Histological Observations on the Schizonts in Cattle Infected with Japanese Theileria sergenti

Masumi SATO, Tsugihiko KAMIO, Shin-ichiro KAWAZU, Toshiaki TANIGUCHI, Tetsuro MINAMI, and Kozo FUJISAKI

National Institute of Animal Health, Tsukuba, Ibaraki 305, Japan

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ABSTRACT. Histological observations were performed on the schizonts of Japanese Theileria sergenti in three calves necropsied 8 and 10 days after application of nymphal Haemaphysalis longicornis infected with T. sergenti. In all the three calves, schizonts were observed in the cytoplasm of huge cells formed in the drainage lymph nodes, liver, and spleen. The huge cells were 50 to 200 μm in diameter. Schizonts had granular appearance and an irregular shape and were 1 to 7 μm in diameter. Ultrastructurally, schizonts had more than one nuclei and were formed in the unit enlarged cell. Schizonts showed a specific reaction against anti-T. sergenti anti serum, therefore, it was concluded that the schizonts were those of T. sergenti histologically.—KEY WORDS: cattle, histology, schizont, Theileria sergenti.


Theileria sergenti is recognized as the causative agent of bovine theileriosis, one of the most serious diseases facing cattle grazing in pastures in Japan [8]. There have been few reports regarding the life cycle of the parasite in cattle but the schizont stage of T. sergenti has not been described until lately.

Schizont stages, macroschizonts and microschizonts, were detected in cattle infected with T. orientalis [14] recognized as the causative agent of benign theileriosis in Korea. Stewart et al. [12] reported the schizonts of T. buffelli, the causative agent of benign bovine theileriosis in Australia, morphologically similar to those of T. orientalis. Recently the schizont stage of Japanese T. sergenti was also described [9]. The schizonts of all the three species have been detected in lymph node biopsy smears from experimentally infected calves. There have been no reports, however, on histological observations of the schizont stage of the three species. In this study, the schizont stage of Japanese T. sergenti is examined histologically.

Though the question on a valid name for the organism designated as T. sergenti in Japan [13] has provoked much discussion, we tentatively use T. sergenti in this paper.

MATERIALS AND METHODS

Animals: Four Holstein calves aged 3 months, were used without splenectomy in the experiments. No parasites were detected in Giemsa-stained blood smears from the calves before use. The absence of antibodies against T. sergenti piromas antigen in the calf sera was also confirmed by the indirect fluorescent antibody test and/or enzyme linked immunosorbent assay (ELISA) [11] before use.

Theileria stock and tick race: The Ikeda stock of T. sergenti isolated in Tochigi Prefecture, Japan [3] was used in this experiment.

The Okayama race of nymphal Haemaphysalis longicornis was used as an inoculation vector of T. sergenti. The procedure for breeding and applying infected H. longicornis for the inoculation of T. sergenti was as described previously [3, 5].

Design of experiment: Three calves were infected with T. sergenti, by feeding of infected nymphal ticks. As a control, uninfected nymphs were applied to one calf. The nymphs were applied to each calf at the left ear by the ear bag method [5]. One (calf No. 1) of the three infected calves was necropsied 8 days after tick infestation. The other two calves (No. 2 and No. 3) along with the control (No. 4) were necropsied 10 days after tick infestation.

After tick application, clinical signs and body temperature were checked every day. Giemsa-stained blood smears were also made every day to check the piromas.

At necropsy all visceral organs were examined microscopically. Samples of the parotid and lateral retropharyngeal lymph nodes were taken for immunohistochemical and electron microscopical observations after draining the site of tick application.

Preparation for light microscopy: The tissues were fixed in 10% phosphate buffered formalin, dehydrated in alcohol, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). Giemsa staining was also performed when necessary.

