Intraerythrocytic Schizogony of *Theileria sergenti* in Cattle

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ABSTRACT. The characteristics of developing intraerythrocytic stages of *T. sergenti* were studied by light and transmission electron microscopy. The parasites with many ribosomes, acristate mitochondria, cytostome, and food vacuoles were morphologically regarded as the trophozoite stage. Although this type of parasites was frequently detected, intraerythrocytic merozoite stage with electron dense cisternae, rhottries and small electron dense bodies was rarely observed in high parasitaemia. The intraerythrocytic stages of *T. sergenti* were divided mainly into four daughters by schizogony, and alternatively into two by binary fission. The daughter parasites in each division had the same ultrastructural features as of merozoites. As a result, it was suggested that *T. sergenti* trophozoites multiplied by schizogony to four organisms or by binary fission in the peripheral erythrocyte, and differentiated to the merozoites which acquired penetrating ability into the erythrocytes.—**KEY WORDS:** merozoite, *Theileria sergenti*, trophozoite.

*Theileria sergenti* is the causative agent of Japanese bovine theileriosis, and the intraerythrocytic stage of parasite causes anemia and pyrexia, which are aggravated by stresses such as other infections, inadequate management, parturition and grazing [17]. The cattle recovered from *T. sergenti* infection have strong protective ability against the challenge infection. Although the detailed mechanisms for parasites to invade host cells and for suppression of their multiplication are obscure, it has been suggested that both humoral and cellular immunity concerns protective activity against the infection with this parasite [17].

Morphological findings on the intraerythrocytic stage, the main causative agent of the disease, show that the parasites with various shapes such as rod- and comma-shaped, ovoid, and tetrad., are observed in the peripheral erythrocytes. The rod- or comma-shapes are suggested to be detected mainly in the acute stage of disease, and the ovoid shape appears in the chronic stage [11, 13]. Few reports are available on the ultrastructure of intraerythrocytic stages of *T. sergenti*, especially with reference to the organelles in the cytoplasm [9, 19], and the serial morphological observations have not yet been reported on the propagation of intraerythrocytic stages. This experiment was undertaken to elucidate the morphological characteristics of dividing intraerythrocytic stages of *T. sergenti*.

MATERIALS AND METHODS

Experimental animal and strain of *T. sergenti*: A Japanese black cow naturally infected with *T. sergenti* in Hokkaido Prefecture was splenectomised to induce high parasitaemia. When parasitaemia reached over 10%, peripheral blood was collected from the jugular vein into 5 ml evacuated tubes containing an anticoagulant (Fig. 1). The blood was thinly smeared on a glass
slide, and stained with 1% Giemsa solution.

**Electron microscopy:** Peripheral blood was fixed in 2.5% glutaraldehyde solution (0.1M PBS pH 7.4) for 1 hour at 4°C. The blood was centrifuged, washed three times with the same buffer and packed in plastic hematocrit tubes. The pellets obtained by cutting the tubes were treated with 1% buffered osmium tetroxide for 1 hour at 4°C, and then they were dehydrated in ethanol series and propylene oxide and embedded in Quetol 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and finally examined under the transmission electron microscope.

**RESULTS**

**Light microscopic observations:** The intraerythrocytic stages of *T. sergenti* showed various shapes; such as anaplasma-like, large and small rod- and comma-shaped, spherical or ovoid, and tetrad (quadruplet forms). Intraerythrocytic parasites multiplied by schizogony into quadruplet configuration and binary fission. Parasites in various stages of division were observed in the peripheral erythrocytes. Four basophilic masses formed in the periphery of parasite (Fig. 2-a), the quadruplet form (Fig. 2-b), and four masses completely separated from each other (Fig. 2-c) were observed. In addition to these types, Y-shaped (Fig. 3-a) and paired piriform organisms (Fig. 3-b) which were connected with the peripheral edge of parasite cytoplasm were observed. But the incidence rate of quadruplet forms was extremely higher than that of the forms in binary fission.

