THE PATHOGENICITY OF RABBIT COCCIDIUM
EIMERIA COECICOLA CHEISSIN, 1947

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Abstract. The pathogenicity of Eimeria coecicola for rabbits has been demonstrated in our experiments. The animals suffered from a prolonged affection of appendix lasting at least 20 days. Pathological changes appeared with the development of emerogony since day 4 post infection (DPI) and were characterized by an inflammatory infiltration and abundant pyogenic component in lamina propria, swelling and coalescence of upper parts of appendix mucosa above atrophied domes, where spaces filled with stagnating inflammatory exudate, endogenic stages of coccidia, and desquamated epithelium are formed. The alteration of the epithelium and exposure of the appendix lamina propria occurs in relation with the gametogony in the period of about 10 DPI. Since 8 DPI, the epithelium of the infected endogenic stages of coccidia becomes hyperplastic, proliferates into lamina propria and is subjected to necrosis. Groups of immature oocysts and their fragments remain in lamina propria and are resorbed, at least for 10 days, by granulomatous inflammatory structures with abundant multinucleate cells of the type of foreign body cells.

The life cycle of Eimeria coecicola has been dealt with by Cheissin (1947, 1967, 1968). According to this author, the developmental stages of this coccidium are situated in the appendix, whereas the meronts can be localized also in the ileum. Cheissin does not give the number of asexual generations. In relation with the pathogenic effect of this coccidium Cheissin (1947) observed enlargement of crypts of caecum and appendix and occurrence of macroscopically visible light spots in the same parts of the intestine.

In our earlier experiments (Pakandl 1988), we attempted to describe the life cycle of E. coecicola. The first meronts were found 4 DPI in the epithelial cells covering the domes in the appendix. The domes are the sites where the lymphatic follicles form approximately hemispherical structures covered with a single-layered epithelium (Pl. I, Fig 1). There arise two generations of meronts, other two generations of meronts appear in the ileum, and the gametogony takes place again in the appendix. The prepatent period lasted 9 days; the same period was reported also by Cheissin (1947, 1967, 1968).

Our previous experiments (Pakandl 1988), the result of which was the description of E. coecicola life cycle, were supplemented and the material from all experiments was used for the evaluation of the pathogenicity of this parasite.

MATERIALS AND METHODS

The methods used are almost identical with those described in our previous paper (Pakandl 1988). The experimental rabbits were 5-week-old hybrids of Large Chinohill and Californian white rabbit and originated from our own breeding.

The experiments were carried out in the following way:

Experiment 1. The doses of oocysts used for the study of the endogenous cycle of E. coecicola were graded as follows: 10^6 for the rabbits killed 2 and 3 DPI, 3 x 10^6 for the rabbits killed 4 and 5 DPI, 2 x 10^6 for the rabbits killed 6 and 7 DPI, 10^6 for the rabbit killed 8 DPI, and 5 x 10^6 for the rabbit killed 10 DPI. The rabbit used as control was killed on day 7 after the beginning of the experiment. The experiment was once repeated using the same arrangement. Altogether 18 rabbits were examined.

Experiment 2. All rabbits were infected with the dose of 25 x 10^6 E. coecicola oocysts. Always 3 rabbits
were killed 4 and 7 DPI. Of the rabbits which should have been killed 10 and 14 DPI, only one of the three animals was killed, so that only 2 rabbits could be used for the histological examinations. Three rabbits were left as controls and were killed on day 7 after the beginning of the experiment.

Experiment 3. Four rabbits were inoculated with the dose of 10^8 oocytes. Two of them were killed on day 11 DPI and the other two on day 17 DPI.

All of the animals were killed by ether. In Experiment 1, samples of small intestine were taken first immediately behind the stomach and then at every 10 cm along its whole length (altogether 13–19 samples). The last sample was taken at 2–3 cm from the ileocecal valve. Four samples were taken from the cecum (1 from the region of ampulla coli, then from the beginning and middle part of corpus cecii and from the viciniy of its transition to appendix). Three samples were taken from the appendix (middle, middle part, and terminal beginning of appendix), and the terminal beginning of the colonic of colon and then at every 10 cm (altogether 5–8 samples). In Experiments 2 and 3, the sample of small intestine was taken immediately behind the stomach, then 30–40 cm from it, in the middle of abdomen, at the distance of 10–15 cm, at the distance of 10–15 cm, and at the distance of 2–3 cm from ileocecal valve. Three samples were taken from the cecum: 1 from the region of ampulla coli, 1 from the middle part of corpus cecii, and 1 from the viciniy of its transition to appendix. The samples from the appendix were taken in the same way as in Experiment 1. One sample was taken from the cranial part of the colon and other 2–3 samples at the distance of approximately 15 cm.

The material for histological examinations was fixed in 10% neutral formaldehyde and further processed by a common paraffin technique. Sections of 4–7 µm were stained with Harris hematoxylin and eosin.

