Tissue Reaction After Subcutaneous Injection of Taenia Saginata Oncospheres

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Abstract. After subcutaneous injection of T. saginata oncospheres, a slight swelling is visible at the site of injection first after 13 days. Conspicuous projecting nodes appear after 3–4 weeks. Histological examinations of the site of injection revealed the following changes: On days 3 and 4 p.i. — edema, focal necrosis and haemorrhage, exudation of leucocytes, activation of monocytes and fibroblasts. On day 7 p.i. — in addition to edema and focal activation of monocytes and fibroblasts, focal accumulation of macrophages, proliferation of blood capillaries and formation of collagen. On day 10 p.i. — eosinophils and light macrophages around the larvae, fibroplastic granulation tissue at the periphery and collagenous connective tissue abundant. Hyperplasia of lymphoid tissue starts to appear at this time. On days 13–14 p.i. — a large amount of newly formed connective tissue in granulation tissue, diffuse infiltration of eosinophils and hyperplasia of lymphatic tissue. On days 19–34 p.i. — haemorrhage around the cysticerci, activation of macrophages, phagocytizing erythrocytes. Endothelia of blood capillaries proliferate; lymphatic follicles are formed at the periphery of nodular affection which appears after inoculation of oncospheres. On day 34 p.i. — distinct formation of a pseudocyst. At the period from 33th to 41st day after injection, there is a mature connective tissue among the cysts, but still strongly infiltrated with cells. Clusters of siderophages are visible in connective tissue septa between the cysts.

After a subcutaneous injection, the oncospheres of T. saginata develop into cysticerci up to the infective stage essentially in the same way as in the intestines (Froyd and Round 1969). The area of pathologic reaction is limited, but sometimes the oncospheres may disperse to other sites (Wikerhäuser et al. 1971, Machnicka and Ślajs 1978). The subcutaneous injection of oncospheres may be used for the studies of early postoncospheral stages and course of pathologic reaction, but also for the studies of the effect of anthelmintics or drugs with a potential cestodicidal effect (Bessonov and Arkhipova 1983). It is possible to use only one experimental animal for injecting the oncospheres into several, previously marked sites and during the experiment, at given time intervals, take the samples for histological and electron microscopy examinations. Some basic features of pathologic resection around the oncospheres and larvae of T. saginata after subcutaneous injection, as revealed in our experiments, are presented in this paper.

Material and Methods

Activated T. saginata oncospheres were injected subcutaneously into 3 male calves weighing 80–100 kg. The sites of injection (7 sites measuring 10 × 10 cm) on both sides of spine were previously shaved and the surface was disinfected. Physiological saline was injected into another site. The oncospheres were activated by Silverman’s (1964) method and injected by a syringe in the dose of 5,000 oncospheres in 0.5 ml sterile physiological saline. Skin biopsies were carried out on days 3, 4, 7, 10, 13, 26, 34, 53 and 61 after injection. The skin and subcutaneous tissue were removed by a routine surgical procedure at local anesthesia by freezing. Procain was used far the suture of the wound.

319
RESULTS

At the site where oncospheres were injected, a slight elevation, visible particularly at oblique lighting, appears only on days 12–13 after injection. About 5 weeks (12–13 days) after injection, a bulge is well visible at the site of injection and the node is filled with a spongy, vascular mass of subcutaneous tissue. The cysts start to appear on day 34 p.i. A week after injection, the node is palpable and the area around it is slightly enlarged. The area around the oncospheres consists of several layers at some places. There is a marked swelling of vessel endothelia and intravascular leukocytosis. The nodular focus contains sometimes also a focal necrosis of subcutaneous connective tissue and often free erythrocytes. There occur oncospheres with an obscure structure, apparently dead, swallowed in a giant multinucleated cell (Plate III, Figs. 2 and 3). However, we found also spherical stages, measuring 0.030–0.040 mm, with large, light cells. Epitheloid cells were accumulated around them. Their structure was quite intact.

On days 13–14 p.i., the histological finding at the site of injection is essentially the same as that by 10 p.i. Although the oncospheres are not significantly larger, the node is more solid and the area around it is slightly more enlarged. The newly formed collagen is abundant and matures. The tissue of the whole node is diffusely infiltrated with eosinophils, but there is also a focal hyperplasia of lymphatic tissue and some clusters of large epitheloid cells or giant multinucleate cells. The blood capillaries are numerous and possess swollen endothelia. T. saginata larvae are spherical or oval, measuring about 0.120–0.130 mm, with large, light cells. They are surrounded by a dense connective tissue layer.

On day 19 p.i. the node consists of mature collagenous connective tissue with star-like fibroblasts and it is scarcely infiltrated with lymphocytes. There are numerous lymph nodes at the periphery and foci with larvae inside the node. It is of interest that the cysticerci are usually surrounded by leukocytes, light macrophages and a large number of lymphocytes. The larval nuclei with numerous mitoses is only more to the periphery (Plate IV, Figs. 1 and 2). The nuclei of cells of histiocytic type are markedly large and bladder-shaped. Whole areas of granulation tissue consist of layers of enormously swollen cells of capillary wall (Plate IV, Figs. 2).

