

Humoral Antibody Responses and Transmission of *Toxoplasma* from Infected Mother Rats to Their Fetuses and Infants¹⁾

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トキソプラズマ感染ラットにおける虫体の胎仔移行
と体液性抗体について

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INTRODUCTION

It is generally considered that congenital toxoplasma infection of infants occurs only when their mothers have suffered acute infection with *Toxoplasma (T.) gondii* during pregnancy, leading to the birth of infected animals.²⁾ It is also reported that the presence of IgM toxoplasma antibody in a newborn infant indicates congenital toxoplasmosis, although in some cases of toxoplasmosis the IgM antibody is absent.³⁾

In previous papers by the authors,^{3,5,7)} the humoral antibodies to toxoplasma in adult rats were observed in IgM alone on the 5th day, in both IgM and IgG on the 7th day, and then in IgG alone from the 28th to the 140th day postinfection. Fetuses and newborn infants from rats with chronic toxoplasmosis showed titers in IgG alone, which remained for 3 weeks after birth, showing a tendency to gradually decrease, disappearing at 5 weeks.

In order to determine the specific period, during which toxoplasma infection in mother rats leads to the positive transmission of parasites to newborn infants, the authors conducted the present study. This study was also performed in order to determine whether the maternal IgG antibody present in newborn infants from chronically infected rats, effects in any way, the antibody responses of the infants to toxoplasma infection.

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MATERIALS AND METHODS

Toxoplasma-infected rats

A total of 60 adult female rats of the Wistar-Imamichi strain over 8 weeks of age were used in the experiments. They were inoculated intraperitoneally with approximately 2.5×10^7 parasites of the RH strain, obtained from the peritoneal fluid of mice on the 2nd day of infection.

Toxoplasma parasites were isolated either from tissues which were taken from the fetuses and the placentas of the mothers, or from pooled samples of the spleen and brain of 3 infants, by mouse inoculation.

Antibody titers to toxoplasma were examined by the dye test and indirect immunofluorescent methods.

Experiment 1

Forty-five adult female rats were divided into 4 groups for the 1st experiment. In group 1, 20 adult rats were examined each week from the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th, 12th, 14th and 16th weeks postinfection (p.i.). They were crossed with male rats on the 6th week p.i., and all got pregnant. Three of them and their fetuses were examined during pregnancy, immediately before delivery. The other rats and their newborn infants were examined on the 1st, 3rd, 5th and 7th weeks after delivery.

In group 2, six adult female rats were crossed with male rats 4 to 7 days p.i., and only two of them got pregnant. These two mother rats and their fetuses were examined to isolate toxoplasma parasites.

In group 3, eleven adult female rats were crossed with male rats 4 to 7 days prior to infection. Four of them and their fetuses were examined one week p.i., and the seven remaining mother rats and their infants were examined periodically to isolate the parasites.

In group 4, eight female rats were crossed with male rats 14 to 18 days before infection with toxoplasma. Four of the eight mother rats and their fetuses were examined one week p.i., and the rest of the rats and their infants were examined periodically. All the infants in group 4 died within 10 days after birth.

Experiment 2

One to two day old infants from normal and toxoplasma chronically infected mother rats were infected intraperitoneally with 10^3 parasites of the RH strain. They were examined on the 1st, 2nd, 3rd, 5th and 7th weeks p.i., respectively.

RESULTS

Humoral antibody responses in fetuses and infants of infected female rats

Toxoplasma antibodies used in the dye test and the indirect immunofluorescent

methods obtained from the rats are shown in Chart 1. In group 1, SF dye titers were shown from the 1st to the 16th week p. i. By the indirect immunofluorescent method, toxoplasma antibodies were observed in both IgM and IgG on the 1st and 2nd weeks, and were later observed in IgG alone from the 3rd to the 16th week p. i. The fetuses of mother rats on the 8th weeks p. i. showed a titer of 1:64 in the dye test and 1:1000 in the IgG immunofluorescence. The antibody activities of newborn infants from mother rats in the 9th week p. i. were observed in IgG alone, showing a tendency to decrease gradually until the 5th week. No titers were detected by the dye and immunofluorescent tests on the 7th week after birth. Toxoplasma parasites were not found in any fetuses or the placentas of 10 mother rats examined on the 7th to 8th week p. i., or in infants from 10 mother rats in the 9th week p. i.

