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Studies on Protective Reaction against *Plasmodium berghei* in Rat Neonates

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Introduction

It is well known that maternal antibody takes a part in the protective reaction against infectious diseases with antigenic specificity (Solomon, 1971). This mechanism seems to be a kind of passive immunity since the protection against the infectious diseases in infants disappears in accordance with decrease of the maternal antibody in the infants. Many reports have been made about a probable collaboration between cell-mediated reaction and humoral antibody in building the protective mechanisms against malaria (Zuckerman, 1977). The protective antibody congenitally transmitted from a hyperimmunized mother to newborn infants via the placental circulation is IgG, and anti-plasmodial protection is generally associated with raised level of IgG. Transient immunity may be conferred by hyperimmunized mother to newborn infants through transfer of such anti-plasmodial antibody.

On the other hand, in our study of an intracellular protozoan parasite, *Toxoplasma gondii* (Tp), neonates born from rats chronically infected with Tp showed high resistance against Tp-infection for a fairly long time after birth (Omata and Suzuki, 1975). Although it is still unknown whether this type of protection against Tp-infection is attributed to humoral antibody or other factors. The reaction is of great interest to analyse the mechanisms of resistance against protozoan infection.

The present study was undertaken to know the role of various factors which probably effected the development of protective reaction to *plasmodium berghei* (Pb) merozoite infection with use of neonates born of chronically infected mother rats.

Material and Methods

Animals

Sprague-Dawley female adult rats were obtained from Dr. Tanabe, Department of Bacteriology, Faculty of Medicine, Osaka University, and ddO strain mice were supplied from the Breeding Station for Laboratory Animals, Osaka University.

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Parasites

Plasmodium berghei (NK 65) was obtained from Dr. Suzuki, Department of Parasitology, School of Medicine, Gunma University, and maintained by serial blood passage in mice.

Detection of parasites and anemia in animals

Blood for hematological and serological studies was taken from the tail vein of test animals. Blood films were fixed in May-Grünwald solution and stained with Giemsa. Parasitemia was determined by counting the number of parasitized red blood cells (RBC) per 10,000 erythrocytes. Blood was collected with a heparinized capillary tube (DADE B4415-20). After sealing one end of the tube, it was centrifuged at 12,000 rpm for 5 min to determine the packed cell volume.

Pooled specimens of the spleen, liver and blood from littermates were homogemized together by crushing with slide glasses and scissors and the parasites in these materials were isolated by inoculation to splenectomized mice.

Fluorescent antibody level was measured by titrating sera from rats using indirect-fluorescent antibody technic with modification of the original method reported by Makimura (Makimura *et al.*, 1974). Fluorescein isothiocyanate conjugated anti-rat IgG and IgM rabbit sera were prepared in the laboratory (Shindo, 1971). Pb-parasitized RBC which were obtained from mice 7–10 days after infection, were washed 3 times in cooled PBS and air dried smears were fixed in acetone and stored at −20 C until use as antigen.

Experimental procedure

Experiment 1

Female adult rats about 8 weeks of age were inoculated intraperitoneally with approximately 8×10^8 parasitized RBC. They were divided into 3 groups. The 1st group was inoculated 5 days after mating with male rats (pregnancy period was approximatelly 15 days), the 2nd group 10–14 days after mating (pregnancy period was 5–10 days) and the 3rd group was mated 5 weeks after parasite inoculation (pregnancy period was 21 days). The mothers and their neonates were subjected to test for the detection of parasite and for measuring packed cell volume. Malarial fluorescent antibody was titrated immediately after the birth of neonates. Infants in group 3 were used in the succeeding experiments (Exp. 2, 3 and 4) and these infants were tentatively called "immunized infants" or "infants of chronically infected mothers".

Experiment 2

At delivery, normal and immunized infants were inoculated with approximately 4×10^6 parasitized RBC and thereafter examined for parasitemia. Packed cell volume and anti-Pb IgM and IgG titer were also determined.

Experiment 3

Re-inoculation test was tried 7 weeks after birth to immunized infants which had been inoculated with parasites at delivery. The inoculum size was approximatelly 1.5×10^7 parasitized RBC intraperitoneally. Normal and immunized infants which were not inoculated with parasites at birth were used as control animals.

