ULTRASTRUCTURE OF BOVINE SPLEEN CELLS PARASITIZED BY THEILERIA PARVA

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ABSTRACT

Kimeto, B.A., 1981. Ultrastructure of bovine spleen cells parasitized by Theileria parva. Vet. Parasitol., 8: 13-21.

The ultrastructure of spleen cells parasitized by *Theileria parva* has been described. All the cells were lymphoblasts. Schizonts were always observed close to mitochondria. In most cases they were located in the region of the golgi apparatus. The majority of non-parasitized lymphoid cells were also lymphoblastic. Plasma cells with well developed ergastoplasm and unparasitized were also observed.

INTRODUCTION

Theileria parva is a protozoa which causes East Coast Fever (E.C.F.), a disease which is transmitted by *Rhipicephalus appendiculatus* ticks. The parasite proliferates within the lymphoid cells and causes lymphoid cell hyperplasia. Ultrastructural studies of the lymph node (De Martini and Moulton, 1973) and the skin (Kimeto, 1978) have been done. The present paper is an electron microscopic study of the parasitized bovine spleen cells.

MATERIALS AND METHODS

Animals

Three Ayrshire steers were used, ranging in age from 14 to 18 months. The animals were examined for the presence of haemoparasites before the beginning of the experiment and proved to be negative.

Adult female ticks infected with *Theileria parva* were attached to the animals as previously described by Bailey (1960). Blood and lymph node smears were prepared and temperatures were recorded every day following tick attachment.

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Electron microscopic studies

Animals were killed in extremis and their spleens were removed and processed for electron microscopy. The organs were fixed according to the method of Ito and Karnovsky (1968) in a formaldehyde—glutaraldehyde mixture containing 0.02% trinitrocresol for 2 h at 4°C, washed 3 times (5 min each time) with 0.2 M phosphate buffer (pH 7.2), and post-fixed with 1% osmium tetroxide in 0.2 M phosphate buffer (pH 7.2) for 3-5 h. Block were washed 3 times (5 min each) in isotonic saline solution, dehydrated in acetone series and embedded in Durcupan.

Thin sections (60 nm) were cut, mounted on polyvinyl formol resincoated copper grids, and stained with uranyl acetate for 5 min and with lear acetate for 3 min. The sections were then examined with an electron micro scope and photographed.

RESULTS

Clinical features and lesions

The clinical signs and lesions observed in all the three animals included lymphadenopathy, fever, petechial haemorrhages in the tongue, leukopenia pulmonary edema, lymphoid cell hyperplasia with mitosis in lymphoid tissues, and lymphoid cell infiltration into non-lymphoid organs. A 50% parasitaemia was observed in two animals, and 60% in the other animal, which was also slightly anaemic.

Electron microscopic studies

Parasitized spleen cells were large, and mostly oval with irregular outline Some showed elongated, drawn-out cytoplasm but phagocytosis was not observed. Numerous polyribosomes were diffusely distributed in the cytoplasm of the cell. Endoplasmic reticulum was scanty and often granular. Mitochondria were multiple, oval, enlarged and with distinct cristae. The golgi apparatus was conspicuous and in some cells the centrioles were observed (Fig. 1). The nuclei were mostly oval and large, and some showed in

Fig. 1. Parasitized lymphoblast from the spleen in ECF. A macroschizont is close to ge apparatus (G), centricle (Ce) and mitochondria (M). It has three nuclei (SN) and two vacuoles (V). Polyribosomes (PR), endoplasmic reticulum (ER), plasma cell (PC) and 1 phocyte (L) are shown. \times 21 000.

Fig. 2. Parasitized lymphoblast from the spleen in ECF showing a macroschizont in the region of nuclear indentation. The schizont has several nuclei (SN) and vacuoles (V). Golgi apparatus (G), mitochondria (M), polyribosomes (PR) and endoplasmic reticulu (ER) are shown. \times 21 000.





Fig. 3. Parasitized (PL) and non-parasitized lymphoblasts (NPL) and a non-lymphoblast (NL) (reticulum cell) from the spleen in ECF. The non-parasitized lymphoblast at the bottom shows multiple indentations of nucleus (N) and cytoplasmic processes (CP). \times 6400.

dentations (Fig. 2). The nucleoplasm contained a lot of euchromatin and little heterochromatin. The nucleoli were prominent in some of the cells.

