The Korean Journal of Parasitology
Vol. 36, No. 4, 227-234, December 1998

Distribution of actin and tropomyosin in *Cryptosporidium muris*

Jae-Ran YU*

Department of Parasitology, College of Medicine, Konkuk University, Chungju 380-701, Korea

Abstract: Actin and tropomyosin of *Cryptosporidium muris* were localized by immunogold labeling. Two kinds of antibodies for actin labeling were used. The polyclonal antibody to skeletal muscle (chicken back muscle) actin was labeled on the pellicle and cytoplasmic vacuoles of parasites. The feeder organelle has showed a small amount of polyclonal actin antibody labeling as well. Whereas the monoclonal antibody to smooth muscle (chicken gizzard muscle) actin was chiefly labeled on the filamentous cytoplasm of parasites. The apical portion of host gastric epithelial cell cytoplasm was also labeled by smooth muscle actin together. The polyclonal antibody to tropomyosin was much more labeled at *C. muris* than host cells, so it could be easily identified even with low magnification (×2,000). The tropomyosin was observed along the pellicle, cytoplasmic vacuoles, and around the nucleus also. The skeletal muscle type actin seems to play a role in various celluar functions with tropomyosin in *C. muris*; on the other hand, the smooth muscle type actin was located mainly on the filamentous cytoplasm and supported the parasites' firm attachment to host cells. Tropomyosin on the pellicle was thought to be able to stimulate the host as a major antigen through continuous shedding out by the escape of sporozoites or merozoites from their mother cells.

Key words: skeletal muscle type actin, smooth muscle type actin, tropomyosin, *Cryptosporidium muris*

INTRODUCTION

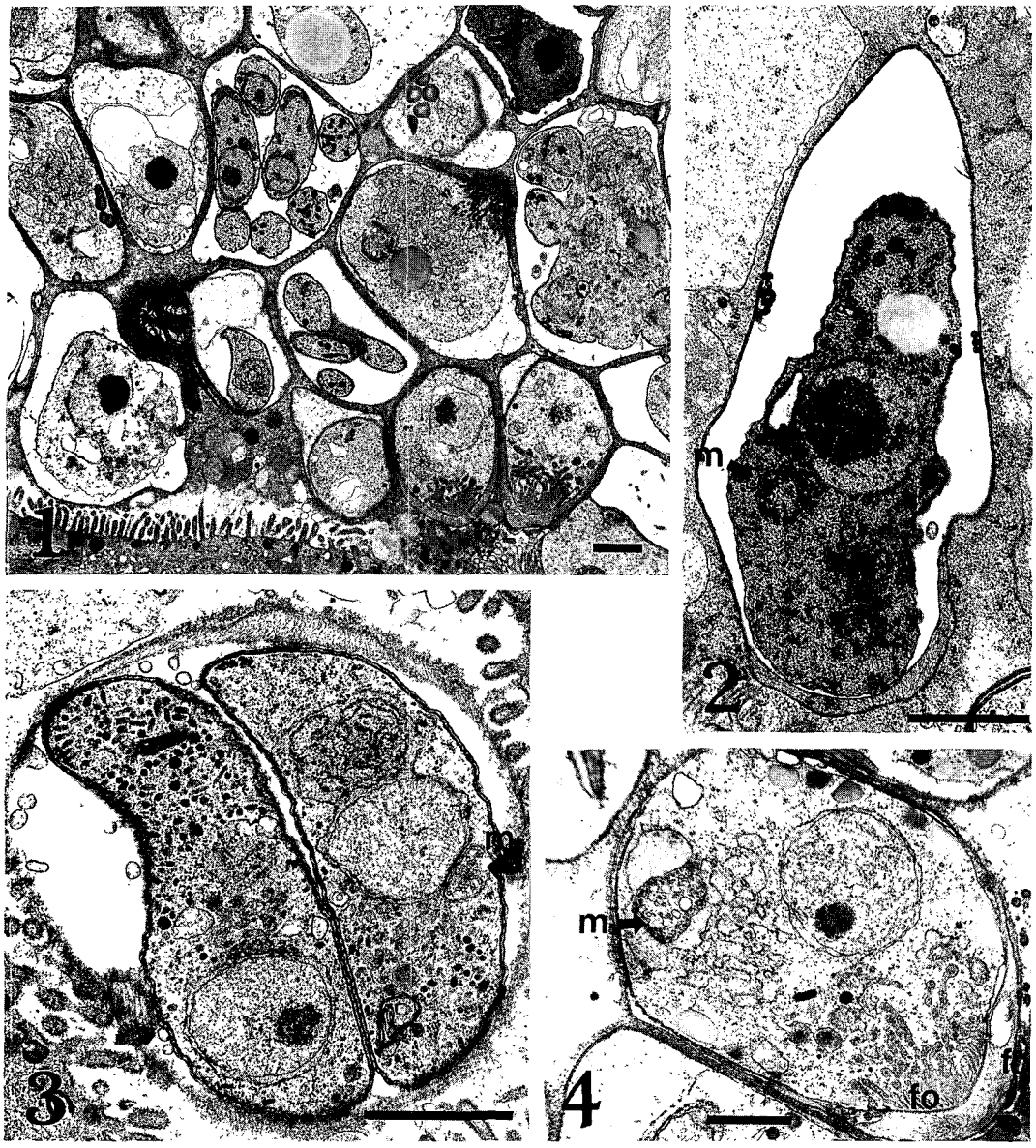
The first description of *Cryptosporidium muris* was by Tyzzer in 1907 and 1910. Since then three kinds of *Cryptosporidium* have been considered as possible human parasites: *C. parvum*, *C. muris*, and *C. beileyi* (Levine, 1984; Current and Reese, 1986). As *Cryptosporidium* has no special motile structure like many other coccidian parasites, it is unclear how it can invade the host epithelium. *Cryptosporidium parvum* has been known to have a lot of actin and tropomyosin in its cytoplasm and on the pellicle, although the functions of those proteins are yet unknown (Yu and Chai, 1995; Yu and Lee, 1996). As another species belonging to *Cryptosporidium*, it would be very valuable to localize the microfilament and its binding protein in *C. muris* to get the hint of its motile system related with invasion.

