Pathological Findings in Mice Immunized with Gamma Irradiated Third-Stage Larvae of Angiostrongylus Cantonensis

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Mice infected with UV-treated third-stage larvae of *Angiostrongylus cantonensis* then induced good protective efficacy against subsequent infection were observed by KAMATH et al.(1986). LEE (1969)also obtained a substantial protective immunity in rats against a lethal challenge with *Angiostrongylus cantonensis* by immunization with gamma-irradiated *Angiostrongylus cantonensis* larvae. Protective antibodies induced in mice infective with gamma-irradiated larvae were also detected by WANG (1990). Obviously, the immunization in experimental animals by infection with attenuated larvae treated with gamma-irradiation showed one of the effective methods to prevent hosts from suffering infection. In order to evaluate the reaction of the hosts after infection with gamma-irradiated larvae, pathological changes of their brains are studied.

Key words: *Angiostrongylus cantonensis.*, γ **-irradiation, Incomplete protection**

Third-stage larvae of *Angiostrongylus cantonensis* were exposed to 100 Krad gamma-irradiation, and then orally introduced to $C_{57}BL/6$ strain (B6) mice once or thrice. The B6 mice were sacrificed three weeks post-infection and brains were glossly and microscopically observed for pathological changes. The observation of these brains tissue was very similar to that of the brains from uninfected mice except slight petechiae and few invaded granulocytes being found. Furthermore, the pathological changes were also observed in brains of mice infected with gamma-irradiated third-stage larvae and subsequently challenged with lethal dose of infective larvae. Althouth it exhibited minimal eosinophilic meningitis and few meningeal petechiae in mice brain. How ever, these pathological changes were sig-

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nificantly less severe than those in mice directly infected with third-stage larvae. Thus, immunization with gamma-irradiated third-stage larvae of *Angiostrongylus cantonensis* through oral infection in B6 mice is a secure and effective route, and the produced antibodies to the homologous infection is incomplete protection.

Materials and Methods

$C_{57}BL/6$ (B6) strain mice weighing 24. 0 ± 1.8 gm and 12 ± 2 weeks old were used in this study. Mice were fed with sufficient food and water in animal cages in air conditioned room (25℃± 1℃). *Achatina fulica,* naturally infected *Angiostrong-gylus cantonensis,* were exposed to 100 Krad gamma-ray and subsequently digested with artificial gastric juice for collecting the third-stage larvae to infect mice by an oral-stomach tube, according to the method described by YEN et al.(1987). Nine groups of mice were designed as A to I and 5 mice were involved in each group. Mice in group A was infected with 30 irradiated third-stage larvae once, and challenged with 30 third-stage larvae 2 weeks after the last infection. Mice in group B was infected with 30 irradiated third-stage larvae thrice in 2 weeks interval, and challenged with 30 third-stage larvae 2 weeks after the last infection with irradiated larvae. Mice in group C and D were challenged with 80 third-stage larvae 2 weeks after the last infection with irradiated larvae as the treatment of group A and B respectively. (The infection schedules as in mice of group A and B instead of 80 third-stage larvae were challenged). Mice in group E was infected with 30 irradiated larvae once. Mice in group F was infected with 30 irradiated third-stage larvae thrice in 2 weeks interval. Mice in group G and H were infected with 80 and 30 larvae respectively. Mice in group I was uninfected as the controls. All mice were sacrificed three weeks after the last infection. The hematoxylin stain of brain tissues was prepared for microcopic pathological changes. Mice infection schedule:

Group	A	B	C	D	E	F	G	H	I
Treat									
infected with 30 irradiated larvae once	+		+		+				
infected with 30 irradiated larvae thrice		+		+		+			
challenged with 80 AcL3			+	+				+	
challenged with 30 AcL3	+	+					+		
uninfected as the controls									+

AcL3: *Angiostrongylus cantonensis* third stage larvae

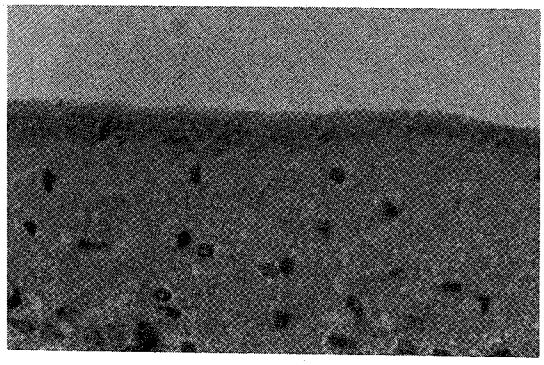


Fig. 1. Meninges of normal mouse. (H.E. stain 400x)