**RESULTS**

Since the results of Experiments 1 and 2 were identical from the viewpoint of pathological changes, they are further evaluated together. Significant pathological changes were observed in the appendix. On the contrary, in the ileum, where *E. coccidocola* stages are also localized, the changes were only slight. About 7 DPI, the villus architecture does not exceed the morphological norm and there is no oedema in lamina propria. About 10 DPI, there is an inflammatory exudate with a marked proportion of neutrophils and eosinophils. Pathological changes observed in the appendix are described in the following survey.

4 DPI — *E. coccidocola* meronts appear selectively in the epithelium of domes above lymphatic follicles which are enlarged and their germinal centres are activated.

5 DPI — In addition of first-generation meronts there appear also meronts of the second generation, which, in addition to the dome epithelium, infest also the epithelium in deeper parts of the mucosa around the domes (Pl. 1, Fig. 2). The mucosa of the appendix is swollen, with oedematous infiltrations, and often completely covers the domes above the markedly activated follicles in the submucosa.

6 DPI — the mucosa of appendix is swollen and infiltrated with hyperplastic glandular epithelium in its upper parts. There are numerous meronts in mucosa epithelium. The domes above the activated lymphatic tissue are often atrophied. The oedematously infiltrated lamina propria contains an inflammatory infiltration with a marked pyogenic component, in which eosinophils are often present.

7 DPI — the mucosa of appendix is thickened and oedematously infiltrated. An inflammatory infiltration, with prevailing neutrophils and eosinophils is visible in lamina propria. The other two appear young gamonts, which are localized mainly in crypts, sometimes also in the superficial epithelium and in the epithelium covering the domes.

8 DPI — the appendix contains only the gamonts, the meronts are almost absent at this time. The mucosa of appendix is thickened and oedematously infiltrated and there is an oedema and inflammatory infiltration with prevailing supplicative component. In lamina propria. Some of the enlarged crypts contains thickened crypt infiltration. Single microabscesses are present in lamina propria. The swollen mucosa closes the spaces above the domes containing, in addition to desquamated epithelium, a stagnating inflammatory infiltration.

10 DPI — the mucosa of appendix is slightly thickened and infiltrated by abundant hyperplastic glandular epithelium. The infected glandular epithelium is significantly metaplastically transformed or it is sometimes absent. In some parts, the basal lamina is disintegrated and immature, often destructed oocytes are embedded in lamina propria, which is infiltrated by abundant inflammatory infiltration with prevailing pyogenic component. There are numerous epithelial erosions on the surface of mucosa and numerous gamonts in the remnants of epithelium (Pl. 1, Fig. 1). The immature oocytes in lamina propria are usually accumulated in clusters and there is a pyogenic inflammatory exudation with numerous eosinophils in their vicinity (Pl. 2, Fig. 2).

Some domes with epithelium infiltrated with developmental stages of coccidia are endemically infiltrated with cavities formed by adhesions of upper parts of the swollen mucosa. The cavities above the domes are filled with a stagnating contents consisting of desquamated epithelium, endogeic stages of coccidia, and purulent discharge. The epithelium and lymphatic tissue of the domes are often atrophied and necrotic.

The lymphatic follicles in the submucosa are swollen and lymphopenia occurs in their central parts (Pl. III, Fig. 1).

14 DPI — the inflammatory structures in the appendix lamina propria get a granulomatous character. In the vicinity of ooyest clusters in lamina propria, there are inflammatory structures, consisting mainly of lymphocytes, neutrophil leucocytes, macrophages, and histiocytes with light plasma (Pl. III, Fig. 2). Some of the oocysts lie in lamina propria in the cytoplasm of giant, multinucleate cells with nuclei arranged in circles at the periphery of cytoplasm (Pl. IV, Figs. 1, 2). The formation of cells of this type is known as a part of the host tissue reaction to a foreign body. The domes are often atrophied and promote into the cavities with the stagnating parasitic and cellular contents. Lymphatic follicles in the submucosa are slightly swollen and well separated from one another.

20 DPIs were found in the feces of rabbits killed 21 and 24 DPI (Experiment 3). Developmental stages of *E. coccidocola* did not occur in the epithelium of appendix. In lamina propria, in the upper and middle parts of appendix mucosa, there were extensive and coalescing granulomatous inflammatory structures with a marked proportion of giant, multinucleate cells, the cytoplasm of which could be recognized in their fragments.

24 DPI — granulomatous inflammatory structures with prevailing macrophages and histiocytes are present in lamina propria of the appendix mucosa; giant multinucleate cells are localized in the vicinity of destructed clusters of oocytes.

**DISCUSSION**

The developmental stages of *E. coccidocola* appear, in addition to the appendix, also in the epithelium of villi and ileum crypts (Pak & d1 1988). The presence of the endogenic stages of coccidia in the epithelium of this small intestine region does not induce the atrophy of villi, not even a mere marked epithelial changes, only a repletion of the inflammatory infiltration in the lamina propria. These slight, almost nonspecific pathological changes in the ileum contrast with the considerable changes caused by the same coccidium in the appendix.