One week later, i.e. about 26 days after injection, a widespread haemorrhage surrounds the cysticerci (1.4–1.0 mm) with soecul anlage. At the border of the zone of erythrocytes and haemorrhagic tissue, there are macrophages migrating from the granulation tissue (Plate V, Figs. 1 and 2). Most probably transformed endothelial cells of blood capillaries or transformed pericytes are involved in the origin of these macrophages. The haemorrhage contains large macrophages with ingested blood cells (Plate V, Fig. 3). The whole system of focus around the cysticerci is surrounded by mature connective tissue with clusters of lymphatic foci.

Also on day 34 p.i., the bladder (measuring more than 1 mm) of the cysticerci with soecul with sucker anlagen is surrounded by a haemorrhage, but it is visible that a cavity — pseudocyst is already forming around it. Its wall consists of a granulation tissue containing histiocyes and bundles of activated and proliferating endothelia of blood capillaries. Solitary, unusually large macrophages separate from the granulation tissue into the cavity of the arising pseudocyst and there they phagocytize the erythrocytes and leukocytes. Resting fibrocytes are arranged in circles in the outer zone of granulation tissue. There are also bundles of collagen fibres and numerous lymphatic foci (Plate VI, Figs. 1 and 2).

On days 53 and 61 p.i., the cysticerci measure 1.7–4.0 mm × 2.3–4.5 mm and possess a soecul (0.9–1.0 mm) with suckers (Plate VI, Fig. 3). They are localized in pseudocysts lying close one to another and separated mostly by narrow connecti-
The results of our studies show that the inflammatory reaction in the subcutis around early postonospherical stages and larvae of T. saginata can be identified by adseption and palpation no sooner than on day 12 after injection. Only about 20–22 days p.i. it is more marked, in form of bulges projecting above the surface, as it was described also by other authors (Macnieska and Slais 1978, Bessono and Archipova 1983). In no case a swelling was observed 6 h after injection of one cercaria, as it was recorded by Storba et al. (1981), not even at simultaneous peroral and subcutaneous infections. Such a reaction might occur if a material heavily contaminated with bacteria was used for the injection, but it is little probable even in this case. It is necessary to stress this fact, since the data published in the above paper might lead to a distortion in the evaluation of the effect of infections on the host.

It is remarkable that the nodular affections at the site of injection may decrease in size already on day 34 p. i. In our experiments, these affections could be identified by adseption only as moderate elevations of skin at the site of injection and by palpation as flat structures in the deep subcutis 7 weeks after injection. The paradox finding that later, e.g. 46 or 53 days p.i., the foci do not enlarge, but their size remains the same or decreases, though the cysticercus continues to grow, may be explained by the fact that the inflammation gradually ceases and the newly formed collagen disappears from about 6 weeks after injection. That is the case also in cysticerci localized in muscles (Blazek et al. 1981). In contrast to them, however, a surprisingly stronger cellularity was observed in the cysts and their vicinity in subcutaneous infections.

The subcutaneous injection of oncospheres also allows very well to observe the onset of individual components of cellular reaction. Around the early postonospherical stages and young larvae, there appear first (in addition to leucocytes) the monocytes turning to macrophages and these seem to be the first to get into contact with the larva. Besides the monocytes, also some cells of the surrounding tissue, e.g. endothelium of capillaries and pericytes, turn to macrophages. Simultaneously with or immediately after the appearance of macrophages there occurs a marked activation of fibroblasts and violent formation of collagen. The vacuolation of their plasma, visible in the light microscope in histological paraffin sections and particularly in semithin sections through the material embedded in resin, is the manifestation of this process. A granular endoplasmic reticulum widened in form of cisterns is involved. This initial phase of granulomatos reaction around C. bovis is thus similar to that in muscles after porical infection (Blazek and Schramlova 1980a, Blazek et al. 1981).

In general it corresponds to the character of tissue reaction when a granuloma arises and develops in case of latent hyperreactivity known, e.g. in schistosomiasis (Boros et al. 1983), even in the experiments in vitro (Bentley et al. 1989).

In relation with the violent activation of fibroblasts it should be noted that a certain part of antigens from Taenia solium larvae has the properties similar to those of fibronectin (Planarte et al. 1982), which is capable of affecting the growth of transplanted cells and is closely associated with the formation of collagen. The observations of early phases of tissue reaction to the presence of T. saginata larvae, particularly the observations of a markedly increased activity of fibroblasts, suggest that the antigens of T. saginata larvae produce an analogical effect. It is also evident that an important role in the initiation of violent activation of fibroblasts is played by the mutual relation between the fibroblasts and macrophages, which (in addition to other functional relations) increase the activity of fibroblasts (Boros et al. 1983).

Of interest is the question how the haemorrhage occurs in the vicinity of cysticercus, so conspicuous about 19–34 days after subcutaneous injection of the oncospheres. It is no accidental finding, since also Macnieska and Slais (1978) state that erythrocytes and fibrin coagula occur in some parts of the inner layer of reaction around the cysticercus on day 30 after subcutaneous injection. However, the authors did not interpret this finding.