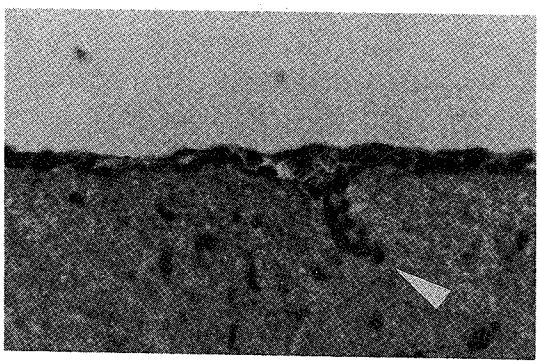


Fig. 2. Light engorgement of vessels in mouse meninges. (H.E. stain 400x)

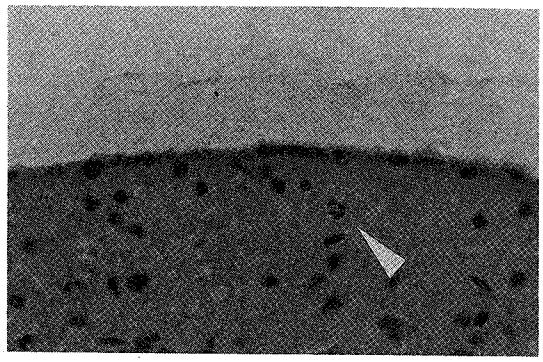


Fig. 3. A few granulocytes in mouse meninges. (H.E. stain 400x)

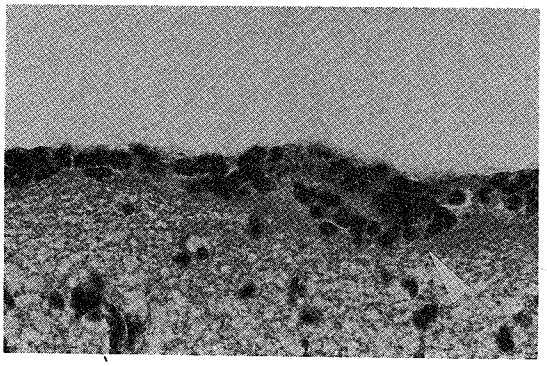


Fig. 4. Light inflammatory reaction in mouse brain. (H.E. stain 400x)

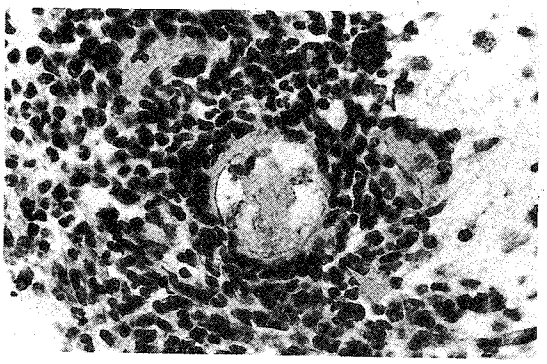


Fig. 5. Worm migrated tract on mouse brain. (H.E. stain 400x)

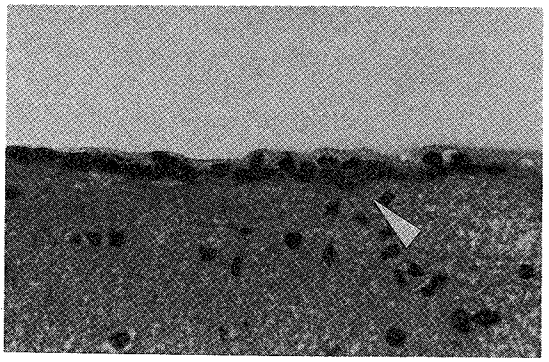


Fig. 6. A few engorgement of vessels in mouse brain. (H.E. stain 400x)



Fig. 7. Granulocytes invaded on mouse meninges. (H.E. stain 400x)

