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INFLUENCE OF PROSTAGLANDIN E_2 (PGE_2) ON T CELLS IN MICE INFECTED WITH *TRICHINELLA SPIRALIS* *

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In studies on the mechanism of "self-cure" in the course of intestinal helminthoses, many authors ascribe an important role to DH [1], i.e. T lymphocytes. (Larsh, 1969, 1975; Kelly, Ogilvie, 1972; Kelly, Dineen, 1973a, b; Ruitenberg, 1974; Vakelin, Lloyd, 1976).

Recently, Kelly et al. (1974) obtained violent expulsion of *Nippostrongylus brasiliensis* from the intestines of rats treated with prostaglandin E_2. Next they found that the levels of this hormone in the intestinal walls increased very markedly during the period proceding expulsion (Dineen, Kelly, 1976). Since in our previous study (Karmańska, Michalska, in printing) we did not observe any mobilization of T cells proceding expulsion of *T. spiralis* from the intestines of mice, we decided to study the behavior of T lymphocytes in animals treated with prostaglandin E_2 **.

Material and methods

Experiments were conducted with 160 female mice of the CFW strain weighing 20 ± 0.8 gm, aged about 3 months, maintained under constant experimental conditions.

T cells were indentified in organ sections by the histochemical method of Mueller et al. (1975).

Other procedures such as manner of infecting the animals and

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[1] delayed hypersensitivity.

counting adult trichinellae and muscular larvae were described in a previous paper (Karmańska et al., 1977).

Results

The animals were divided into two groups: one group (controls) were infected with *T. spiralis* in doses of 200 larvae/mouse; and the second group was injected between days 1 and 30 p.i.[2] intraperitoneally with prostaglandin E_2 (U.12062, 11465-HAK-52IIB, Upjohn Co., Kalamazoo) every 12 hours in doses of 150 μg/kg/day. PGE_2 was dissolved acc. with instruction of Uphjon Co. Mice of both groups were killed 5, 10, 15, 20, 25, 30, 42 and 60 days p.i., and preparations from slices of the jejunum, masseter muscle, mesenteric and axillary lymph nodes and spleen were made for study of T cells.

In all animals that were killed, intestinal parasites were counted, and at 60 days p.i. also muscular larvae.

Jejunum. In control mice (group 1), during the early period after infection, i.e. between days 5 and 15 p.i., the cellular infiltrates in the intestinal villi contained almost no T lymphocytes. The infiltrates were composed of B lymphocytes and a small number of histiocytes and polymorphonuclear leukocytes. In the deeper lavers of the mucous membrane at the base of the villi there were large aggregates of histiocytes. Beginning on day 20 p.i., about 30% of the villi contained 1-3 T cells. In the stroma at the base of the mucous membrane, T lymphocytes constituted about 5% of the lymphocytes and polymorphonuclear leucocytes. On subsequent days p.i. the numbers of T lymphocytes continued to increase. On day 25 p.i. about 60% of the villi contained from 1 to 6 T cells, and at the base of the mucous membrane about 10% of all cells. Between days 30 and 60 p.i., T cells were present in almost all villi, from 2-6 on day 30, and later as many as 10 or more (on days 42 and 60), while their mobilization continued in the deep layer of the intestinal mucosa.

In the experimental mice which received PGE_2 (group 2) between days 5 and 25 p.i. the numbers of T cells were much lower among the cellular infiltrates in the mucous membrane of the jejunum. At this time, the infiltrates were composed mainly of B lymphocytes, histiocytes and polymorphonuclear leucocytes. Large aggregates of histiocytes and macrophages in the deep layers of the mucous membrane at the base of villi among the intestinal glands were observed.

On days 30 to 42 p.i. in the lamina propria of the mucous membrane about 30% of the intestinal villi contained 1-3 T lymphocytes.

[2] post infection.

Subsequently, on day 60 p.i., T lymphocytes were even more numerous since about 50% of the villi contained 1-5 T cells on average, or sometimes more. From day 30 p.i. to the end of the experiment, loosely scattered T lymphocytes were present in the deep layers of the mucous membrane, constituting about 10% of other cells (day 30), and increasing somewhat on days 42 and 60 p.i.