**Electron microscopic observations:** The intraerythrocytic stages were surrounded by a single unit membrane. The double membranated nucleus had homogenous karyoplasm. Numerous ribosomes, formed polysomes, double membranated acristate mitochondria, cytostome, and food vacuole

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**Fig. 1.** Changes in parasitaemia and hematocrit value after splenectomy. Hematocrit value decreased in inverse proportion to the increase of parasitaemia. Peripheral blood was collected at the points with an arrow.
- ●●●: parasitaemia
- ○○○: hematocrit value

**Fig. 2-a.** Constriction of cytoplasm around four basophilic masses. Giemsa stain×1,350.

**Fig. 2-b.** Quadruplet form. Giemsa stain×1,350.

**Fig. 2-c.** Diffusion of basophilic masses. Giemsa stain×1,350.
were found in the cytoplasm. The cytostome and food vacuole were surrounded by a single membrane. Host cell cytoplasm was taken in by the cytostome and then by the food vacuole through an invagination of the plasmalemmal membrane (Figs. 4 and 5-a). The dense ring around the neck of cytostome was considered to be the terminal bar beneath the plasmalemmal membrane (Fig. 5-b). Food vacuoles occupied a large portion of cytoplasm, but the digestion by the vacuole was not observed. Parasites with the cytostome and food vacuole could be easily detected in the case of highly parasitaemia and took various shapes; such as the rod- and comma-shaped, ovoid, and irregular in the sections.

A few organisms of intraerythrocytic stage had electron dense cisternae, rhoptries, and small electron dense bodies under the plasmalemmal membrane. Rhoptries ranged from one to four in number (Fig. 6) and small electron dense bodies were also recognized in large numbers (Fig. 7). In this experiment, the polar ring, conoid, and subpellicular microtubes, which are the organelles characteristic in other Apicom-
plexa such as *Eimeria* and *Isospora* [12], were not observed in the intraerythrocytic stages of *T. sergenti*.

Extensive examination of serial sections showed that daughter parasites were produced by intraerythrocytic schizogony in the peripheral blood (Figs. 8 and 9). Nuclear division occurred prior to cytoplasmic division. Four daughter parasites produced by schizogony had electron dense cisternae,
rhoptries, and many small electron dense bodies. A residual body of mother parasite was observed in the center of the four daughters (Fig. 9).

The intraerythrocytic parasites in binary fission were of Y-shape, and rhoptries appeared in the daughter parasites (Fig. 10).

DISCUSSION

Conrad *et al.* proposed that the intraerythrocytic forms of *T. parva* [5] and *T. annulata* [4] with the cytostome and food vacuole are referred as trophozoites, compared with that of *Babesia* [8, 15] and *Plasmodium* [1]. In the case of *T. sergenti*, most of intraerythrocytic stages were morphologically similar to the trophozoites of *Babesia* [8, 15] and *Plasmodium* [1], the characteristic structures of which were reported in detail [7, 14, 15]. It is suggested that in the trophozoite of *B. microti* and *B. bigemina* the food vacuole is surrounded by a single unit membrane and this structure expands the surface of parasite to facilitate the ingestion of host cell nutrients [14, 15]. The labyrinthine structure near and/or within food vacuoles is present in *T. parva* of cattle and *Theileria sp.* in waterbucks [7]. This structure is regarded as a digestive organelle. The food vacuole of *T. sergenti* was not observed to have digestive function. Therefore the vacuole was hardly considered the specific organelle with the functions of ingestion, digestion and absorption of host cell cytoplasm, but only an invaginated portion of plasmalemmal membrane. Parasites will directly absorb nutrients of host cell cytoplasm from their surface of bodies. Food vacuoles will be useful, especially for this aim, for expanding the surface which is in direct contact with host cell. 