MATERIALS AND METHODS

Expression of *Cryptosporidium muris* in laboratory mouse

Three-week old mice (C57BL/6J) were immunosuppressed by intramuscular injection of Depomedrol® (10 mg/kg) once a week. After

• Received 5 August 1998, accepted after revision 5 November 1998.
• This study was financially supported by the research grant of Konkuk University in 1997.
* Corresponding author (e-mail: jaeran.yu@kcucc.cj.konkuk.ac.kr)

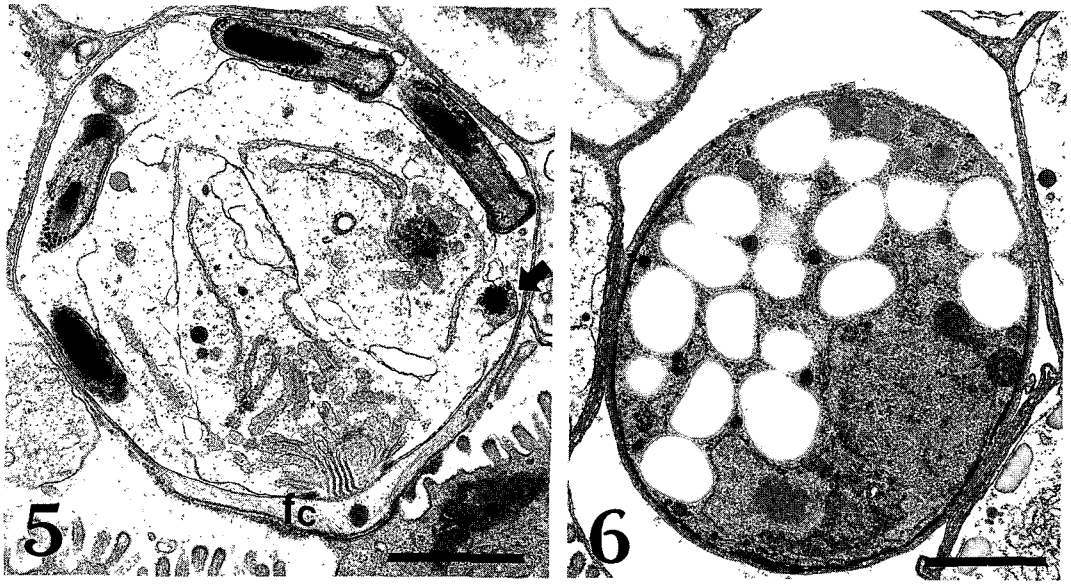


Figs. 1-4. Various developmental stages of *Cryptosporidium muris* observed by transmission electron microscopy (TEM). **Fig. 1.** Various developmental stages of *C. muris* in the gastric gland of mice. **Fig. 2.** Trophozoite of *C. muris*. **Fig. 3.** Merozoites of *C. muris*. **Fig. 4.** Macrogametocytes of *C. muris*. fc, filamentous cytoplasm; fo, feeder organelle; m, mitochondrion; bar, 1 μm.

3 weeks, oocyst production was detected by stool examination using a modified acid fast stain, and the stomach of oocyst-positive mice were detached and fixed at 2% paraformaldehyde and 0.4% glutaraldehyde for about 1 hr at room temperature.

Preparation of tissue antigen

The fixed stomach tissue was washed with 0.1 M PBS, and dehydrated through an alcohol series from 30 to 95%. Dehydrated tissues were embedded in LR gold resin (Electron Microscopy Sciences) and polymerized at -20°C for 72 hrs under UV illumination. The



Figs. 5-6. Another two stages of *Cryptosporidium muris* observed by TEM. **Fig. 5.** Microgametocyte of *C. muris* with six microgametes. Arrow pointed out cross sectioned microgamete showing surrounding tubule-like structures. **Fig. 6.** Underdeveloped oocyst of *C. muris*. fc, filamentous cytoplasm; bar, 1 μm.

