presence of parasites in the mice, but not to parasitic infection of the liver per se. In the intervening 5 decades since their report, most studies on congenital toxoplasmosis have focused primarily on the CNS, with less attention to the liver and/or other tissues.

In the present study, the extraordinary hepatic and renal lesions were not limited to the BALB/c strain. Similar changes were seen in the NYLAR pups as well, but they invariably were of lesser magnitude and restricted to the narrow wedges of tissue at liver margins. The lesions in the NYLAR pups may reflect a midpoint in the development of the massive and destructive terminal changes affecting the BALB/c mice. In the C57 pups, a mouse strain highly susceptible to *T. gondii*, a few small calcifying nodules were found in the liver of 4 of 41 pups, although otherwise the livers appeared normal. It is also intriguing that the CNS changes noted in the pups of the 2 susceptible strains are reminiscent of the vascular, periventricular and cortical lesions observed in the brain of the wasting, terminally ill NYLAR and C57 maternal mice chronically infected for 4 months. In marked contrast, the destructive cerebral and hepatic lesions in the BALB/c pups had no discernable counterparts in the adult BALB/c dams. How then to interpret the striking pathological, and seemingly paradoxical, changes in the congenitally infected pups ? As a working hypothesis, we offer the following tentative sequence of events in explanation of our observations. After inoculation of infective brain emulsion into the gravid dams, parasites reached placental tissues via maternal leukocytes [13, 15]. In the placenta, maternal cytokines, in concert with parasites, fever, circulating toxins (toxoplasma antigens ?), and hormonal and metabolic disequilibrium, may have disturbed placental circulation and adversely affected the uterine environment [10, 22]. Placental insufficiency was indicated by the intrauterine growth retardation, fetal deaths and resorptions, abortions, and stillbirths. In addition, maternal cytokines produced in the course of an infection during pregnancy may have harmful effects on the developing brain of the fetus [1, 10]. From placental foci, parasites were disseminated throughout the fetal vasculature by hematogenous transport, leading to widespread endothelial cell and tissue invasion. Pups surviving the prenatal insult developed a progressive disease syndrome characterized by growth retardation, profound cachexia, hepatosplenomegaly, petechial lesions of skin, bloody nasal exudate, and extensive pathological changes in the CNS, liver, and other organs. The initial cellular damage caused by the parasite stimulated an outpouring of proinflammatory cytokines from endothelial cells, splenic and hepatic macrophages, and T cells which evoked a strong systemic inflammatory response, i.e., a toxoplasmic sepsis. The septic condition, a progressive and injurious systemic inflammatory process affecting the microvasculature of organs throughout the body, is orchestrated by an array of cytokines and other vasoactive mediators which cause vasodilation and loss of plasma volume due to increased vascular permeability [20, 37]. The vascular changes lead to tissue edema, hypotension, and the breakdown of normal

hemostasis, resulting in intravascular coagulation, hypoxic-ischemic death of cells in various tissues, and multiple organ dysfunction [24]. Further, it has been shown that a cellular constituent of *T. gondii* acts as a "superantigen", and activates large numbers of T cells [12]. Whereas conventional antigen specifically stimulates a small percentage of T cells, superantigen non-specifically stimulates massive numbers of T cells to produce equally massive amounts of cytokines [21]. It was postulated [11] that an overproduction of cytokines contributed to the pathogenesis of toxoplasmosis. The hypothesis was based on observations indicating that the high mortality of *T. gondii*-infected gene knockout mice was not due to an increase in parasite burden but to an inability to down-regulate cytokine production, resulting in cytokine overproduction [18, 31]. Pertinent to the sepsis thesis are early case reports describing systemic dissemination of the parasite, endothelial invasion, symptoms suggestive of a septic syndrome, and multi-organ involvement in congenitally infected human infants [13, 27], plus recent descriptions of a severe sepsis associated with disseminated toxoplasmosis in AIDS patients [2-4, 7, 23]. We contend that the numerous cystic cavitations, the most conspicuous pathologic lesions in the postnatal CNS, are not due to the invasion and destruction of brain cells by toxoplasma tachyzoites, but are the sequelae of microinfarcts resulting from a disseminated vasculopathy. Endothelial disruption, caused by parasite invasion, leukocyte adherence, and cytokine-mediated damage, very likely led to breakdown of normal hemostatic mechanisms and the intravascular formation and deposition of thrombi in the microvasculature of the immature pups. The thrombi occluded blood vessels and resulted in the development of ischemic infarcts, not only in the brain but throughout the pup. The subsequent removal of necrotic tissue by macrophages from areas of cerebral infarction left the numerous cavities. The multicystic encephalopathy, present in varying degree in the 3 strains of pups, has been reported as a sequela of other congenital infections [30] and clearly is not unique to congenital toxoplasmosis. The small hemorrhagic petechiae appearing belatedly on the head, ears, and tail of the pups, plus the apparent intrapulmonary hemorrhages evinced by the bloody nasal exudate, implies a bleeding diathesis caused by the consumption of serum clotting factors [26]. The consumption of clotting proteins may lead to bleeding, so both clotting and bleeding can occur simultaneously in the affected mouse. We suggest that the pathogenesis of congenital toxoplasmosis, in our mouse models, can be ascribed to the adverse effects of maternal cytokines and placental insufficiency on prenatal development, and to microvascular dysfunction and loss of hemostatic regulation leading to postnatal perfusion failure. We further suggest that contributing to the severe pathological changes and developmental perturbations in the physiologically immature pups may have been the overproduction, or insufficient downregulation, of 1 or more cytokines orchestrating the putative toxoplasmic sepsis.

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