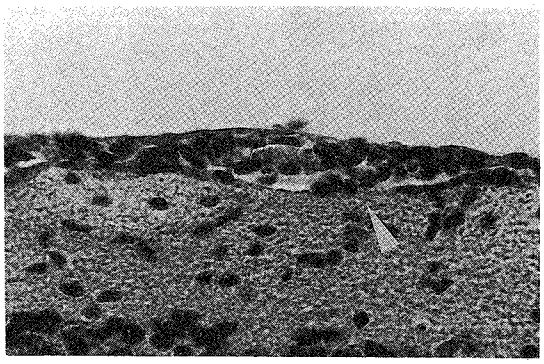


Fig. 10. A few and light engorgement of vessels in mouse meninges. (H.E. stain 400x)

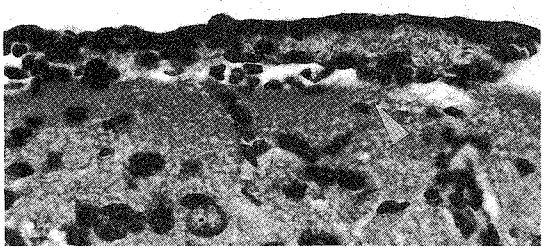


Fig. 8. A few petechiae in mouse meninges. (H.E. stain 400x)

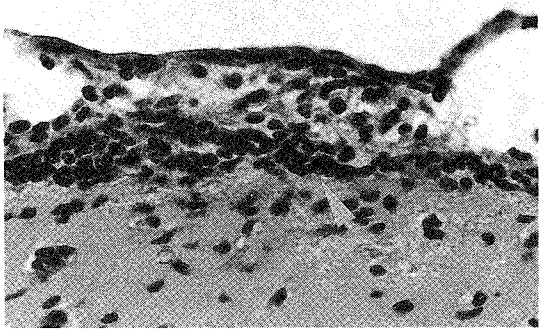


Fig. 11. Inflammatory reaction in mouse meninges. (H.E. stain 400x)

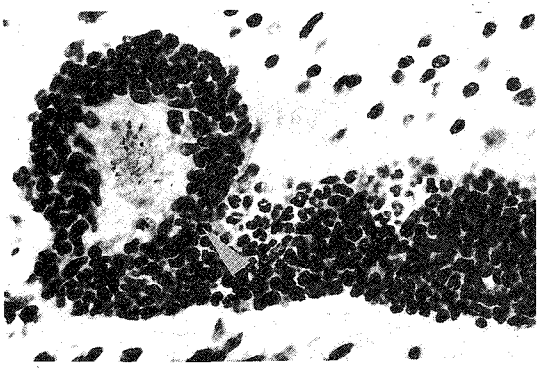


Fig. 9. Worm migrated tract in mouse brain. (H.E. stain 400x)

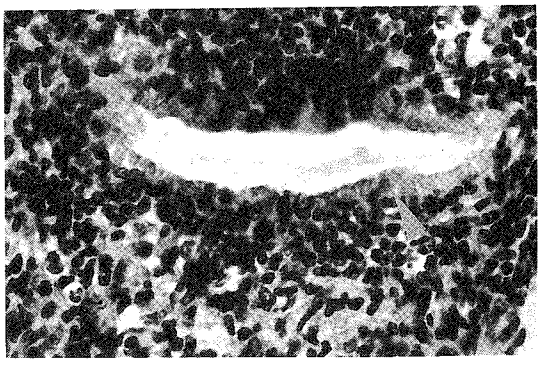


Fig. 12. Inflammatory reaction in mouse brain. (H.E. stain 400x)

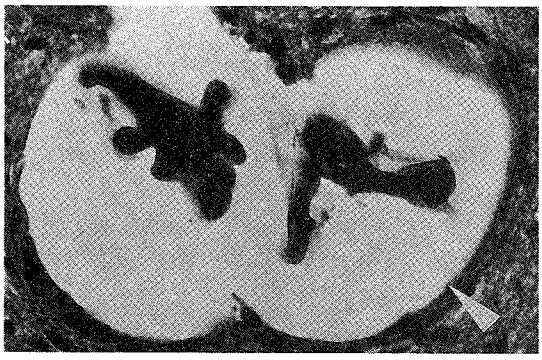


Fig. 13. Worms as an inclusion body in mouse brain. (H.E. stain 400x)