Six adult rats in group 2 were infected with toxoplasma one week before crossing, and 2 of them got pregnant. The two pregnant rats and their fetuses were autopsied on the 3rd week p. i. Antibody activities in the fetuses, from which parasites were isolated, were observed in the titers of IgG immunofluorescent test and the dye test on the 3rd week p. i., showing no titer of IgM antibody in the serum.

Rats in group 3 were infected with toxoplasma on the 1st week after pregnancy. Of the 11 female rats and their fetuses, four were examined in the 1st week p. i. The other seven mother rats and their infants were examined on the 1st to 7th weeks after birth. Antibody titers in the dye and the IgG tests in mother rats showed a tendency to increase to the 3rd week p. i., remaining moderately high until the 7th week p. i. IgM antibody titers were observed in the 1st to 3rd weeks p. i., and then the titer was not again detected until the 7th week p. i.

Toxoplasma antibodies of mother rats in group 4, those infected with toxoplasma in the later stage of pregnancy, appeared rapidly in the IgM immunofluorescent titers up to the 2nd week p. i., and maintained a high activity of the IgG and the dye titer until the 8th week p. i. The infants from which the parasites were isolated, showed a titer of 1:64 in the dye and IgG tests one week after birth, although showing no IgM antibody in the serum. All infants in this group died within 10 days after birth.

Transmission of the parasites to the fetuses and infants in groups 1, 2, 3 and 4

Percentages of the isolation of parasites from fetuses and infants of the toxoplasma-infected mother rats are shown in Table 1. In group 1, 10 mother rats and their fetuses and infants from ten other mother rats were examined. No parasites of toxoplasma were found in any of the fetuses or the placentas in the 10 rats examined from the 7th to the 8th week, nor in infants from the 10 other rats on the 9th week p. i.

Two of the 6 mother rats in group 2 were pregnant, and their fetuses were examined to isolate parasites. Toxoplasma parasites were isolated from the fetus of one rat, indicating 50 percent of parasite isolation.

GROUP HUMORAL ANTIBODY TITERS	Pregnancy Period																Weeks postinfection
	8	7	6	5	4	3	2	1	0	9	10	11	12	13	14	15	
1																	
2.5 × 10 ⁷ RH Infection																	
Mother rats	0	4000	4000	16000	16000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000
SPT	0	256	4000	16000	16000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000
IgM	0	256	16	< 4	< 4	< 4	< 4	< 4	< 4	< 4	< 4	< 4	< 4	< 4	< 4	< 4	< 4
IgG	0	16	1000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000
Fetus and Infants																	
SPT					64						64				16		< 4
IgM					< 4						< 4				< 4		< 4
IgG					1000					1000					256		< 4
2																	
2.5 × 10 ⁷ RH Infection																	
Mother rats																	
SPT																	
IgM																	
IgG																	
Fetus and Infants																	
SPT																	
IgM																	
IgG																	
3																	
2.5 × 10 ⁷ RH Infection																	
Mother rats																	
SPT																	
IgM																	
IgG																	
Fetus and Infants																	
SPT																	
IgM																	
IgG																	
4																	
2.5 × 10 ⁷ RH Infection																	
Mother rats																	
SPT																	
IgM																	
IgG																	
Fetus and Infants																	
SPT																	
IgM																	
IgG																	

64 < 4 § All infants died within 10 days after birth

64

64

Chart 1 Humoral Antibody Responses in Fetus and Infants from Mother Rats Infected with *Toxoplasma gondii*

Table 1. Percentage of the parasite isolation in the fetus and infants from the toxoplasma-infected mother rats

Group	1	2	3	4
Pregnancy rats/No. of rats used	20/20	2/6	11/11	8/8
No. of fetus per mother rat	10-16	6	10-14	10-14
No. of infants per mother rat	6-12		7-12	3-15
Isolation of the Parasites				
No. of isolated case/No. of examined				
Fetus	0/10	1/2	4/4	4/4
Infants	0/10		7/7	4/4
Percentage of the parasite isolation				
Fetus	0	50	100	100
Infants	0		100	100

Note: As for the examination of the parasite isolation, each 3 fetus or infants were pooled as a sample.