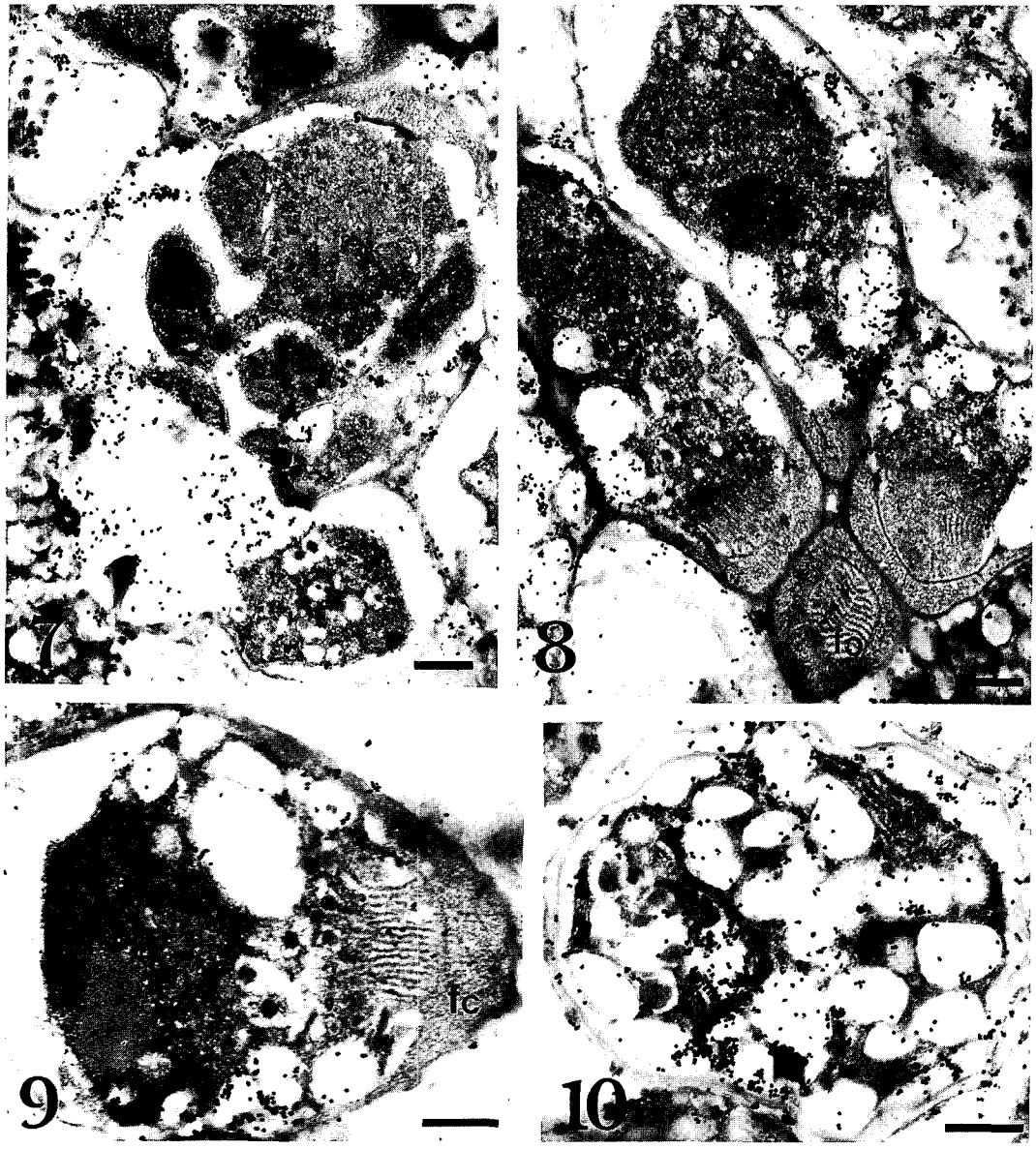
ultrathin section was done at 90 nm thickness and sections were mounted onto nickel grids.

Immunogold labeling

The immunogold labeling procedure followed the methods of Yu and Chai (1995). Briefly, tissue sections were incubated in PBS-milk-Tween (PMT) for 10 min and exposed to primary antibodies diluted with PMT for 2 hrs at room temperature. The primary antibodies used were rabbit anti-tropomyosin (chicken gizzard muscle; Sigma), rabbit anti-actin (chicken back muscle, polyclonal; BioGenex), and mouse anti-actin (chicken gizzard muscle, monoclonal; Chemicon). The sections were washed thoroughly with PBS-BSA-Tween and reincubated with 5 nm gold conjugated goat anti-rabbit IgG (Sigma) and goat anti-mouse IgG (Sigma) overnight at 4°C. Silver enhancement was done with a commercial kit (Amersham) followed by background staining with uranyl acetate and lead citrate. The stained sections were examined under a transmission electron microscope (Jeol 1200 EXII).