Experiment 4

Within 48 hrs after birth, normal and immunized infants were divided into 3 groups as follows; thymectomized, splenectomized and intact groups in littermates (Rozing *et al.*, 1978: Shindo, 1971). They were inoculated intraperitoneally with approximatelly 4×10^5 parasitized RBC one day after the operation. Examination for parasitemia and determination of anti-Pb IgM and IgG titers were done 1, 2 and 3 weeks after inoculation. At the time of death, or

Table 1 Detected parasites and serum antibody titers in *P. berghei*-infected mother rats and their neonates

Group of rats	Mother rats				Neonate rats			
	Rat No.	Parasitemia	PCV*	Antibody titer at delivery (IgG)	Neonate No.	Parasite isolation	Antibody titer at birth (IgG)	(IgM)
I	1	0	39	1,024	6	+	64	4
	2	1.2	34	1,024	7	—	256	4
	3	0	38	1,024	10	—	64	4
	4	1.4	34	1,024	4	—	64	4
	5†	0	38	1,024	9	—	64	4
II	1	9.6	31	4	8	—	4	4
	2	6.8	28	16	10	—	4	4
	3	5.8	30	16	6	—	4	4
	4	5.1	26	16	6	—	4	4
	5	6.2	35	4	10	—	4	4
III	1	0	35	1,024	7	—	256	4
	2	0	43	1,024	5	—	256	4
	3	0	42	1,024	11	—	256	4
	4	0	39	1,024	8	—	256	4
	5	0	40	1,024	10	—	256	4

*: Packed cell voulme

†: Aborted and died before delivery

Abult female rats were inoculated with 8×10^8 parasitized red blood cells 5 days after mating in Group I, 14 days after mating in Group II and 5 weeks before mating in Group III. Parasitemia, PCV and reciprocal of antibody titer were observed on the lst day after birth.

after the experiment these infants were killed, when still alive, to examine the remnants of thymus or spleen.

Results

Experiment 1: Parasitemia, packed cell volume and serum antibody titers in mothers and neonates (Table 1)

Parasitemia in infected mother rats of Group 1 decreased or disappeared and packed cell volume recovered at nearly normal level. Parasite was recovered in one litter. One mother rat aborted and died before the expected date of delivery. Anti-Pb IgG titer of sera in mothers increased to 1 : 1,024 at the time of delivery. In Group 2, parasites were detected in all mother rats, whose average parasitemia was 6.7% and packed cell volume dropped to 30% on an average at the time of delivery. Anti-Pb IgG titer ranged from 1 : 4 to 1 : 16 in mother rat. In Group 3, no parasitemia was found and packed cell volume was in a normal range at delivery. Parasite was not isolated from neonates. Anti-Pb IgG titer in mother rats was at 1 : 1,024 and their neonates showed 1 : 256 of IgG titer.

Experiment 2: Parasitemia, packed cell volume and serum antibody titers in immunized infants inoculated with Pb at birth (Figs. 1, 2)

No parasitemia was observed for 5 weeks after birth in immunized infants. While, in normal infants the peak parasitemia (6.5%) was found 2 weeks after birth, with gradual decrease until the 5th week (Fig. 1).

In immunized infants, anti-Pb IgG titer which was 1 : 256 at birth, elevated to 1 :

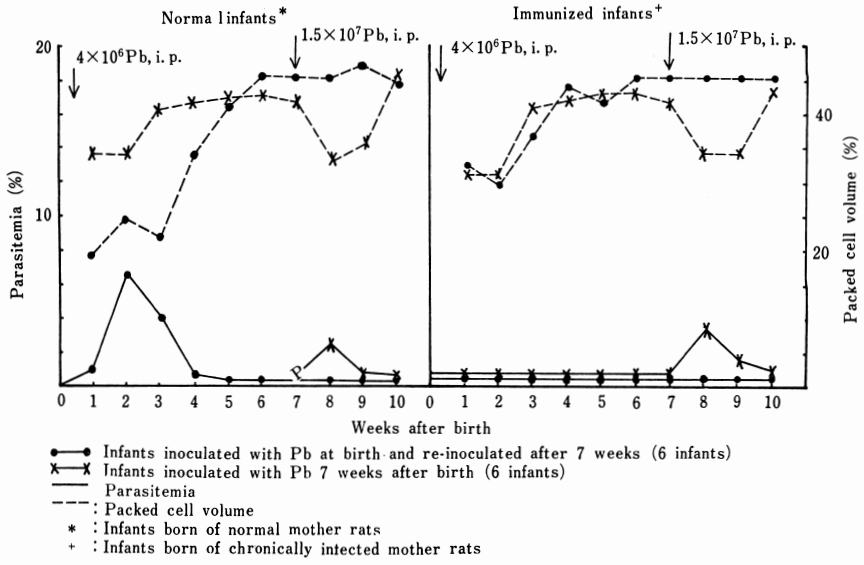

Fig. 1 Parasitemia and packed cell volume in normal and immunized infants infected with *P. berghei*.