Masseter muscle. In the intermuscular infiltrates in control animals (group 1) on days 10 and 15 p.i. T lymphocytes were encountered sporadically. The infiltrates were composed of histiocytes, B lymphocytes and polymorphonuclear leukocytes. The latter became more numerous from the 15th day p.i. Subsequently, between days 20 and 25 p.i., lymphocytes became more numerous in the infiltrates. However, their highest level was observed on days 30, 42 and 60 p.i. Usually they were loosely scattered in the infiltrates, or formed small aggregates in the neighborhood of muscular larvae. Beginning on day 20 p.i. they could be found within the larval capsules, which they penetrated in small numbers. On days 42 and 60 p.i. T lymphocytes within the capsules of larvae were more numerous. In inflammatory infiltrates, composed mainly of eosinophilic leukocytes, few T cells were usually situated at the periphery of granulomas.

In mice treated with PGE_2, sporadic T lymphocytes in the masseter muscle were encountered in the intermuscular infiltrates on days 15 and 20 p.i. At this time, the infiltrates were formed predominantly by histiocytes and by smaller numbers of B lymphocytes and polymorphonuclear leukocytes. Somewhat more numerous T cells were seen after the 25th day p.i. In the following period their numbers increased, and on days 42 and 60 p.i. they were already numerous. In some parts of the intermuscular infiltrates T lymphocytes accounted for on half or more of the lymphocytes, as well as at sites of decided predominance of B lymphocytes and polymorphonuclear leukocytes (the latter in granulomas). Toward the end of the experiment (days 42 and 60, and occasionally day 30 p.i.) single or small groups of T lymphocytes could be observed within the larval capsules.

Lymphoid organs. In the mesenteric lymph node from the control mice (group 1) between days 20 and 25 p.i. T lymphocytes were rather scarce. The largest number was in the subcortical layer of the node, intermingled with B lymhpocytes. In the central parts of germinal centers and in the medullary cords in the deeper parts of the lymph nodes T cells were encountered sporadically. A slight increase in numbers of T cells began on days 25-30 p.i., and larger numbers appeared after 42 days to 60 days, forming large groups especially in the sub-

cortical layer and less often in the periphery of the follicles, and occasionally in their center. The smallest increase in T cells was in the cords of the medullary part. Toward the end of the period of observations (days 42-60 p.i.) an increase in histiocytes and large macrophages was noted in the lymph nodes.

In animals treated with PGE_2 (group 2), T lymphocytes were not very numerous in the mesenteric lymph node during the first half of the period, i.e. between days 5 and 30 p.i. The largest number was in the subcortical layer in small groups. In the cortex they were rare in the peripheral parts of the follicles, and none were seen in its center. Only a few cells were seen in the medullary cords. Similarly to the control mice, the number of T cells increased after 42 and 60 days p.i. mostly in large subcortical groups. Scattered cells in the periphery of the germinal centers and within the follicles were also present besides numerous histiocytes. In the medullary cords, T cells were still scarce.

In the axillary lymph nodes from the control animals (group 1) at all intervals of observation, T cells behaved similarly to the cells in the mesenteric lymph node, except that mobilization after 42 and 60 days was weaker.

In mice treated with PGE_2 (group 2), T cells behaved similarly in the axillary and mesenteric lymph nodes. At thirty days p.i. their numbers were small, mainly in the subcortical layer in small groups, and began to increase after 42 days p.i. However, mobilization of these cells was weaker than in the meseneteric lymph node.

In the spleens of control mice (group 1) at 5 to 20 days p.i. the numbers of lymphocytes were small.

In the rich white pulp, in which B lymphocytes, histiocytes and macrophages predominated, T cells were present mainly in the peripheral parts of the Malpighian follicles, and exceptionally in their interior. Red pulp also contained them. Beginning 25 days p.i., the number of T lymphocytes slowly increased, especially in the peripheral parts of the follicles and in their center, besides in the red pulp. At this time the white pulp of the spleen contained numerous histiocytes and macrophages.

In the spleens of experimental mice treated with PGE_2 (group 2), at 30 days p.i. T lymphocytes were few in number, situated in the neighborhood and peripheral parts of the lymphatic follicles of the spleen and in the red pulp. Similarly to the mesenteric and axillary lymph nodes, a distinct increase in T cells was observed on days 42 and 60 p.i. Mobilization was most pronounced in the white pulp of the spleen, not only in the neighborhood and peripheral parts of the Malpighian fol-

licles, but also in their interior. In the red pulp, the number of these cells was smaller.

Parasitologic studies. In control mice killed 5 days p.i. the mean number of adult parasites was 99; on day 10 p.i. there were 55, and on day 15 p.i. 15.3. No parasites in the intestines were found after 20 days p.i.