Almost all non-parasitized spleen cells (Fig. 3) showed similar ultrastructural changes as described for the parasitized cells. Some of the cells possessed multiple nuclear indentations. Other non-parasitized cells were small with relatively large nuclei and little cytoplasm. These were tentatively identified as being lymphocytes. Plasma cells contained well developed ergastoplasm with many ribosomes. Some of the ergastoplasmic cisterns were dilated. Oval electron lucent bodies were observed in some cells (Fig. 1). Plasma cells were not parasitized.

Schizonts were always located close to mitochondria and in most cases they were in the region of the golgi apparatus. These parasites varied in shape size and structure. Each parasite possessed a unit membrane. Uninucleate schizonts (Figs. 4 and 5) possessed large oval nuclei surrounded by narrow and granular cytoplasm. The granules resembled ribosomes found in host-

Fig. 4.(a) Parasitized lymphoblast from the spleen in ECF. The macroschizont (S) is close to mitochondria (M) but not in the region of golgi apparatus (G). The host cell nucleus (HN) is euchromatic. Mitochondria (M), polyribosomes (PR) and endoplasmic reticulum (ER) are shown. \times 21 000.

(b) The schizont has a large nucleus (SN), narrow cytoplasm (C) and a vacuole (V). $\times~70~000.$





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cell cytoplasm. Several schizonts were multinucleated and multivacuolar. Round electron-dense bodies were observed in some parasites (Fig. 6). Other schizonts were elongated (Fig. 7) and in this particular cell the heterochromatin appeared to be dividing into two parts. Some cells (Fig. 8) contained several parasites.

DESUSSION

The parasites observed within splenic cells were macroschizonts, and they were embedded in host-cell cytoplasm. The majority were located in the region of the golgi apparatus of the host cells. Similar observations were made by Hilliger (1965) in parasitized spleen cells grown in vitro, and the author suggested this may be related to cell division. Schizonts were always found close to mitochondria in all of the parasitized cells, possibly indicating that the energy requirements of the parasite were derived from the host cell. The parasite may have found it unnecessary to develop its own energy sources as long as it was intracellular. Those parasites close to the centrioles may be expected to divide when the host cell undergoes cell division, as has been observed before in studies in vitro (Hulliger, 1965; Moulton et al., 1971a).

The ultrastructural changes in parasitized spleen cells were similar to those in parasitized lymphoblasts in the lymph node reported by De Martini and Moulton (1973). These parasitized lymphoblasts originate by transformation from reticulum cells after phagocytosis of *T. parva* (Barnett, 1960; Moulton et al., 1971a; Kimeto, 1978) and are capable of multiplying continuously in vitro (Malmquist et al., 1970; Moulton et al., 1971a). These cells multiply in vivo and migrate to many organs. Similar ultrastructural changes were observed in most of the non-parasitized spleen cells. These changes could be attributed to the presence of parasite antigen in tissue fluids, a phenomenon similar to the in vitro stimulation of lymphoid cells by phytohemagglutinin (De Martini and Moulton, 1973).

Since almost all of the spleen cells observed were lymphoblasts, the spleen may have lost some of its functions, one of which is phagocytosis. The nonparasitized lymphoblasts will not be infected, since infection is initiated by phagocytosis. In vitro studies have also shown no infection of new lymphoblasts (Hulliger, 1965; Moulton et al., 1971b).

Fig. 5. Parasitized lymphoblast from the spleen in ECF showing uninucleate macroschizont with a double-membraned vacuole (DV) close to a mitochondrion (M). Nucleus (N) and cytoplasm (C) are indicated. \times 57 000.

Fig. 6. Parasitized lymphoblast from the spleen in ECF showing a macroschizont with a round electron dense body (db). Vacuole (V), nucleus (N), mitochondria (M) and endoplasmic reticulum (ER) are shown. \times 35 000.

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Fig. 7. Lymphoblasts from the spleen in ECF. Parasitized lymphoblast in the left shows an elongated schizont (S), and the host-cell chromatin seems to be dividing into two. There is also a parasitized red blood cell (E). Lymphocyte (L) is marked. \times 8000.



Fig. 8. Parasitized cell from the spleen in ECF showing several parasites (P). Mitochondria (M) and host-cell nucleus (HN) are shown. \times 21 000.

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