EFFECT OF TOXOPLASMA GONDII ON HISTOPATHOLOGY AND HISTOCHEMISTRY OF RETICULOENDOTHELIAL SYSTEM IN EXPERIMENTAL ANIMALS


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Abstract. The effect of acute and chronic infections by Toxoplasma gondii on the spleen, thymus and liver of mice and rats, and on their antibody response has been studied. In acute infection while the splenic weight increased the thymic weight decreased. The histopathological studies demonstrated lymphocytic depletion of splenic follicles and thymic cortex. Numerous phagocytes and plasma cells were seen in the red pulp and thymic medulla. Vascular congestion and haemorrhages were marked. The liver cells showed degeneration which progressed from hydropic to fatty. The succinic dehydrogenase activity of damaged cells was decreased, while the phosphatase activity was increased. The parasites were seen in some liver cells. Marked cellular infiltration was observed around the blood vessels in the form of granulomata. The reticuloendothelial cells, Kupffer cells and phagocytes showed high alkaline phosphatase activity. In chronic infection the thymus showed early lymphocytic depletion then returned to normal. The splenic weight was increased and the follicles were enlarged with the presence of immunoblasts in the germinal centres. The cords of the red pulp were thickened and contained numerous plasma cells. Most of the liver cells were normal with normal enzymatic activity but small foci of necrosis were seen. There was a gradual increase in antibody response in both acute and chronic infections. It was concluded from the results that acute infection mainly produced toxic effects, whereas chronic infection produced immunological responses.

Toxoplasma gondii is an intracellular parasite that can invade any nucleated mammalian cell (Remington et al. 1960).

In acute infection the parasite undergoes an obligatory period of intracellular division within the lymphoid-macrophage system. The pseudocyst produced is of a temporary nature and soon breaks down to liberate the parasites, which then penetrate new cells. In chronic infection this pseudocystic phase lasts for about 9—11 days then dies down to be replaced by a cystic phase which takes place in the deeper tissues, e.g. anals, central nervous system and musculature (Lainson 1958). In acute infection the destruction of the infected cells is followed by the appearance of parasites in the blood stream. They disappear from the blood with the appearance of antibodies (Hutchison and Work 1969).

In a case reported by Pinkerton and Weinman (1940), Toxoplasma was found in the enlarged liver and spleen, which therefore proved to be involved early in acute infection. Also Brown and Jacobs (1958) commented that splenomegaly may stand beyond the acute and subacute infection. Similarly, Quintao (1963), Pierini (1968), Wishahy et al. (1971), Tsai (1972) and Rifaat et al. (1973, 1976), stated that hepatosplenomegaly is one of the manifestations of acquired toxoplasmosis in cases with or without signs and symptoms.

In the present investigation an attempt is made to throw more light on the effect of the parasite on the reticuloendothelial system and to correlate serological, histopathological and histochemical findings.
MATERIALS AND METHODS

Animals. Non-inbred albino mice and rats free of Toxoplasma infection and weighing 25—30 g and 50—60 g respectively were used. The mice served as models for the acute infection while the rats were used for the chronic infection.

Inoculation. The highly virulent RH strain was used for infection of experimental models. The peritoneal exudate of mice infected 3 days previously with the RH strain was diluted with penicillin — streptomycin saline and the dose of the inoculum adjusted so that each mouse received 80 000 and each rat received 120 000 free extracellular parasites. The inoculation was done by the subcutaneous intrascapular route. The mice were examined on days 3, 5 and 7 and the rats on days 10, 20, 30 and 40 after infection. Control non-infected mice and rats were examined on the same days as the test animals.

Serological examination. The mice were lightly anaesthetised with ether and exsanguinated by severing the axillary vessels. The blood was collected separately and the serum separated and stored at —20 °C until use. The antibodies were assessed by the indirect fluorescent antibody test (Kramat 1963). The antigen was prepared according to Goldman (1957). The fluorescein labelled anti-mouse and anti-rat globulins were obtained from Burroughs and Wellcome. A Zeiss photomicroscope III equipped with an Osram lamp HBO 200, W 4 was used.

Tissue examination. Spleens and thymuses were removed and weighed. Parts of spleen, thymus and liver were fixed in acetone and processed for histochemical localization of the alkaline phosphatase enzyme by Gomori (1952) technique. The site of enzymatic activity appears as black deposits.

Other parts of the same organs were fixed in 10 % formalin and paraffin sections 64 thick were prepared for histopathological study. They were stained by:


Fresh frozen sections 20 μm thick were prepared from the liver to study the succinic dehydrogenase activity (SDH) according to the Nachlas technique (Pearse 1960). Normally the site of enzymic activity appears as purple deposits in the cytoplasm of hepatic cells.