In mice treated with PGE_2 the mean number of parasites on the 5th day p.i. was 109; on day 10 up to 78.6, on day 15 p.i. 42. 3, and on day 20 up to 22.5. On day 25 p.i. the mean number of intestinal parasites was 9, on day 30 — 4.75 and on day 42 — 1.5. No parasites were found after 60 days.

The data are illustrated in a figure.

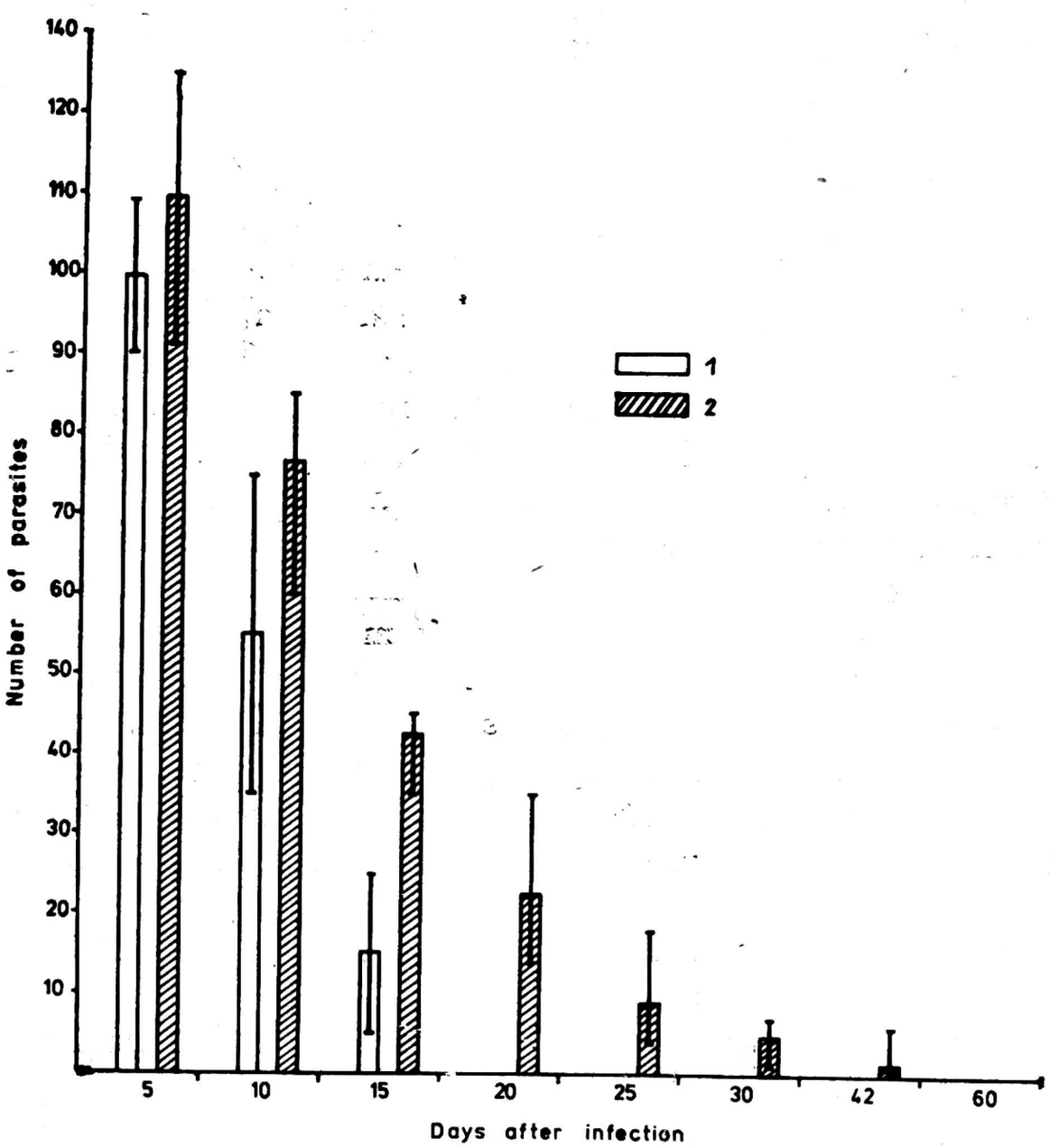

Fig. Mean number of *T. spiralis* in the intestines
1 — control group, *2* — experimental group

The mean number of muscular larvae on day 60 p.i. in controls was 39.150 (S.D. = 7,415.1), and in mice treated with PGE_2 29.428 (S.D. = = 7,101.0).