As it was mentioned above, at a certain stage of pathological process around the cysticercus blood capillaries are newly formed in the granulatous tissue and they form bands of cellular clusters situated close to one another. Later the clusters get a lumina and it is probable that the extensive haemorrhage around the cysticercus is produced just by them. Such a haemorrhage is not caused even by the traumatization of tissue by the injection of the infective material. The haemorrhage may also be induced by a certain movement of the larva. The developing larvae seem to require just the blood medium at a certain stage of development. Later there appear large macrophages in the haemorrhage which phagocytize the erythrocytes and other blood cells and perform thus a cleaning function. Then they migrate to the periphery of the haemorrhage and to the surrounding granulatous tissue where they get the characteristic form of granulatous reaction in the tissue around the pseudocysts even after the florid process has been terminated. They accumulate there and store the hemoserin. The dynamics of tissue reaction around the T. saginata larva developing in the subcutis includes that the pseudocyst, in which the cysticercus is localized, arises either by reconstruction (if it is demonstrated that the inner surface is covered with endothelium) of a blood capillary, as it was assumed earlier (Blazek et al. 1981) or that a posthaemorrhagic pseudocyst is essentially involved in this localization.
На 3—4-й день — опухоль, фокальный некроз и кровотечение, эскалация лейкоцитов, активация монокитов и фибробластов. На 7-й день — кроме опухоли и очагов эозинофильных лейкоцитов, активация монокитов и фибробластов, фокальное некрозирование микрососудов, пролиферация кровяных капилляров и образование капсулы. На 10-й день — вокруг лимфоцитов эозинофильные лейкоциты и светлые микрогранулы. В то же время имеются гиперплазия лимфатических тканей. На 13—14-й день — в лимфатических тканях находится большое количество новообразованной соединительной ткани, диффузная инфильтрация эозинофилов и гиперплазия лимфатической ткани. На 19—34-й день — вокруг капилляров наблюдается пролиферация и активация микрогранулем, фрагментирующиеся гранулы. Эндоциты кровяных капилляров пролиферируют, на периферии образуются лимфатические фолликулы. На 34-й день видимо образование сокращений. На 53—61-й день среди гранулем соединительная ткань, но все время в больших степенях инфильтрированы клетками. В соединительных тканях и некрозах между клетками встречаются инфильтрации гранулем.

REFERENCES


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Oocysts of *T. saginata* on day 4 after subcutaneous injection. Fig. 1. Oocyst on the margin of a large focus of eosinophils and histiocytes. (HE, ×300). Fig. 2. Only moderate activation of subcutaneous connective tissue cells in the vicinity of the oocyst. (HE, ×300). Figs. 3, and 4. Detail of the oocyst. Typical luminescent halo corresponds to the zone of microvilli projecting from the surface of the oocyst. (HE, ×300).

Tissue reaction in the subcutis in the first week after injection of oocysts. Fig. 1. On day 4 p.i., hyperemia and exudates in subcutaneous connective tissue, early exudation of mononuclear cells, activation of fibroblasts and cells of blood vessel wall. (Semithin section, toluidine blue, ×300). Fig. 2. On day 7 p.i., centre of the focus with erythrocytes (ER) and macrophages with vacuolated plasma (arrow); some of them at mitotic division. Marked activation of fibroblasts and proliferation of cells of blood capillary wall at the periphery of the focus (double arrow). (Semithin section, toluidine blue, ×300). Fig. 3. Giant multinuclear cell of Langhans’s type in young granulation tissue. Day 7 p.i. (HE, ×250). Fig. 4. Detail of activated fibroblast. Day 7 p.i. (HE, ×600).
Fig. 1. On day 10 p.i. cysticercus (C) is surrounded by eosinophils (EO), zone of macrophages (MP) and fibroplastic granulation tissue (GT) more to the periphery. (HE, ×120). Figs. 2 and 3. Tissue reaction around the oncosphere (O), dead at the stage of early postoncosporal development. Day 10 p.i. (HE, ×300).

Fig. 1. Granulation tissue with conspicuous activation of capillary endothelia and formation of multinuclear synplasms. Day 10 p.i. (HE, ×300). Fig. 2. Detail of cellular proliferation from Fig. 1. (HE, ×800).
On day 20 p.i., Fig. 1. Cysticercus (C) surrounded by haemorrhage (HR). Granulation tissue (GT) and numerous macrophages with ingested erythrocytes (arrow) at the periphery of the haemorrhage. (HE, ×120). Fig. 2. Detail of the haemorrhage from Fig. 1. Free erythrocytes (arrow), macrophages (double arrow), fibroblasts (F). (HE, ×300). Fig. 3. Detail of the macrophage with ingested erythrocytes (arrow). (HE, ×900).

Fig. 1. Nodular hyperplasia of lymphocyte tissue occurs later at the periphery of the focus with cysticerci. Day 34 p.i. (HE, ×75). Fig. 2. Detail of a lymphoid focus from Fig. 1. (HE, ×100). Fig. 3. Cysticercus from subcutaneous localization on day 61 after injection of oncospheres. (HE, ×30).