RESULTS

Effect of acute and chronic infections on the splenic and thymic weights

In acute infection there was an increase in the weight of mouse spleen. The average weight at 7 days was 0.17 g, whereas the average weight of normal mouse spleen was 0.13 g. On the other hand, the thymic weight showed gradual decrease. The average weight was 0.03 g at 7 days compared to 0.0104 g of control thymus.

In chronic infection the splenic weight gradually increased. The average weight of normal rat spleen was 0.2 g. The increase in weight reached its maximum of 0.262 g at 30 days. Thymic weight did not show any appreciable changes.

Effect of acute and chronic infections on the immunofluorescent antibody titre

A gradual increase in the antibody titre was observed, rising from 1/64 after 3 days to 1/512 after 7 days of acute infection, and from 1/512 after 10 days to 1/4096 after 20 days decreasing to 1/1024 after 40 days of chronic infection.

Effect of acute infection on the spleen of mouse

At 3 days: The spleen showed mild congestion. The lymph follicles were normal. No organisms were seen.

At 5 days: Vascular congestion was marked, with rupture of the blood sinusoids. Wide areas of haemorrhages were seen in the red pulp. The lymph follicles showed marked lymphocytic depletion (Plate I, Fig.1) compared to a control section (Plate I, Fig. 2). The red pulp contained numerous large acidophilic multinucleated phagocytic cells and polymorphonuclear leucocytes in groups (Fig. 1). The organisms were not seen. The reticuloendothelial cells in the red pulp were enlarged and showed high alkaline phosphatase activity (Plate II, Fig. 1).

At 7 days: The vascular congestion and lymphocytic depletion were more marked than at 5 days. The red pulp contained numerous polymorphs, phagocytic cells and plasma cells.
Effect of chronic infection on the spleen of rat

At 10 days: Vascular congestion and areas of subcapsular haemorrhages were seen. The lymph follicles were normal. Few phagocytic cells were seen in the red pulp.

At 20 and 30 days: The lymph follicles were enlarged and packed with lymphocytes. The germinal centre contained numerous cells with basophilic cytoplasm (immunoblasts) and so it appeared dark. The cords of the red pulp were thickened, with increase in their lymphocyte content. Many plasma cells were seen in the red pulp.

Fig. 1. Acute infection of spleen after 5 days with red pulp containing numerous polymorphs.

At 40 days: The lymph follicles were well formed and many plasma cells were present in the red pulp.

Effect of acute infection on the thymus of mouse

At 3 days: Focal areas of lymphocytic depletion were seen in the cortex (Plate I, Fig 3), compared to a control section (Plate I, Fig. 4). The reticular epithelial cells were hypertrophied and showed high alkaline phosphatase activity, with numerous plasma cells and phagocytic cells seen in the medulla. Vascular congestion was marked.

At 7 days: The lymphocytic depletion became more marked with marked proliferation of the reticular epithelial cells. They showed high alkaline phosphatase activity.

Effect of chronic infection on the thymus of rat

At 10 days: The thymus showed a moderate degree of focal cortical lymphocytic depletion. There was no cell phagocytosis.

At 20, 30, and 40 days: The thymus was normal. There was no increase in the alkaline phosphatase activity of the reticular epithelial cells.

Effect of acute infection on the liver of mouse

At 3 days: The liver cells showed hydropic degeneration and decreased succinic dehydrogenase activity compared to a control section. The glycogen content was also decreased. The alkaline phosphatase activity was increased (Plate II, Fig. 2) compared to a control section (Plate II, Fig. 3). The blood vessels were dilated and engorged.
with blood. Cellular infiltration formed of lymphocytes, histiocytes, polymorphs and large phagocytic cells was seen.

At 5 days: The liver cells showed marked fatty degeneration (Plate II, Fig. 4) and the organisms were seen in the degenerated cells (Fig. 2). The succinic dehydrogenase activity and glycogen content were much decreased. Cellular necrosis was observed around the haemorrhagic areas and cellular infiltration became extensive around the blood vessels in the form of granulomata. Numerous phagocytes were present among the infiltrating cells. The endothelial cells lining the blood sinusoids and von Kupffer cells were enlarged and showed high alkaline phosphatase activity (Plate II, Fig. 2).

At 7 days: The cellular degeneration and necrosis were marked with wide areas of haemorrhages. Cellular infiltration was evident.

Effect of chronic infection on the liver of rat

At 10 days: Vascular congestion was evident, with localized areas of haemorrhages. The liver cells around the haemorrhagic areas were necrosed and brown pigments were seen. However, most of the liver tissue was normal and the succinic dehydrogenase activity and glycogen contents were normal.