RESULTS

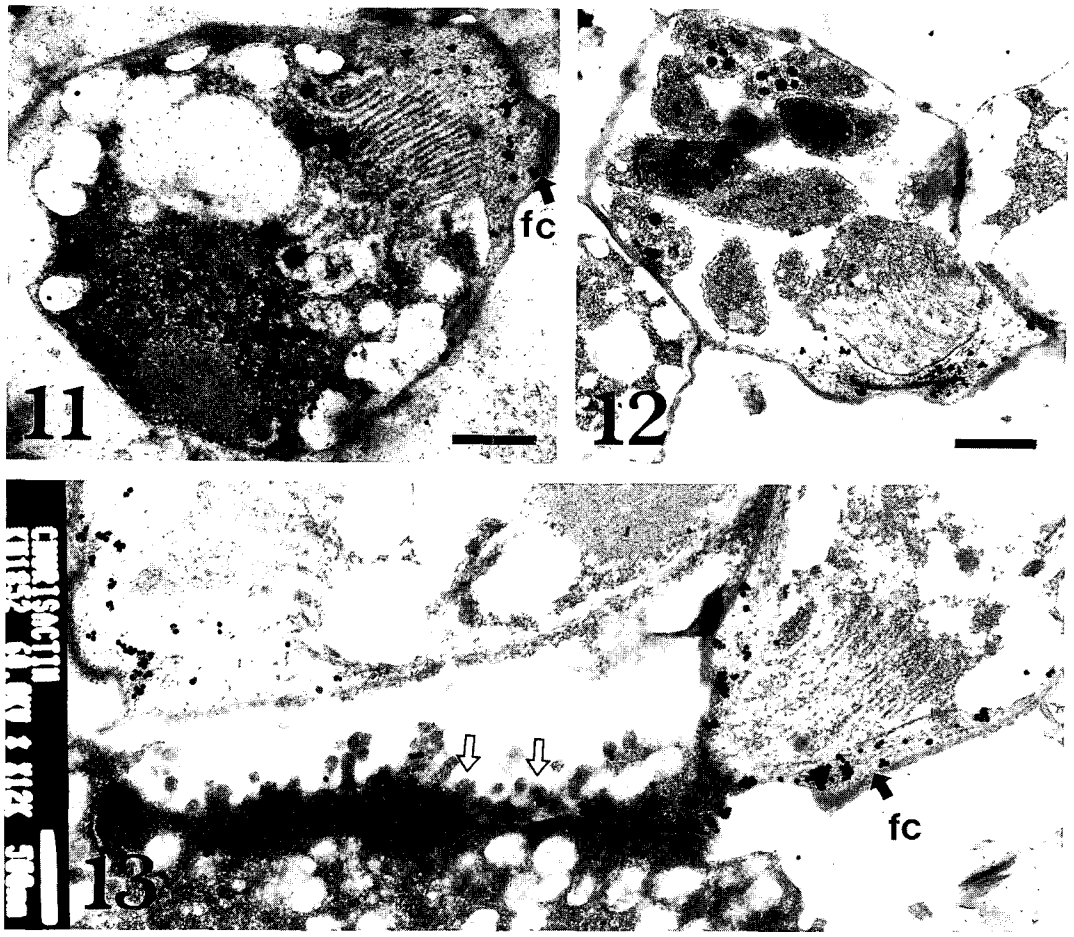
Almost all examined stomach glands were filled with various developmental stages of *C. muris* (Fig. 1). The population of macrogametocyte was more dominant than other forms. Each asexual and sexual form had a mitochondrion in its cytoplasm near the nucleus (Figs. 2-4). The filamentous cytoplasm under the feeder organelle that has thought to be originated from the host, was well developed (Figs. 2, 4 & 5). The microgametocyte containing 6 microgametes was observed. Each microgamete was enveloped by 2 membranes, one from the residual body and the other from its own cytoplasmic membrane (Fig. 5). The postmost part of the microgamete was coated with a thick layer whose nature is not exactly known. Many tubule-like structures were also found in the cytoplasm of microgametes by longitudinal and cross sectional views. These tubules surrounded the black nucleus portion of microgametes (Fig. 5). An underdeveloped oocyst was enveloped by five membranous structures; two layers were originated from the host, two were oocyst shell structure and the other one was a cytoplasmic



Figs. 7-10. Immunogold localization on the skeletal muscle type actin of *Cryptosporidium muris*. **Fig. 7.** Localization of skeletal muscle type actin on the pellicle of meront of *C. muris*. **Fig. 8.** Another developing meront showing many labeled skeletal muscle type actin on its feeder organelle as well as the pellicle. **Fig. 9.** Macrogametocyte of *C. muris*. **Fig. 10.** Oocyst of *C. muris* showing many labeled skeletal muscle type actin. fc, filamentous cytoplasm; fo, feeder organelle; bar, 0.5 μm.

membrane of parasite (Fig. 6). The oocyst shell was almost twice as thick as the others.