Fetuses and infants from 11 and 8 mother rats in groups 3 and 4, respectively, were examined. Toxoplasma parasites were isolated from all fetuses and infants in groups 3 and 4, showing 100 percent parasite isolation.

Antibody responses to toxoplasma in newborn infants

As shown in Chart 2, all infants, 1 to 2 days after birth, from normal and toxoplasma chronically infected mother rats were inoculated with 10^3 parasites. In 21 infants from normal mother rats, toxoplasma IgM antibody immunofluorescent titers were first detected in the 2nd week p.i., showing a maximum titer of 1:256 in the 3rd week p.i. Thereafter the titers showed a tendency to decrease gradually until the 5th week p.i., showing no titer in the 7th week p.i. The dye test and the IgG im-

Group	*Tp infection					
	Delivery	1	2	3	5	7
1 Infants from chronically infected mother rats						
Antibody titers						
SFT		1:4000	1:4000	1:4000-256	1:4000-64	1:1000-4
IgM	<1:4	<1:4	≤1:4	<1:4	<1:4	<1:4
IgG	1:1000	1:1000	1:1000-256	1:1000-16	1:1000-4	1:1000-4
Isolated case of Tp/ No. of examine	6/6	5/5	5/5	4/5	0/5	
2 Infants from normal mother rats						
Antibody titers						
SFT	1:4	1:256	1:1000-256	1:4000	1:4000	1:4000
IgM	<1:4	1:16	1:256-64	1:4	<1:4	<1:4
IgG	1:4	1:256	1:1000	1:1000	1:4000	1:4000
Isolated case of Tp/ No. of examine	6/6		5/5	5/5	5/5	

*All infants were infected with 10^3 trophozoites of the RH strain within 2 days after delivery.

Chart 2. Humoral Antibody Responses in Infants from Normal and Toxoplasma Chronically Infected Mother Rats

munofluorescent antibody titers showed a tendency to increase gradually from the 1st week p. i., maintaining a plateau from the 5th to the 7th weeks p. i. Toxoplasma parasites were isolated from all infants until the 7th week p. i.

In infants having maternal IgG antibody from chronically infected mother rats, with the exception of a single case which showed a titer of 1:4 on the 3rd week p. i., toxoplasma IgM immunofluorescent antibody was not detected at all up to the 7th week p. i. As for the dye test and the IgG antibody titers, a tendency to decrease gradually from the 3rd to the 7th week p. i. was observed. Toxoplasma parasites were isolated from all the infants up to the 3rd week p. i. Although parasites were found in one infant in five on the 5th week p. i., none were found in infants on the 7th week p. i.

DISCUSSION

In normal adult female rats infected with *T. gondii*, remarkable increases of toxoplasma antibody titers in the dye and IgG immunofluorescent tests were observed in the acute to chronic phases. Toxoplasma IgM antibody appeared within one week p. i. in adult rats, and then decreased gradually until the 3rd week p. i. These IgM and IgG antibody responses to toxoplasma in adult rats, as well as those reported by other authors,^(1,5,7) indicate that antibodies against toxoplasma, as detected by the indirect immunofluorescent tests, were produced in general response to infection with per-acute toxoplasmosis or with encysted or chronic toxoplasmosis.

The IgM antibody response in infants from normal mother rats was first detected in the 2nd week p. i., showing a maximum titer on the 3rd week, and towards a gradual decrease up to the 5th week p. i. There was a clear tendency of delay in appearance of IgM toxoplasma antibody in newborn infants as compared with that in adult female rats.