Discussion

The mechanism of "self-cure" in intestinal helminthoses has been studied intensively by many authors. Some, including Larsh (1969, 1975), Kelly, Ogilvie (1972), Kelly, Dineen (1972), Dineen et al. (1973a, b), Ruitenberg (1974) and Vakelin, Lloyd (1976) believe that the main cause of expulsion of intestinal parasites is cell-mediated immunity (i.e. DH).

Kelly et al. (1974) found that PGE_2 injected intraperitoneally in rats infected with *Nippostrongylus brasiliensis* accelerates expulsion of intestinal parasites. Dineen, Kelly (1976) noted that in the period preceding expulsion, levels of this hormone in the intestinal walls increase. We therefore decided to study the behavior of T lymphocytes in mice infected with *T. spiralis* and treated with PGE_2.

In contrast to Kelly et al. (1974) we injected PGE_2 intraperitoneally in order to avoid a direct effect on the intestinal mucosa.

We identified T cells in sections of various organs by the histochemical method of Mueller et al. (1975) based on modification of the reaction for nonspecific esterase, in which the cytoplasm of T cells contains spots of reddishbrown reaction product.

We obtained results different from those of Kelly et al. (1974). In mice treated with PGE_2, expulsion of adult parasites was delayed. Stimulation of T lymphocytes was also delayed in all the studied organs. Despite delay of both phenomena, we do not think that the two phenomena are related. Our earlier studies (Karmańska, Michalska, in printing) and the results in the group of control mice show mobilization of T cells after expulsion of the parasites from the intestines. We assumed that the weaker stimulation of T lymphocytes was due to the fact that PGE_2 acting on adenyl cyclase causes a rise in the intracellular levels of cAMP, equivalent to lowered activity of the cells (Quagliata et al., 1973). Raised levels of intracellular cAMP inhibit degranulation of mast cells, as has long been known (Lichtenstein, Bernardo, 1971; Perper et al., 1972; Kimura et al., 1974; Sullivan, 1975).

In a previous study (Karmańska, Michalska, 1977), we found that PGE_2 lowers the levels of degranulating cells in the intestinal mucosa very distinctly. As is known, rapid degranulation of mast cells usually accompanies the beginning of the process of expulsion. We concluded that delayed expulsion of intestinal parasites in animals treated with

PGE_2 is connected with the smaller numbers of mast cells undergoing degranulation, respectively that both phenomena are regulated by third, hitherto unknown factor.

The reported studies indicate that PGE_2 administered parentally acts only by raising levels of intracellular cAMP, because we observed no effect of a direct action on the parasites, or on the environment, as described by Dineen, Kelly (1976). The raised levels of PGE and PGF in the intestinal walls of rats infected with *N. brasiliensis* described by these authors could have been a result of an inflammatory process since it is known that levels of mediators such as histamine, serotonin, kinins and prostaglandins are elevated in this process.

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WPŁYW PROSTAGLANDYNY E_2 (PGE_2) NA LIMFOCYTY T U MYSZY ZARAŻONYCH *TRICHINELLA SPIRALIS*

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Autorki podawały prostaglandynę E_2 (PGE_2) myszom szczepu CFW (samicom o wadze około 20 g, w wieku 3 miesięcy) zarażonym 200 larwami *T. spiralis*. Preparat stosowano dootrzewnowo w okresie między 1-30 dniem p.z., co 12 godz., w dawce 150 µg/kg/dobę. Myszy sekcjonowano 5, 10, 15, 20, 25, 30, 42 i 60 dnia po zarażeniu (p.z.), po czym z wycinków jelita czczego, mięśnia żuchwowego, węzłów limfatycznych: krezkowego i pachowego, oraz ze śledziony sporządzano preparaty według histochemicznej metody Muellera i wsp. Równocześnie liczono pasożyty w jelitach, w 60 dniu inwazji także larwy w mięśniach.

W wyniku przeprowadzonych badań stwierdzono, że jakkolwiek początek mobilizacji limfocytów T u zwierząt, które otrzymywały PGE_2 był opóźniony, a szczyt pobudzenia wyrażony słabiej niż w kontroli, to jednak faktów tych nie można łączyć z przedłużonym przeżywaniem włośni w jelitach, ponieważ mobilizacja limfocytów T w grupie zwierząt kontrolnych wystąpiła także już po usunięciu pasożytów.