Preparation for immunohistochemical staining: The parotid and lateral retropharyngeal lymph nodes were cut into about 0.5 cm cubic blocks, which were frozen immediately in n-hexane at −80°C and sectioned 8 μm thick with a cryotome. The sections were fixed in cold acetone and stained by the direct immuno-peroxidase (DPO) method using horseradish-peroxidase conjugate in the same procedure as described previously [6]. The substrate used for DPO staining was 0.1% hydrogen peroxide containing 0.05% 3,3'-diaminobenzidine-tetrahydrochloride (DAB) in Tris buffer at pH 7.6.

Preparation for electron microscopy: For the electron microscopical observation, the parotid and lateral re-
tropharyngeal lymph nodes were cut into 1.5 mm cubes, pre-fixed in 2% phosphate buffered glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated and embedded in Epon mixture. Ultra-thin sections were stained with uranyl acetate and lead citrate before examining with a JEOL 1200 EX electron microscope.

RESULTS

Clinical features: Pyrexia (41.2°C) was observed in calves No. 2 and 3 between 9 and 10 days after tick application. Piroplasms were detected in blood smears collected from the calves 10 days after infestation. Swelling was observed in the parotid area on the left side, the site of tick infestation, of all infected calves from 8 days after infestation. No clinical changes were observed in uninfected calf No. 4.

Gross lesions: In the infected calves, the left parotid and lateral retropharyngeal lymph nodes were three times larger than those on the right side. The left parotid lymph node in the control calf was slightly larger than the right one. No macroscopical changes were observed in other visceral organs of all the calves.

Microscopical observations: Table 1 shows the summarized data on the experimental calves.

In the local drainage lymph nodes, the left parotid and retropharyngeal lymph nodes, of the three infected calves, the lymph nodes were obscure in structure due to the infiltration of the cortex with large lymphocytes. In the lymph nodes of calves No. 2 and 3, the number of large round cells, resembling lymphoblasts, with a single bright nucleus and scant cytoplasm remarkably increased in the paracortical zone. Infiltration with macrophages and swelling of the reticulendothelial cells were also prominent in the lymphatic sinus. Numerous huge cells were observed in the subcapsular and medullary sinuses and lymphatic tissue of the infected calves (Fig. 1). Their nuclei were enlarged irregularly and frequently twisting. Their cytoplasm was rich and stained basophilic. The huge cells were approximately 50 μm in diameter in calf No. 1, while in the lymph nodes of calves No. 2 and 3 they reached about 100–200 μm in diameter (Fig. 2). Most of the cells contained numerous basophilic granules, which varied coarse to fine in appearance, in the cytoplasm. The coarse granules were 3 to 7 μm in diameter, while the fine ones were about 1 μm and sometimes uniform in size. Clusters of uniform granules were observed in the sinuses and lymphatic tissues of calves No. 2 and 3.

Huge cells, of the same structure as observed in the lymph nodes, were also found in the liver of calves No. 2 and 3 (Fig. 3). The granules found in the liver were about 1 μm in diameter. The huge cells containing fine granules were sometimes degenerative and the granules seemed to be released from them. Clusters of the granules were frequently found in the sinusoid or central vein and the granules themselves contacted the erythrocytes directly (Fig. 4).

Huge cells were also observed in the spleen. None of the prominent changes described above were seen in the control calf.

Immuno histochemical observations: The granules in the huge cells in the lymph nodes showed a specific reaction against anti-*T. sergenti* antibodies after DPO staining (Fig. 5).

Ultrastructural observations: Electron microscopical observation revealed granules 3 to 7 μm in diameter in the cytoplasm of huge cells (Fig. 6). The granules showed electron-dense appearance and had more than one nucleus and membranous structure on each surface. The granules also had morphological features such as lamellar structure and vacuoles. The nucleus of huge cells was enlarged irregularly and had a great deal of chromatin. An unit cell membrane was observed on the surface of the huge cell.