During the life-cycle of *Eimeria coccidocola*, the mucosa becomes swollen and an extensive hyperplasia of the epithelium infected by endogeic stages of coccidia occurs about 8 DPI. The hyperplastic epithelium then proliferates into lamina propria, where it becomes necrotic and the endogeic stages of coccidia, mostly immature oocysts, remain in lamina propria. Already about 10 DPI, neutrophils accumulate...
in lamina propria around the groups of immature oocytes and microabscesses develop there.

Granulomatous inflammatory structures appearing 14 DPI are a reaction to foreign bodies, which are immature oocytes or their fragments enclosed in lamina propria after disintegration of hyperplastic gland structures. Numerous giant, multinucleate cells from foreign bodies occur particularly around the oocytes which often lie in their cytoplasm. The intensive granulomatous inflammatory reaction in lamina propria of appendix remains up to 24 DPI, i.e. at least for 10 days; further course of this infection was not observed. Embedding of E. coeciloaca oocytes into lamina propria of appendix and the following giant-cell granulomatous inflammatory response result in a considerable prolongation of the pathogenic effect of this coccidium.

Cheissin (1947) reported the endogenous cycle of E. coeciloaca to terminate about 13 DPI. This is in agreement with our coprophilic examinations, during which no oocytes were found in rabbit faeces in the period of 14 DPI — 24 DPI. In spite of this, E. coeciloaca oocytes persist even for 13 DPI in lamina propria of appendix where they induce a giant-cell granulomatous inflammatory reaction as a reaction to the presence of foreign bodies. The oocytes embedded in lamina propria are not released into intestinal lumen and seem to be resolved during the inflammatory process in lamina propria. According to our results, the resorption of oocytes and their fragments last at least 10 days and the pathogenic effect of E. coeciloaca is thus considerably prolonged, going beyond the limit of its life-cycle.

Gregory and Catchpole (1986) recorded a granulomatous inflammation following E. flavescens infection in caecum 20 DPI. Granulomatous structures occurred simultaneously also in lymphatic follicles of the appendix.

The alteration of epithelium and uncovering of lamina propria occur in relation with the gametogony, 10 DPI. This period appears to be most important from the viewpoint of a possible secondary infection. Coudert (1978) considers E. coeciloaca, as well as E. perforans, to be little pathogenic for rabbit. However, Cheissin (1947) observed pathological changes in the appendix. Our experiments evidence that Eimeria coeciloaca is pathogenic for rabbit inducing in it a prolonged affection of the appendix.

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НАТОГЕННОСТЬ КОЦИДИИ EIMERIA COECICOLA CHEISSIN, 1947 У КРОЛИКОВ

И. ВИТОВЕЦ И М. ПАКАНДА

Резюме. Была изучена натогенность коцидии Eimeria coeciloaca для кроликов, у которых в наших экспериментах выясняли их высокоочное заболевание анапсидоз, продолжающееся минимум 20 дней. Патологические изменения появились в развитии мерогонии на 4-й день после заражения. Они характеризовались воспалительной инфильтрацией и обильным интенсивным компонентом в lamina propria, инфильтрацией и развитием вторичных структур, расположенных на поверхности воспалительных экссудатов, внутренних структур коцидий и окружающих анапсидоз. Изменения эпителия и открывшиеся lamina propria анапсидоза происходит в связи с гистогенезом в периоде около 10 дней после заражения. От 16 до 20 дней после заражения происходит превращение анапсидоза в иные стадии, которые пролиферируют в lamina propria и инфицируются кроликом. Группы нервных волокон и их фрагменты остаются в lamina propria в те же периоды минимально 10 дней резорбируются через пороги воспалительных структур с многочисленными клетками типичных чумных клеток.
Fig. 1. Dome in the appendix mucosa of control rabbit. Histological section stained by Harris’ haematoxylin and eosin (HE, 300×). Fig. 2. 5 DPI. Meronts in the dome epithelium (HE, 750×).

Fig. 1. 10 DPI. Numerous gamonts and oocysts in the epithelium and lamina propria of appendix mucosa (HE, 160×). Fig. 2. 10 DPI. Oocysts and gamonts in pyogenic infiltrate in lamina propria (HE, 1,000×).
Fig. 1. 10 DPI. Lymphoid exhaustion in lymphatic follicles in appendix submucosa. (HE, 100 x).
Fig. 2. 14 DPI. Granulomatous inflammatory structures with numerous multinucleate giant cells and histiocytes with light plasma in appendix mucosa. (HE, 180 x).

Fig. 1. Oocysts and their fragments in the cytoplasm of giant multinucleate cells in appendix lamina propria. (HE, 640 x).
Fig. 2. Granulomatous inflammatory structures with oocysts and their fragments in appendix lamina propria. (HE, 1,600 x).