Fig. 7. Ultrastructure of quadruplet form with the same features as of merozoite. Electron dense cisternae (arrow head), rhoptry (R), and small electron dense bodies are present in cytoplasmic hemisphere. ×26,000. Scale bar=1 μm.
Figs. 8 and 9. Serial sections of quadruplet form composed of four merozoites. Electron dense cisternae (arrow head), low density rhoptry (R). A lot of small electron dense bodies are present in merozoites. Nucleus division is already completed and there is a residual body (RB) of mother organism in the center portion. ×44,000. Scale bar=500 nm.
cytoplasm. Both cytostome with the terminal bar at the neck and food vacuole took in cytoplasm of host cell, and seemed to be the stable organelles differentiated from the plasmalemmal membrane of parasite. The organisms with the cytostome and food vacuole were regarded as the trophozoite of intraerythrocytic stage in *T. sergenti*.

Mature trophozoites of *T. sergenti* became irregular in shape and formed daughters in the cytoplasm. Four daughter parasites produced by intraerythrocytic schizogony had the same morphological characteristic features as of merozoites: electron dense cisternae, rhoptries and small electron dense bodies. Electron dense cisternae appeared in the inner membrane precursor. The inner membrane is also observed in the merozoite of *Babesia* [14, 15, 18] and *Plasmodium* [1], but this membrane is not cleared to be composed of either a thickened single membrane or double membranes. In *T. sergenti*, electron dense cisternae composed the single thickened membrane. The function of rhoptries and small electron dense bodies is suggested to be related for parasites to penetrate into the erythrocytes [2, 3, 6]. Small electron dense bodies appeared to be the transsections of rhoptry ducts or micronemes. These structures are easily observed in exoerythrocytic merozoites produced by schizonts in the lymphocytes [16]. Exoerythrocytic merozoites of other *Theileria* species have a comparatively and extremely small numbers of rhoptries and small electron dense bodies, respectively [16]. But in this experiment intraerythrocytic merozoites had many small electron dense bodies and a few rhoptries. Rhoptries and small electron dense bodies seem to preserve the substance discharged by parasites during their penetration into the erythrocytes [2, 3]. When the number of rhoptries decreased,
the amount of the discharged substance will be supplemented by the small electron dense bodies. The rhoptries of young merozoites were often observed as low electron dense bodies because of scantiness of the preserved contents (Figs. 8 and 9). Conrad et al. reported that these structures are formed during the schizogony of intraerythrocytic stages of *T. parva* [5] and *T. annulata* [4]. *T. sergenti* merozoites with rhoptries, small electron dense bodies and electron dense cisternae were few in number in the peripheral erythrocytes in high parasitaemia. Judging from the function of these structures, it is suggested that the merozoites are the invasive stage to the erythrocytes.

Binary fission can be widely observed to occur in *Babesia* [8, 10, 15], but in *Theileria* it occurs only in *T. annulata* [13]. By light and electron microscopies of *T. sergenti* it was suggested that binary fission was not the main but only one method of intraerythrocytic multiplication. The daughter parasites in binary fission also had the same ultrastructural feature as of merozoites.

As a result, in *T. sergenti* most of the intraerythrocytic stages were morphologically regarded as trophozoites. They were divided into a quadruplet form of merozoites by schizogony or binary fission, and merozoites had rhoptries, small electron dense bodies and electron dense cisternae. It was suggested that only merozoites had an ability for penetration into the erythrocytes.

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要約

Theileria sergenti 赤内型原虫の分裂様式；川本 哲・高橋清志・黒沢 隆・其田三夫・小沼 操1) (北海道大学家畜伝染病学教室) —— T. sergenti の赤内型原虫の分裂様式について光顕的および電顕的観察を行った。赤内型原虫は単一の核を有し、細胞質にはリボソーム、クリステのないミトコンドリア、細胞口および食胞が認められ、形態的に栄養型 trophozoite stage と見なされた。末梢血中に観察される赤内型原虫は、ほとんどものが、この形態を有し、まれに rhoptry, small electron dense body および electron dense cisternae を有する原虫が観察され、これは形態的に感染型の merozoite stage と考えられた。T. sergenti の赤内型原虫は主経路である四つの娘細胞をつくる schizogony により、あるいは副経路である二分裂によって増殖し、いずれの分裂経路においても娘細胞は merozoite であった。このことから T. sergenti 末梢血内で schizogony あるいは二分裂によって増殖し、娘細胞は merozoite に分化し、赤血球への感染性を有するようになることが示唆された。