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γ-Glutamyl-transpeptidase in lymphatic tissues of *Mastomys natalensis* during an infection with *Acanthocheilonema viteae* *

S. N. Singh[a], A. K. Srivastava, S. C. Gupta, R. K. Chatterjee[a] and K. C. Saxena

Divisions of Biochemistry and [a]Parasitology, Central Drug Research Institute, Lucknow 226001 (India)
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Summary. During *Acanthocheilonema viteae* infection, the specific activity of γ-glutamyltranspeptidase (γ-GT) increased in peritoneal exudate cells and bone marrow and decreased in lymphnodes of *Mastomys natalensis* throughout the course of infection. However, though there was an increase in specific activity of γ-GT in thymus and spleen during the prepatent phase of *A. viteae* infection, the level either returned to normal or decreased during the latent phase of infection. A close correlation was observed between the host's immune status during *A. viteae* infection and the level of γ-GT in lymphoid tissues.
Key words. Acanthocheilonema viteae; Mastomys natalensis; γ-glutamyl-transpeptidase; lymphoid tissues; filarial infection; immunosuppression.

The enzyme γ-glutamyl transpeptidase (EC 2.3.2.2.; γ-GT), which catalyses the initial step in the breakdown of glutathione, i.e. the transfer of the γ-glutamyl moiety of glutathione to a variety of amino acids and other peptides, has attracted attention because of its presence in lymphoid cells[1,2]. The activity of γ-GT was found to be 2–3 times higher in B-lymphocytes than in T-lymphocytes, and an enhancement of the activity of γ-GT was also reported after mitogenic stimulation of lymphocytes[1]. Marked variations in the γ-GT activity in lymphoid cells derived from patients with various neoplastic and lymphoproliferative diseases have also been reported[2]. So far, there has been no report on γ-GT activity in the host's lymphoid tissues during parasitic infections. *Acanthocheilonema viteae* infection in *Mastomys natalensis* is a chronic filarial infection, and resembles human onchocerciasis. We examined the specific activity profile of γ-GT in certain lymphoid and non-lymphoid tissues of *M. natalensis* at various stages of *A. viteae* infection. The results obtained are summarised in the present communication.

Materials and methods

Mastomys natalensis 'GRA', Giessen strain, were infected with *Acanthocheilonema viteae* through the infective bites of the tick vector *Ornithodoros moubata*. Details of maintenance and monitoring of the infection were as described elsewhere[3]. At all stages of infection non-infected animals of matched age were used as controls.

For tissue collection, the animals were killed by cervical dislocation. Lymph nodes (popliteal and mesenteric), spleen, thymus and liver of groups of animals which had prepatent, patent and latent *A. viteae* infections, as well as control animals, were excised, washed in cold 150 mM KCl and homogenized in the same medium (10 %, w/v). Exudate cells from the peritoneal cavity, and bone marrow from femur and tibia bones, were collected in phosphate buffered saline (pH 7.2). Peritoneal exudate cells (PEC) and bone marrow cells (BM) were recovered by centrifugation at low speed (500 × g 10 min) and suspended in 2.0 ml of 150 mM KCl. The homogenates of thymus, spleen, lymph nodes and liver, as well as PEC and BM suspensions, were sonicated at 1.5 Å output in a Cell Disruptor Model W-220 F (Heat Systems – Ultrasonics, USA) for 1 min and centrifuged at 10,000 × g for 30 min, and the supernatants were assayed for γ-GT activity.

The specific activity of γ-glutamyl transpeptidase was determined by previously described methods using the artificial substrate γ-L-glutamyl-p-nitroanilide[4]. 1 ml of assay mixture contained 100 mM Tris (pH 8.0), 20 mM glycylglycine, 5 mM γ-glutamyl-p-nitroanilide and a suitable amount of the enzyme preparation. The reaction vessels were incubated at 37 °C for 30 min. After inhibition of the reaction with 1.5 N acetic acid, the formation of p-nitroaniline was monitored at 405 nm and the enzyme activity expressed as μg p-nitro-aniline liberated $min^{-1}mg^{-1}$ protein.

γ-L-glutamyl-p-nitroanilide and glycylglycine were purchased from Sigma Chemical Company, USA. Protein in preparations was quantitated according to the Lowry method using bovine serum albumin as standard. Student's t-test was used for statistical analysis of the results.

Results

Table 1 shows the number of microfilariae present in tail blood, and the number of adult *A. viteae* recovered from the body of the infected animals, at various phases of infection. Table 2 depicts the specific activity profile of γ-GT in spleen, thymus, lymph nodes, PEC, BM and liver of *M. natalensis* during the prepatent, patent and latent phases of *A. viteae* infection.

In BM and PEC, the specific activity of γ-GT was found to be high throughout the infection, the maximum being in the latent phase (around 110 and 325 % in BM and PEC, respectively), when there were no detectable microfilariae in the peripheral circulation. At this stage, the worms recovered had degenerated and disintegrated. In lymph nodes, the specific activity of γ-GT was found to be low at all the stages of infection. Here, the maximum decrease was at the patent phase (around 60 %) when there was a heavy infection. In the thymus, the specific activity of γ-GT showed a marked increase (around 30 %) during the prepatent phase of the infection when the worms were in the developing stages, and this pattern was continued until the start of the patent phase. However, in the latent stage of the infection, the specific activity of γ-GT was slightly lower than, or of the same order as that in the uninfected animals of the same age group. As in thymus, BM and PEC, the specific activity of γ-GT in spleen also exhibited an increasing trend at the prepatent phase (around 30 %) of infection but was more or less normal during the patent and latent stages of the infection. In spite of a large change in the specific activity of γ-GT of the lymphatic tissues of *M. natalensis* during the course of *A. viteae* infection, the specific activity of γ-GT in liver remained unchanged.