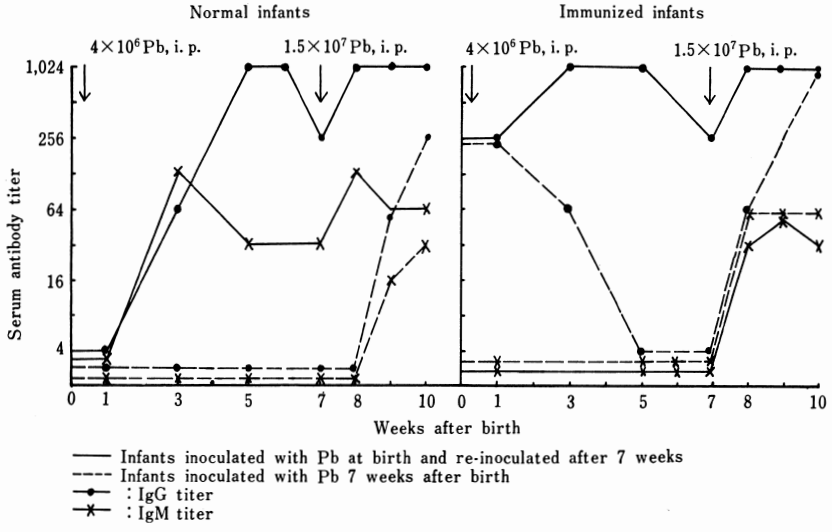

Fig. 2 Serum antibody titers against *P. berghei*-infection in normal and immunized infants, 3 infants of each group were bled and pooled their serum.

1,024 in the 3rd week and was maintained until the 5th week. On the other hand, IgM titer was not detected for 5 weeks after inoculation. In normal infants, anti-Pb IgG titer boosted to 1 : 1,024 in 5 weeks, then turn to 1 : 256 in the 7th week. Anti-Pb IgM was also detected at 1 : 128 titer 3 weeks after inoculation, then the titer decreased to 1 : 32 (Fig. 2).

Experiment 3: Results of re-inoculation test (Figs. 1, 2)

In normal and immunized infants which were inoculated with Pb at birth were challenged again with 1.5×10^7 parasitized RBC intraperitoneally 7 weeks after birth. No parasitemia appeared for 3 weeks after challenge. In control infants, normal and immunized, which were not inoculated at birth but after 7 weeks, parasitemia was found for 3 weeks after challenge. These results were shown in Fig. 1.

Serum antibody titers after inoculation were demonstrated in Fig. 2. IgG titer in re-inoculated normal and immunized infants, which was 1 : 256 at the time of re-inoculation, rose to 1 : 1,024. IgG titer in immunized infants, not inoculated with Pb at birth, which fell to zero at the time of challenge, elevated increase to 1 : 1,024 after challenge. IgM titer in immunized infants which did not manifest any increase after Pb-inoculation at birth, rose gradually to 1 : 64 after re-inoculation. IgM titer in normal infants after re-inoculation showed a tentative increase to 1 : 128, then maintained 1 : 64. IgM titer in normal and immunized infants which were not inoculated at birth, elevated gradually after challenge.

Experiment 4: Effect of thymectomy and splenectomy upon parasitemia and antibody production (Figs. 3, 4)

Immunized infants which were thymectomized, showed parasitemia 1 week after Pb-inoculation, becoming more intensive up to the 3rd week and resulted in death. In splenectomized immunized infants, profile of the development of parasitemia was

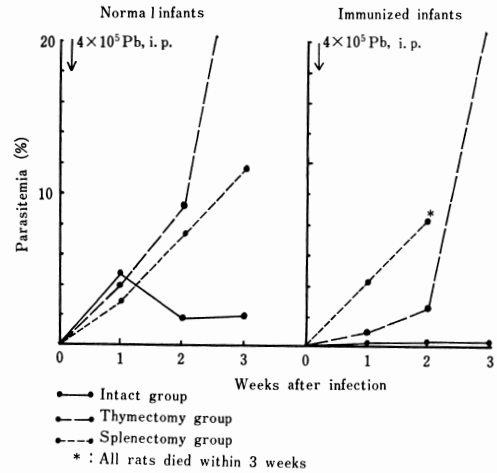

Fig. 3 Parasitemia after *P. berghei*-infection in normal and immunized infants which were thymectomized of splenectomized.