The polyclonal antibody to chicken back muscle actin was labeled on the pellicle and cytoplasmic vacuoles of parasites (Figs. 7-10). The feeder organelle was labeled by a small amount of actin also (Fig. 8), whereas labeling pattern with the monoclonal antibody to chicken gizzard muscle was completely different from the polyclonal antibody to chicken skeletal muscle. The monoclonal antibody to chicken smooth muscle was chiefly labeled on the filamentous cytoplasm of the parasite (Figs. 11-13). The apical portion of



Figs. 11-13. Filamentous cytoplasm of macrogametocyte **(Fig. 11)** and type I meront **(Fig. 12)** labeled by monoclonal antibody to smooth muscle type actin. **Fig. 13.** The apical portion of gastric epithelium (white arrows) was also labeled together. fc, filamentous cytoplasm; bar, 0.5 μm.

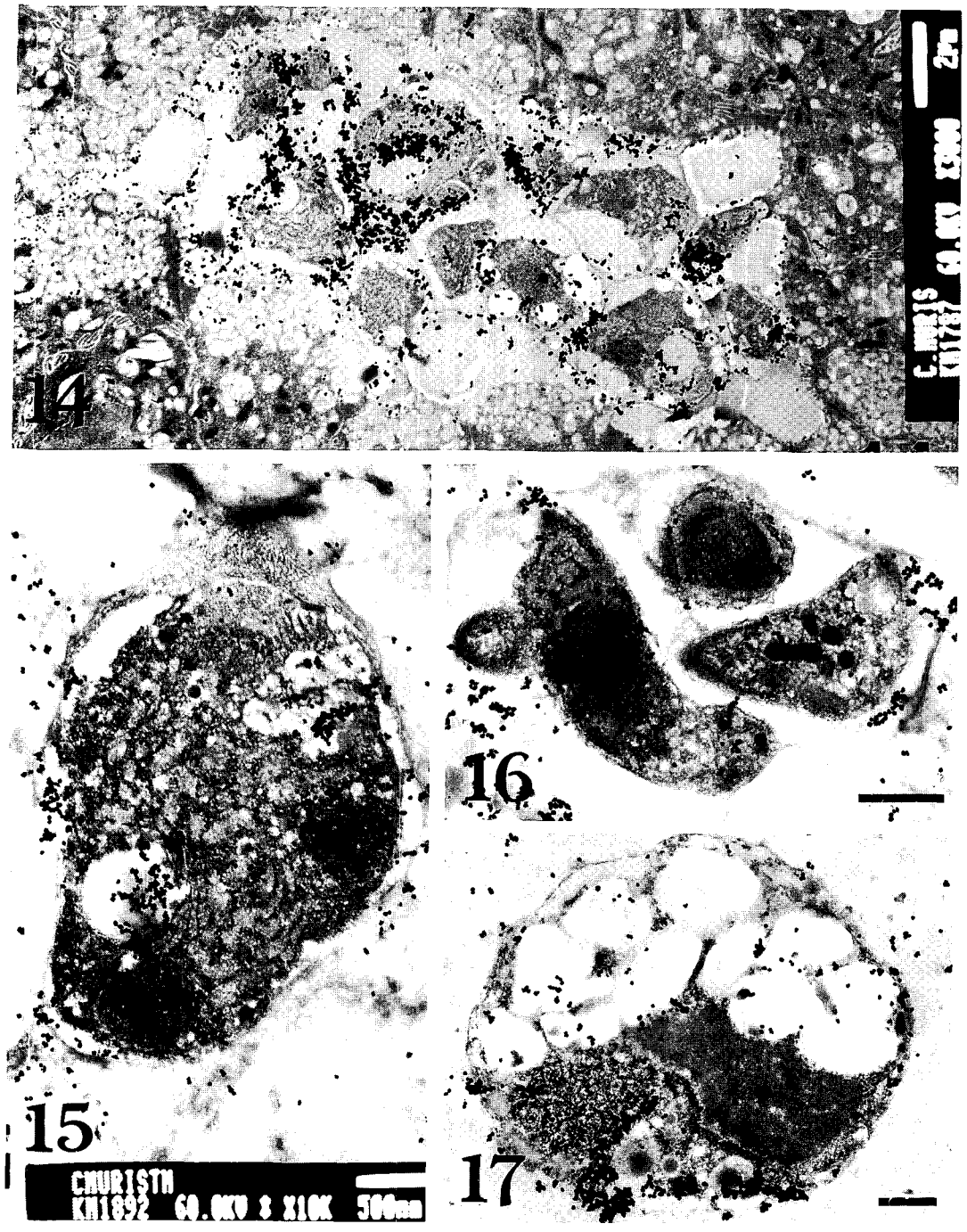
host gastric epithelial cell cytoplasm was also labeled along with filamentous cytoplasm (Fig. 13).