Table 1. Summarized data on experimental calves infected with the Ikeda stock of *Theileria sergenti* by feeding of *Haemaphysalis longicornis*

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Tick application</th>
<th>Stage of ticks</th>
<th>Number of ticks</th>
<th>Necrospy (Days after tick application)</th>
<th>Detection of huge cells containing schizonts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Parotid lymph node Left</td>
</tr>
<tr>
<td>1</td>
<td>Infected nymphs</td>
<td>200</td>
<td>8</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Infected nymphs</td>
<td>200</td>
<td>10</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Infected nymphs</td>
<td>200</td>
<td>10</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Uninfected nymphs</td>
<td>200</td>
<td>10</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

Degree: -; Negative, +; Small number, ++; Large number.
Fig. 1. Huge cells (arrows) formed in the sinus of the parotid node of *T. sergenti*-infected calf No. 2, and containing schizonts in the cytoplasm. Giemsa, × 100.

Fig. 2. A huge cell in the left parotid node of *T. sergenti*-infected calf No. 2. Note the coarse granules (schizonts, arrow heads) around the enlarged nucleus (arrow) of huge host cell. Giemsa, × 400.

Fig. 3. A huge host cell in the liver of *T. sergenti*-infected calf No. 2, containing schizonts (arrows) in the cytoplasm. H&E, × 200.

Fig. 4. Cluster of the uniform fine granules observed in the central vein of *T. sergenti*-infected calf No. 2. Note the granules contacting erythrocytes. Giemsa, × 1,000.

Fig. 5. A huge cell in the parotid node. Note the granules showing the specific reaction against anti-*T. sergenti* antibodies. DPO, × 400.

Fig. 6. Granules (G) around the enlarged nucleus (N), having an electron-dense appearance.
DeMartini and Moulton [1] described that in the drainage lymph nodes the changes attributed to *T. parva* infection are extreme enlargement of the paracortical zone due to infiltration and proliferation of parasitized lymphocytes, often in mitosis, and there are very large numbers of lymphoblasts in the sinuses and medullary cords. Parasitized lymphocytes are also recognized in other nodes. In the present study, increase in the number of large round cells was observed in the paracortical zone. The cells, however, were not parasitized by the parasite, so this may be attributed to the response to antigenic stimulation [2].

In the present study, a lot of huge cells containing granules in the cytoplasm were characteristically observed in the local drainage nodes such as the parotid and retropharyngeal nodes, livers and spleens. It was confirmed that the granules were exo-erythrocytic forms of *T. sergenti* because they showed a specific immunohistochemical reaction against anti-*T. sergenti* antibody. In addition, ultrastructural findings of the granular bodies suggested that they were the schizont stage of *T. sergenti* and existed in the cytoplasm and that the huge cells were derived from the host cells.

Moreover, a single schizont of *T. sergenti* develops into two forms: during the phase of nuclear division it appears to be a 'macroschizont' and during merozoite formation, to be a 'microschizont' with rosette-like appearance [10, 11]. According to this definition, the schizonts of *T. sergenti* observed in this microscopical study were considered the 'macroschizont' because they were multinuclear and produced no merozoites.

Schizonts of *T. parva* and *T. annulata* are formed in lymphocytes or monocytes and mature only in lymphoid cells [1, 13]. Those of *T. mutans* are also formed in the lymphoid cells [15]. Schizont stage of *T. sergenti* was observed in a unit cell as well as those of the other three species, although the schizonts detected in lymph node biopsy smears [9] were morphologically similar to those of *T. orientalis* and *T. buffeli* [12, 14]. The host cells of *T. sergenti* are apparently stimulated to be enlarged, while those of *T. parva* or *T. annulata* are stimulated to be divided into daughter cells [4].

Although there is not yet obtained enough evidence of the process to the schizont stage of *T. sergenti*, it might be speculated that the parasites invade into host cells by infestation of infected ticks and the cells are stimulated to become huge. Schizonts are formed in the cytoplasm of the resultant enlarged cells and mature therein. Finally the huge cells containing schizonts are broken and the parasites are released.

REFERENCES