At 20, 30 days: The liver was normal, except for small foci of necrosis. The enzymatic activity and glycogen contents were normal.

DISCUSSION

In acute infection with the RH strain of Toxoplasma, a gradual decrease in the thymic weight was observed, whereas the splenic weight was increased. The decrease in thymic weight was due to focal areas of cortical lymphocytic depletion. A similar
lymphocytic depletion was observed in the lymph follicles of the spleen. The depletion reached its maximum by the 7th day in both the thymus and spleen. Henry et al. (1973) observed depletion of lymphocytes from the cortex of lymph nodes of rabbits infected with a highly virulent strain of *Toxoplasma*. Similarly, Huldt et al. (1973), in a study of neonatal *Toxoplasma* infection in mice, found depletion of the thymic cortical lymphocytes.

This lymphocytic depletion is probably due to death of the lymphocytes in the thymus and spleen as evidenced in this work by the presence of numerous phagocytic cells. Phagocytosis of host cells is not found solely in toxoplasmosis but it is also seen in glandular fever and in the cortex of thymus during acute involution (Henry et al. 1973). The lymphocytolytic effect of *Toxoplasma* may be due to a toxic factor liberated from the organism. Weinman and Klatenko (1950) have demonstrated a toxin produced by this strain which in intravenous injection kills mice very quickly. Another possible explanation for this cellular destruction is the liberation of lysosomal enzymes present in the organism (Hansson and Sourander 1968). On the other hand, lymphocytic depletion may be the result of transfer of lymphocytes from the thymus and spleen to focal lymphocytic infiltration sites which occur in lesions of other tissues (Henry and Beverley 1969). In the present study granulomata of infiltrating cells, mainly of lymphocytes, were seen in the liver.

The increase in splenic weight inspite of the lymphocytic depletion in acute infection can be attributed to its congestion and infiltration with large numbers of polymorphonuclear leucocytes.

The cytotoxic effect of acute infection was evident in the liver, which progressed from hydropic to fatty degeneration. These degenerative changes were due to the multiplication of the organism intracellularly (Pulvertaft et al. 1954), causing damage to the cell organoids. Mitochondrial damage was proved by the decrease in succinic dehydrogenase activity.

The decreased liver glycogen can be attributed to the action of glycolytic enzymes produced by the toxoplasms (Lund et al. 1966). Another possible cause of glycogen depletion is the increased activity of the organisms leading to its utilization. This was substantiated by Mira Gutiérrez and Del Rey Calero (1966) who demonstrated the presence of PAS-positive substance in the organisms. At the same time it must be noted that the degeneration of liver cell organoids prevents new glycogen formation.

The alkaline phosphatase activity was increased in the degenerated liver cells. Sherlock and Walshe (1947) and Meyer and Williams (1948) described similar increase in alkaline phosphatase activity in nuclei and cytoplasm of degenerated liver cells and attributed this increase to failure of the damaged cells to excrete the enzyme. But the increased alkaline phosphatase activity found to occur in the reticuloendothelial cells of spleen, Kupffer cells and endothelial cells lining the liver sinusoids rather indicates increased activity of reticuloendothelial system (Weir 1973). This activity was found to return to normal in chronic infection. Similarly, Ruskin et al. (1969) found that mice chronically infected with *Toxoplasma* did not exhibit appreciably increased reticuloendothelial system activity as reflected by carbon clearance testing.

The increased alkaline phosphatase activity occurring in the reticular epithelial cells of the thymus is probably due to a rapid proliferation (of these cells). This is substantiated by Pearse’s (1960) statement that apart from the nuclei and brush border of kidney tubules, this enzyme seldom occurred in the cytoplasm except in rapidly regenerating cells, especially in cells that form fibres.

In the present investigation the organism was detected in liver cells but was.
neither seen in the lymphocytes nor in the phagocytes. This coincides with the electron microscopic studies performed by Henry et al. (1973), who found that the phagocytic activity which was seen histologically appeared to be macrophage ingestion of the host cell materials and not of Toxoplasma.

In chronic infection, the thymus showed early slight lymphocytic depletion, then returned to normal. But the main reaction was in the spleen where large lymph follicles were formed. Numerous plasma cells were seen in splenic follicles and red pulp and in the thymic medulla, and the antibody titre reached its maximum with the maximum follicular reaction. These findings suggest the presence of an immunological response.