The polyclonal antibody to tropomyosin was labeled much more to *C. muris* than host tissue, so it was very easy to identify the worms with low magnification (× 3,000) (Fig. 14). The tropomyosin was observed along the pellicle, cytoplasmic vacuoles, and around the nucleus (Figs. 15-17).

DISCUSSION

It is well known that actin has six isoforms; among these the actin of *Cryptosporidium* is known as a γ-isoform (Kim *et al.*, 1992). Actin has many kinds of binding proteins, and the tropomyosin is one of them. The role of tropomyosin in skeletal muscle is to stabilize actin, making it easy to bind myosin onto actin filament. Nonmuscle type tropomyosin is thought to be related to various cellular functions such as cell mitosis, phagocytosis, pinocytosis, excretion, and cytoplasmic movement (Alberts *et al.*, 1994). This actin and tropomyosin system is controlled by calcium-dependent phosphorylation of myosin light chain and the troponin is excluded from that system (Lau *et al.*, 1985).

In this study the mice showed very heavy infection, so most of the gastric glands contained worms. Among those worms, macrogametocytes were the most prominent forms. The large percentage of macro-



Figs. 14-17. Immunogold localization on the tropomyosin of *Cryptosporidium muris*. **Fig. 14.** *Cryptosporidium muris* has a lot of tropomyosin in its body, so it was very easy to notify the worm with lower magnification. **Fig. 15.** Tropomyosin labeling was shown around nuclei, cytoplasmic vacuoles, and on the pellicle. **Figs. 16-17.** Merozoite **(Fig. 16)** showing labeled tropomyosin around its nucleus and oocyst **(Fig. 17)** around vacuoles and pellicle. Bar, 0.5 μm.

gametocyte may explain the high infection intensity, because thin-walled oocysts originating from macrogametocytes can start the internal autoinfection that causes an enormous increase in parasites number. *Cryptosporidium muris* has two structures not found in *C. parvum*. It has a filamentous cytoplasm under the feeder organelle and mitochondrion in its cytoplasm. So, two species could be differentiated by these two morphological structures by electron-microscopy.

With two kinds of anti-actin antibodies, it was found that each kind of actin localized at a different locations. Skeletal muscle type actin localized mainly in parasites, but smooth muscle type actin was exclusively on the filamentous cytoplasm and the apical portion of host epithelium. In regard to this differences, it was suggested that the skeletal muscle type actin may be related with the various cytoplasmic functions of parasites such as mitosis, vacuolar movement, surface movement, and so on, whereas the smooth muscle type actin may be related to the attachment of the parasite and may support the worms' parasitism.

Cryptosporidium muris was found to have as much tropomyosin as in *C. parvum* (Yu *et al.*, 1996). Because of the location of tropomyosin on the pellicle, it was thought that tropomyosin might be able to stimulate the host as a major antigen through continuous shedding out of pellicles and residual bodies by escape of sporozoites or merozoites from their mother cells. But the cytoplasmic functions of parasite might be related to tropomyosin as well as to actin because the distribution of tropomyosin around the vacuoles or the nuclear membrane is very similar to that of actin.

Conclusively, in this study it was found that *C. muris* has two kinds of actin which showed different localizations in the worm. The skeletal muscle type actin was located mainly on the pellicle and cytoplasm of worm and may play a role in various celluar functions with tropomyosin; on the other hand, the smooth muscle type actin was located mainly on the filamentous cytoplasm and may support the parasites for firm attachment to the host.

