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Research Note

Invasion and Development of Sporozoites of a Chicken Embryo Passage Line of *Eimeria tenella* in Cell Culture

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Attenuation of pathogenicity of *Eimeria mivati* (Long, 1972; Long et al., 1982), *E. tenella* (Gore et al., 1983; Long 1972), and *E. necatrix* (Gore et al., 1983; Shirley, 1980) has been reported after serial passages of the parasites in chicken embryos. We have serially passaged a strain of *E. tenella* of Japan origin in chicken embryos. The strain after passage 51 times showed lower pathogenicity in chickens than before (Nakai and Ogimoto, 1987a). Sporozoites of the passage line contained smaller amounts of amylopectin and consumed the amylopectin reserve in a shorter time as compared to the original strain (Nakai and Ogimoto, 1987c), which was considered to be a cause of the low pathogenicity. In the present study invasion of sporozoites of the passage line into a cell culture and the development in cells were investigated.

E. tenella K-2 strain and the passage line were used (Nakai et al., 1982; Nakai and Ogimoto, 1983b). In the passage line, oocysts were obtained from chicken embryos at each passage, and sporozoites prepared from the oocysts were used for the next passage. Oocysts from a culture serially passaged 60 times (P60) and once (P1) were prepared under the same condition in chicken embroys. That is, the embryos were inoculated with sporozoites from oocysts of a culture serially passaged 59 times in chicken embryos in the former and with the original strain which was maintained by peroral infection in chickens in the latter (Nakai and Ogimoto, 1987c). Sporozoites were prepared as mentioned previously (Nakai and Ogimoto, 1983a), and suspended in Eagle MEM (Nissui) to inoculate into Leighton tubes of 2-day-old HeLa S-3 monolayer cell culture. Coverslips recovered from the tubes were stained with the hematoxylin-eosin stain.

Trophozoites were detected at 24 hr of incubation, and typical second generation schizonts were observed at 144 hr. There was no difference between P1 and P60 in morphology and timing of appearance of developmental stages of the parasites. Further development of the parasite was not investigated, because of the difficulty of maintenance of the cell culture.

Numbers of sporozoites which invaded cells were counted at 2 hr and 24 hr of incubation by observing 1,000 cells in each culture (Table 1). At 2 hr there was little difference between the number of invaded sporozoites of P1 and that of P60. After 24 hr incubation, the number of invaded sporozoites of P1 increased significantly, but that of P60 did not. The number of P60 was significantly lower than that of P1 at 24 hr ($P < 0.05$). These results showed that P60 sporozoites invaded into cells with a similar rate as that of P1 only in the initial course of incubation.

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Table 1 Rate of invasion of sporozoites into HeLa S-3 culture cells.

	incubation time	
	2 hr	24 hr
P1*	10.8 ± 1.0^{a}	$18.1 \pm 1.3^{b\dagger}$
P60	8.9 ± 1.4	11.6 ± 0.7^{c}

* Sporozoites of the lines passaged once (P1) or 60 times (P60) in chicken embryos were inoculated into 2-day-old HeLa S-3 monolayer cell culture.

† Mean percentage ± SE (n=3). From each culture, 1,000 cells were chosen at random, and the numbers of sporozoites invaded in cells were counted. There were significant differences between a and b ($P < 0.05$) and b and c ($P < 0.05$).

A similar experiment was performed in the cell culture with the medium containing 1.0 ppb monensin (Table 2). There was also little difference between the number of invaded sporozoites of P1 and that of P60 at 2 hr incubation. Although the number of P1 sporozoites-invaded-cells increased significantly by 24 hr, that of P60 did not increase. The difference between the number of P1 and that of P60 at 24 hr was significantly great ($P < 0.01$).

Monensin is known to accelerate sodium influx into the sporozoite by stimulating (Na^{+}-K^{+})-ATPase. This reaction requires energy supplied by glycolysis from amylopectin reserve (Smith and Galloway, 1983). It was observed that amylopectin had close relation with viability and infectivity of *E. tenella* sporozoite (Nakai and Ogimoto, 1983a, 1987b). The sporozoites of the passage line were observed to have less amylopectin reserve than the original strain (Nakai and Ogimoto, 1987c). It is considered that with the presence of monensin the amylopectin reserve in the sporozoites of P60 disappeared in a shorter time, or the rate of amylopectin consumption of the sporozoites was accelerated as compared to the original strain. The possibility, however, that the passage line has different sensitivity to monensin by some altered properties, e.g., membrane permeability, enzyme activity, etc., might be also suspected.

Table 2 Rate of invasion of sporozoites into HeLa S-3 culture cells under treatment of monensin.

	incubation time	
	2 hr	24 hr
P1*	8.8 ± 0.9^{a}	$12.2 \pm 0.9^{b\dagger}$
P60	6.3 ± 1.2	5.6 ± 0.6^{c}

* Sporozoites of the lines passaged once (P1) and 60 times (P60) in chicken embryos were inoculated into 2-day-old HeLa S-3 monolayer cell culture of which medium contained 1.0 ppb monensin.

† Mean percentage ± SE (n=3). From each culture, 1,000 cells were chosen at random, and the numbers of sporozoites invaded into cells were counted. There were significant differences between a and b ($P < 0.05$) and b and c ($P < 0.01$).

From these data it is suggested that the lower ability of the sporozoites to invade host cells may be one of the causes of the attenuation of pathogenicity of the passage line, and the different content and metabolism of the amylopectin may be a significantly contributing factor to the low pathogenicity of the line.

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