It is known that parasitaemia may persist in spite of high titres of circulating antibody. It was suggested that it was because the parasites are protected within the leucocytes from the effect of antibodies (Jacobs 1962). Ham (1974) explained that the antigenic stimulation of T lymphocytes stimulates the formation of small lymphocytes that increase the size of lymph follicles and the thickness of the cords of the red pulp, while the stimulation of B lymphocytes induces the formation of plasma cells. It was stated further that plasma cells are not normal constituents of the thymus for two reasons: firstly, T lymphocytes cannot develop into plasma cells, secondly, if any B lymphocytes should penetrate a capillary and enter the thymic tissue it would be in an environment in which it would be unlikely to encounter an antigen. This is because of the blood-thymic barrier that prevents blood-borne antigens from escaping into the perivascular tissue (Ham 1974). However, recent studies by Ravioila and Karnovsky (1972) showed that the blood thymic barrier is effective only in the cortex and not in the medulla.

The liver in chronic infection was nearly normal except for small foci of necrosis. The necrotic foci may be due to a delayed hypersensitivity that results from occasional rupture of some cysts and liberation of their organisms, because the elastic tough membrane surrounding the cyst protects the liver cells from the harmful effect of the organism (Lainson 1958).

It can be concluded that acute infection with highly virulent Toxoplasma gondii produced cytotoxic effects, increased activity of reticuloendothelial system and stimulated antibody production. Chronic infection, on the other hand, produced mainly immunological responses.

ВОЗДЕЙСТВИЕ TOXOPLASMA GONDII НА ГИСТОПАТОЛОГИЮ И ГИСТОХИМИЮ РЕТИКУЛОЭНДОТЕЛІАЛЬНОЙ СИСТЕМЫ У ПОДОПЫТНЫХ ЖИВОТНЫХ

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Резюме. Изучено воздействие острой и хронической инфекции Toxoplasma gondii на селезенку, тимус и печень мышей и крыс и на их иммунный ответ. При острой инфекции повышается вес селезенки, тогда как вес тимуса понижается. При гистопатологическом исследовании обнаружено лимфоцитарное опорожнение фолликулов селезенки и кортика тимуса. Многочисленные фагоциты и клетки плазмы обнаружены в красной пульпе и мозговом веществе тимуса. Встречалось выражительное васкулярное скопление и геморрагия. Дегенерация клеток печени продвигалась от водянистой к жировой. Активность сукицинатдегидрогеназы поврежденных клеток понижалась, тогда как активность фосфатазы повышалась. Паразиты были обнаружены в некоторых клетках печени. Выявлена значительная клеточная инфильтрация вокруг кровеносных сосудов в виде гранулем. Ретикулоэндотелиальные клетки, купферовские клетки и фагоциты проявляли высокую
активность щелочной фосфатазы. При хронической инфекции в тимусе обнаружено сначала лимфоцитарное опорожнение и после того он опять стал нормальным. Вес селезенки повысился и фолликулы увеличивались с присутствием иммунобластов в геморрагических центрах. Капилляры сердечной жидкости утолщались и содержали многочисленные плазматические клетки. Клетки печени были большей частью нормальные, с нормальной энзиматической активностью, но обнаружены небольшие очаги некроза. Постепенно повысился иммунный ответ, при острой и хронической инфекциях. Из результатов вытекает, что острыя инфекция имеет главным образом токсическое действие, тогда как хроническая инфекция вызывает иммунный ответ.

REFERENCES


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Fig. 1. Acute infection of spleen after 5 days. The follicles show marked lymphocytic depletion (H & E). (× 100). Fig. 2. Spleen of normal mouse showing a splenic follicle with a germinal centre. The red pulp is formed of blood sinusoids surrounded with lymphocytes (H & E). (× 100). Fig. 3. Acute infection of thymus after 3 days. Focal areas of lymphocytic depletion are seen in the cortex. (H & E). (× 100). Fig. 4. Thymus of normal mouse. The cortex is heavily infiltrated with lymphocytes. The medulla contains fewer lymphocytes. (H & E). (× 100).
Fig. 1. Acute infection of spleen after 5 days showing increased alkaline phosphatase activity in the reticuloendothelial cells and phagocytic cells (Gomori). (× 100). Fig. 2. Acute infection of liver after 3 days showing increased alkaline phosphatase activity in the cytoplasm and nuclei. The Kupffer cells and infiltrating cells show high activity. (Gomori). (× 100). Fig. 3. Liver of normal mouse showing alkaline phosphatase activity. The nuclei are well stained while the cytoplasm is faintly stained. (Gomori). (× 100). Fig 4. Acute infection of liver after 5 days. The liver cells show fatty degeneration. Marked cellular infiltration is also seen. (H & E). (× 100).