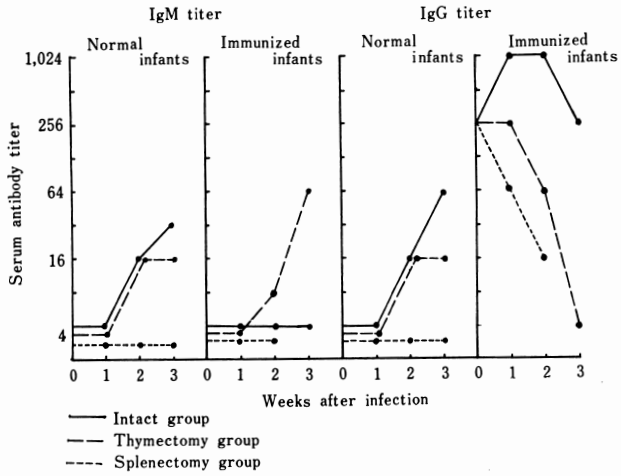

Fig. 4 Serum antibody titers against *P. berghei*-infection in normal and immunized infants which were thymectomized or splenectomized. 4×10^5 parasitized RBC were inoculated i. p. a day after the operation.

similar to those shown by thymectomized new born animals. Some of the infants died before the 3rd week in spite of mild parasitemia, probably due to other causes than Pb-infection (Fig. 3).

Fig. 4 shows IgG and IgM titers in thymectomized and splenectomized infants after Pb-inoculation. In thymectomized normal infants, both IgG and IgM antibodies were found at lower titer than in intact infants. In splenectomized normal infants, however, neither IgG nor IgM production was found.

In immunized infants, IgG titer was 1 : 256 at birth. After Pb-inoculation following to thymectomy or splenectomy, the IgG titer was lowered rapidly as compared with that in intact infants in which IgG after Pb-inoculation was increased to 1 : 1,024.

It was noted that in thymectomized immunized infants, IgM titer showed a tendency of slight increase after the 2nd week of Pb-inoculation. While in intact and splenectomized immunized infants, IgM titer did not show any increase after inoculation.

Discussion

It has been demonstrated that the protection against Pb in the neonates born of mother rats with chronic Pb infection was attributable to maternal anti-Pb IgG which could be transmitted through the placental barrier and also to immunoglobulin contained in milk, as well as soluble Pb antigen which could be transmitted through the placenta, and partially to nutritional condition through the diet which could suppress the development of Pb (Zuckerman, 1977). From these facts, it was thought that the main effect of protection would be due to maternal antibody or activation of immune system in neonates.

The protective antibody to Pb is generally inherent in the IgG fraction which contains anti-Pb precipitin but not in IgM fraction (Hamburger and Zuckerman, 1976). Since the IgG class immunoglobulin is able to pass through the placental barrier, it is believed that anti-Pb IgG which can be transmitted from immunized mothers may destroy the parasites in infants.

Desowitz (1971) reported that infants born of immune mother rats had a significantly higher level of immunity to Pb after vaccination with non-living Pb antigen than unvaccinated littermates or vaccinated infants born of normal mothers. He also postulated that the combined agencies of maternally derived immunity and immunization with non-living vaccine caused the development of the long-term functional immunity. Desowitz (1973) further reported that Pb soluble antigen might cross the placental barrier and sensitize fetal immunocompetent cells. However, according to our previous observations in neonates, the protective response was still present when the maternal Pb antibody had disappeared 5 weeks after birth. In this case, we could not consider the possible effect of transmission of soluble Pb antigen. High Parasitemia in immunized infants, with thymectomy or splenectomy was observed, which resulted in death. This fact would reason a speculation that not only maternal antibody but neonate immune responses took a part in their protective reaction until the 5th week after birth when the maternal antibody had disappeared.