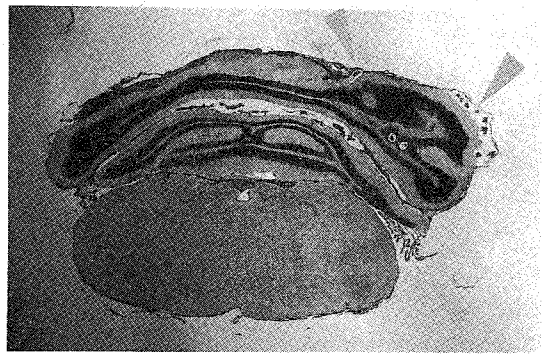


Fig. 14. Worms gathering in mouse meninges. (H.E. stain 5x)

Results

The observation of brains of mice from group A and B, except slight engorgement and few granulocytes on meninges, was very similar to normal mouse(Figs. 1,2 and 3). In group C mice, brains were found with light inflammatory reaction in the meninges and encephalon and with migrated track by the worms (Figs. 4 and 5). The findings from group D mice revealed a few engorgement, petechiae and granulocytes (Figs. 6 and 7). In group E mice, slight engorgement of the vessels, light inflammation, a few petechiae, and invaded granulocytes in the meninges and worm migration tract were found (Figs.8 and 9).The brains of group F mice were found with a few engorgement of vessels in meninges (Figs. 10). Apparent gross changes findings hemorrhage, vessel engorgement, tortuosity on meninges were observed from contrast group G and H mice. In microscopic finding, brain tissues of mice from these two groups showed worms gathered on meninges and causing eosinophilic meningitis and meningoencephalitis, otherwise, around the worm cuticle in the brain tissue, they were adhered by many granulocytes, and granulomatous reaction around the worms was seen with forming a nodular like changes (Figs. 11-14).

Discussion

Protective antibodies induced in B6 mice infected with gamma-irradiated third-stage larvae of *Angiostrongylus cantonensis* have been deteceted by WANG (1990). It is suggested that the migrating gamma-irradiated larvae might supply an efficient stimulation for production of antibodies. The pathological changes in the brain tissues of mice infected with gamma-irradiated larvae is still unclear. In this study,there were no obvious pathological changes in brain tissues of mice infected once or thrice gamma-irradiated larvae. Thus, for producing antibodies to resist the subsequent infection with gamma-irrediated *Angiostrongylus cantonensis* third-stage larvae, the infection by oral route is more effective and safer.

LEE (1969) and WANG (1990) reported that experimental animals immunized with gamma-irradiated third-stage

larvae through oral infection then challenged with lethal dose of infective third-stage larvae,the animals did not die after challenge, moreover, the protection was above 87 %. Although a few worms might develop to the fifth stage larvae, the size of the worm was smaller and less in numbers. The reason is that these survived worms have been exposed to the protective antibodies. In comparing to mice infected with larvae without immunization, the pathological findings in the brain of mice infected with gamma-irradiated larvae then subsequent challenged with non-irradiated larvae were almost the same as those findings in normal mice. Thus, the immunization with gamma-irradiated third-stage larvae through oral infection can induce incomplete protection.

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經伽瑪射線照射過的廣東住血線蟲第三期幼蟲感染小鼠後的腦部病理發現

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廣東住血線蟲第三期幼蟲經100 Krad伽瑪射線照射後，分別以口胃管餵入C57BL／6（B6）品系小鼠胃內一次或三次。於感染後第三週將B6小鼠解剖，觀察其腦組織大體變化及病理病變，這些腦組織的病理變化與未感染的對照組十分相近，僅有輕微的微血管出血及少許顆粒性白血球侵潤現象被發現。此外，小鼠經由伽瑪射線照射過的廣東住血線蟲第三期幼蟲免疫後，再感染致死或次致死劑量的感染性幼蟲，雖然顯現出局部的嗜伊紅性腦膜炎及些許腦膜出血點在小鼠的腦部，但以上小鼠腦部的病理病變很明顯地比直接感染廣東住血線蟲第三期幼蟲後引起之病變輕微。因此，經伽瑪射線照射過的廣東住血線蟲第三期幼蟲經口感染B6小鼠為一安全而有效之途徑，且能引發抗體，對同源性的感染能具有不完全的保護作用。

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