As for the transmission of toxoplasma infection from chronically infected rats to their fetuses and infants, no case of congenital toxoplasmosis was detected in 63 pooled samples born from mother rats chronically infected with *T. gondii*. Parasites were isolated in the fetus of one of the 6 mother rats impregnated 4 to 7 days p. i. When mother rats were infected with toxoplasma during pregnancy, the parasites were transmitted to the fetuses and the infants in 100 percent of the cases. Taking into consideration the present results, it is the authors' opinion that congenital toxoplasmosis in infant rats would be induced by the time of toxoplasma infection, that is the period of pregnancy.

The present studies were performed on fetuses *in utero* and newborn infants collected from rats with chronic toxoplasmosis possessing antibodies of IgG origin. Only IgG antibody to toxoplasma was demonstrated in the fetuses and infants, from which no parasites were isolated. Thus, such infants, when they were infected with

T. gondii, did not produce any IgM toxoplasma antibody until the 7th week p.i. Although the dye and IgG antibody titers showed a tendency to maintain high levels up to the 7th week p.i., these antibodies in some cases decreased gradually from the 3rd week p.i. In consequence, the humoral antibody response in the newborn infants with maternal IgG antibody may partially or totally suppress their IgM response to infection. The maternal IgG may also totally suppress the survival of toxoplasma parasites in the infants on the 7th week p.i., at which time no parasites were isolated. However, it is not known whether the maternal IgG alone totally prevent proliferation of toxoplasma in the infants. Further research is necessary in order to clarify the relationship between the maternal IgG and suppressor T-cells in the sensitized lymphocytes concerning to the cell mediated immunity.

SUMMARY

Humoral antibodies to toxoplasma in adult female rats were produced in IgM initially followed by IgG in general response to infection with peracute toxoplasmosis or encysted or chronic toxoplasmosis. In normal newborn infants there was a clear tendency of delay in appearance of IgM antibody as compared to adult female rats. When infants from chronically infected mother rats were infected with *T. gondii*, IgM antibody was not detected until the 7th week postinfection. In the transmission of toxoplasma infection from chronically infected rats to their fetuses and infants, no cases of congenital toxoplasmosis were detected. When mother rats were infected with toxoplasma during pregnancy, the parasites were transmitted to the fetuses and infants in 100 percent of the cases.

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摘 要

トキソプラズマ (Tp) 虫体の感染に対して、極めて抵抗性の強いラットを用いて Tp 虫体の母仔間移行の有無と、それらのラットにおける体液性抗体の出現について検討した。すなわち、実験は Wistar-Imamichi 雌成熟ラットの妊娠直前 (Group 2)、妊娠初期 (Group 3)、妊娠後期 (Group 4) に Tp 2.5×10^7 個感染したものと慢性期妊娠 (Group 1) に分け、各 Group の胎仔・新生仔について行った。

Group 2 は 6 例のうち 2 例が受胎し、その 1 例の胎仔から虫体を分離した。Group 3 と 4 では、検索妊娠母ラット 19 例全例の胎仔・新生仔へ Tp 虫体の移行を認めた。Group 3 での胎仔・新生仔出生頭数および出生後の存命も、対照健全群と相異なかったが、Group 4 では出生頭数が極めて少い例および出生後 10 日以内に全例死亡・食殺された。Group 1 では、胎仔・新生仔から虫体は分離されなかった。

一方、血清中 Tp 抗体では虫体移行新生仔は生後 7 週まで、IgG 抗体の高値と、3 週目を頂点とする IgM 抗体が出現した。虫体移行が認められない慢性 Tp 症母ラットより出生した新生仔では、IgM 抗体は検出されず母仔間移行 IgG 抗体が出生後 5 週目まで漸減する傾向を認めた。

ついで、移行 IgG 抗体の新生仔 Tp 感染に対する抗体 Response を検討するために、生後 1~2 日の移行抗体所有新生仔および健康新生仔に 10^3 個の Tp 虫体を感染した。健康新生仔では、Tp 感染後 2 週目より 3 週目を頂点とする IgM 抗体の出現と IgG 抗体の漸増が観察された。しかるに、移行抗体所有新生仔では、IgM 抗体の出現は 1 例も観察されなかった。IgG 抗体は 6 例のうち 4 例では実験期間中、高値を保持した。残りの 2 例では IgG 抗体の漸減が観察された。