Thymectomy caused the impairment in the development of T-cell population. Neonatal thymectomy can therefore help to define the relative resistance of T-cells to later challenge with Pb. Brown *et al.* (1968) reported that in Pb-infected rats, the reactions most consistently depressed by neonatal thymectomy were cell-mediated immunity and possibly humoral immune responses, especially to particulate antigens, and also other mechanisms through which the thymus might conceivably alter phago-

cytosis and hemopoiesis.

Zuckerman (1977) summarized the various functions of the spleen in plasmodial infection, especially erythrocyte-phagocytosis and splenomegaly characterized by raised IgM titer which would be commonly related to the initial protective function of the spleen. While, Makimura *et al.* (1974) observed that adult splenectomized rats infected with Pb showed anemia and unabated parasitemia until the rats succumbed to death. An early rise in both IgM and IgG antibody titers were also noted. In the present study, however, anti-Pb IgM was not detected in the splenectomized infants until their death. Rozing *et al.* (1978) reported that the spleen provided a highly efficient environment for the differentiation of IgM producing plasma cells during neonatal time. Araujo and Remington (1974, 1975) observed that maternally transmitted IgG suppressed the IgM antibody response in the fetus and newborn infants in Toxoplasmosis.

Our present study suggests that not only the maternal antibody but the neonate host immune systems take a part in protective reaction to Pb infection. It is also postulated that thymocytes during neonatal life can be stimulated by specific antigen and antibody complex to induce the proliferation and maturation of peripheral immune system, especially spleen lymphocytes, or that the peripheral immune systems containing reticuloendothelial cells during neonatal life can recognize the IgG-parasite complex and concomitantly induce to switch on the secondary immune responses. It was revealed in this experiment that thymocytes and spleen cells contributed to the development of protective reaction in infants born of immune mothers. However, further investigation into defined mechanisms of immune response in neonatal period which would be induced by parasite-IgG complex must be undertaken.

Summary

Transmission of malaria parasites from pregnant mothers to fetuses was thought to be very rare, even in case of parasite infection during pregnancy.

In infants of *P. berghei* (Pb) chronically infected mother rats (immunized infants), high level anti-Pb IgG antibody which was transmitted from the mothers was observed at birth and the titer decreased gradually until the 5th week. These infants showed high resistance against parasite infection just after birth but lost the resistance in the 7th week. After Pb-inoculation into immunized infants just after birth, IgG titer elevated further, although no parasitemia appeared and no IgM production was detected.

Immunized infants, if thymectomized, completely lost resistance against Pb-infection showing severe anemia and parasitemia which consequently resulted in death. It is postulated that maternal IgG antibody plays an important role in Pb-infection in rats. On the other hand, thymus and spleen also give significant influences to the development of resistance in rats born of Pb-infected mothers.

This study was reported at the 1st Japanese-German Cooperative Symposium on Protozoan Diseases, Tokyo, 18–22 October, 1977, and at the 48th Annual Meeting of the Japanese Society of Parasitology, Tokyo, 5–6 April, 1979.

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Plasmodium berghei 感染に対するラット新生児の防御反応に関する研究

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一般に，感染症においては胎盤移行抗体の減少に伴なって，新生児の防御能が漸次低下していくことが知られている．今回われわれは，マラリア慢性感染ラットから得られた新生児を用いて，その防御応答に及ぼす種々の要因について検討した．

マラリア原虫の胎児への移行は稀で特に慢性感染ラット母体より生れた新生児からは原虫を検出することはできなかった．マラリア慢性感染ラットから生れた新生児において，移行 IgG 抗体は出産時に最高値を示し，その力価は5週目まで漸次減少した．これらの新生児は出産直後のマラリア接種に対して明瞭な抵抗性を示し，IgG 抗体力価はさらに上昇し，血中に原虫は出現せず，また IgM 抗体の産生は見られなかった．

出産直後に胸腺，あるいは脾臓を摘出した慢性感染ラット由来の新生児は，マラリア感染に対して抵抗性を失い，高度の貧血，および原虫血症を示し死に至った．

移行 IgG 抗体がマラリア感染ラットにおいて感染防御機能に重要な役割を有し，また，マラリア感染母ラットより生れた新生児ラットにおいては胸腺，脾臓が，マラリア感染母体由来の感染防御能の発現に影響を与えていると思われた．