ACKNOWLEDGEMENT

I would like to thank Mr. Byong-Hwa Chang in Division of Electronmicroscopy, College of Medicine, Konkuk University, for his quite nice technical support to get nice pictures.

REFERENCES

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1994) Molecular Biology of the Cell, 3rd ed. p787-861. Garland Publishing Inc, New York & London.

Current WL, Reese NC (1986) A comparison of endogenous development of three isolates of *Cryptosporidium* in suckling mice. *J Protozool* **33**(1): 98-108.

Kim K, Gooze L, Petersen C, Gut J, Nelson G (1992) Isolation, sequence and molecular karyotype analysis of the actin gene of *Cryptosporidium parvum*. *Mol Biochem Parasitol* **50:** 105-114.

Lau SYM, Sanders C, Smillie LB (1985) Amino acid sequence of chicken gizzard γ-tropomyosin. *J Biol Chem* **260:** 7257-7263.

Levine ND (1984) Taxonomy and reveiw of the coccidian genus *Cryptosporidium* (Proto-zoa, Apicomplexa). *J Protozool* **31**(1): 94-98.

Tyzzer EE (1907) A sporozoan found in the peptic glands of the common mouse. *Proc Soc Exp Biol Med* **5:** 12-13.

Tyzzer EE (1910) An extracellular coccidium, *Cryptosporidium muris* (gen. et sp. nov.), of the gastric glands of the common mouse. *J Med Res* **23:** 394-413.

Yu JR, Chai JY (1995) Localization of actin and myosin in *Cryptosporidium parvum* using immunogold staining. *Korean J Parasitol* **33**(3): 155-164.

Yu JR, Lee SH (1996) Localization of cytoskeletal proteins in *Cryptosporidium parvum* using double immunogold lableling. *Korean J Parasitol* **34**(4): 215-224.

초록=

쥐와포자충에서 acin과 tropomyosin의 분포

유재란

건국대학교 의과대학 기생충학교실

쥐와포자충의 운동에 관여하는 구조에 대하여 다른 구충류에서와 마찬가지로 알려진 바가 없다. 이 연구에서는 쥐와포자충에서 microfilament와 그 결합단백질의 분포를 관찰하여 이 기생충의 운동기전에 대한 이해를 돕고자 하였다. Actin의 분포를 보기 위해 두 종류의 actin, 즉 닭 골격근과 평활근의 actin에 대한 항체를 사용하였고, tropomyosin의 관찰을 위해서는 닭 골격근의 tropomyosin에 대한 항체를 이용하여 면역황금표지법으로 관찰하였다. 관찰된 모든 발육단계의 쥐와포자충이 actin과 tropomyosin을 가지고 있었는데 두 종류의 actin은 서로 다른 부위에서 관찰되었다. 즉, 골격근에 대한 항체는 주로 세포질과 세포막 구조에 표지되었고, 평활근에 대한 항체는 feeder organelle과 숙주세포 사이의 섬유질 구조 (filamentous cytoplasm)에 주로 표지되어 서로 다른 actin이 상이하게 분포하고 있음을 알 수 있었다. 분포 위치로 미루어 볼 때 골격근형 actin은 기생충의 세포질 내 여러 가지 현상에, 평활근형 actin은 쥐와포자충과 숙주세포 부착을 유지시키는데 주요 역할을 할 것으로 생각된다. Tropomyosin은 쥐와포자충 모든 발육단계에서 관찰되었는데 세포막에 주로 분포하였고 세포질 내의 소공포 (vacuole) 막 주위 및 핵 주위에서도 관찰되었다. Tropomyosin은 쥐와포자충의 발육단계가 변함에 따라 끊임없이 분포를 달리하는 것으로 생각되며 특히 막구조에 다수 분포하므로 항원으로서 숙주세포를 자극할 가능성이 있는 것으로 보인다.

〔기생충학잡지 36(4) : 227-234, 1998년 12월〕