Table 1. Recovery rate of microfilariae and adult worms from *Mastomys natalensis* infected with 3rd stage larvae of *Acanthocheilonema viteae* (mean with range)

Stage of infection	Days post infection	Microfilariae/ 20 mm^3 tail blood [+]	Adult worms recovered [+]	Status of the worms
Prepatent	60	0	12 (7–19)	Live
Patent	130	73 (30–102)	19 (5–45)	Live
Latent	210	0	Degenerated and disintegrated worms	–

[+] There were six animals in each group.

Discussion

The present study demonstrates the presence of a substantial amount of γ-GT in different lymphoid tissues of *M. natalensis* and thus supports the view that γ-GT can be regarded as a lymphoid cell marker [1, 2]. The study also indicates an increasing amount of γ-GT activity in PEC and BM throughout the course of *A. viteae* infection. In spleen and thymus, however, there was an increase in γ-GT activity in the initial stages followed by a progressive decline in the later stages of the infection. In lymph nodes, on the other hand, a decrease was observed during the entire course of the infection.

PEC contains predominantly macrophages, which are known to be activated during parasitic infections. The macrophages play an important role not only in the removal of infection by non-specific and antigen-specific mechanisms, but also through induction of cell-mediated and humoral immune responses by producing monokines and processing of antigens. The prolonged enhancement of γ-GT in PEC could be due to the increase in synthesis of monokines and increased functional activity of peritoneal macrophages. Bone marrow is the primary site of origin of all the lymphoid cells. The continued increase in BM γ-GT may be ascribed to an increase in the production of progenitors.

Spleen and lymph nodes house lymphocytes and are the primary sites of development of the immune response, while the thymus possesses thymocytes, the precursors of T-lymphocytes. Increase in γ-GT in spleen and thymus in the initial stages of infection could possibly be due to increased replication of lymphocytes/thymocytes and enhanced synthesis of monokines/immunoglobulins in these tissues in response to the infection. Decrease in the γ-GT level in spleen in patent and latent stages and in lymph nodes during the entire course of infection could

Table 2. Activity of γ-glutamyl transpeptidase* in tissues/cells of control and *Acanthocheilonema viteae* infected *Mastomys natalensis* at various stages (mean ± SD)

Tissues/ cells	Prepatent		Patent		Latent	
	Control	Infected	Control	Infected	Control	Infected
Spleen	1.74 ± 0.11	2.22 ± 0.07 $^{++}$	2.67 ± 0.13	2.30 ± 0.18 ^{ns}	2.83 ± 0.12	2.63 ± 0.10 ^{ns}
Thymus	3.57 ± 0.34	4.55 ± 0.48 $^{+}$	1.81 ± 0.20	2.37 ± 0.26 $^{+}$	1.49 ± 0.11	1.21 ± 0.12 ^{ns}
Lymph nodes	3.45 ± 0.26	2.99 ± 0.40 $^{+}$	3.97 ± 0.04	1.55 ± 0.15 $^{++}$	4.36 ± 0.32	2.48 ± 0.16 $^{+++}$
PEC	6.02 ± 0.18	8.97 ± 0.39 $^{++++}$	6.96 ± 0.63	9.60 ± 1.06 $^{++++}$	7.18 ± 0.32	30.35 ± 1.55 $^{++++}$
Bone marrow	5.57 ± 0.89	8.44 ± 1.45 $^{+}$	6.12 ± 0.17	9.10 ± 0.61 $^{+++}$	4.79 ± 0.05	10.12 ± 0.38 $^{++++}$
Liver	0.67 ± 0.09	0.59 ± 0.06 ^{ns}	0.56 ± 0.08	0.47 ± 0.05 ^{ns}	0.67 ± 0.05	0.64 ± 0.07 ^{ns}

*μg p-nitroaniline liberated/min/mg protein; $^{+}p < 0.05$; $^{++}p < 0.025$; $^{+++}p < 0.005$; $^{++++}p < 0.001$; and ns = not significant on applying Student's t-test. There were six animals in each group.

be a reflection of the decreased synthesis of lymphokines and immunoglobulins as a result of immunosuppression, which is known to be prevalent in clinical and experimental filariasis especially in the chronic/patent stages [5-13]. The above postulate is supported by the observation of Novogrodsky et al. [1] of a direct correlation of γ-GT activity with synthesis and secretion of lymphokines/immunoglobulins from T and B-lymphocytes, respectively. Furthermore, the production of interleukin and interferon has been shown to be appreciably decreased in lymphocytes derived from microfilariaemic individuals [6]. It seems possible that the enzyme γ-GT in the host's immune system could very well be utilized as a marker of immunosuppression and/or immunostimulation. Further work on monitoring γ-GT in immunosuppressed and immunostimulated hosts is in progress, which should help to show whether this is indeed the case.

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Effects of a juvenile hormone mimetic, fenoxycarb, on post-embryonic development of the European corn borer, *Ostrinia nubilalis* Hbn.

C. Gadenne, S. Grenier*, G. Plantevin* and B. Mauchamp

*Laboratoire de Physiologie de l'Insecte, INRA, route de St-Cyr, F-78026 Versailles cedex (France), and *Laboratoire de Biologie Appliquée, Bât. 406, INRA-INSA, 20 avenue Albert Einstein, F-69621 Villeurbanne cedex (France)*
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Summary. The effect of a juvenile hormone mimetic, fenoxycarb, Ro 13-5223, was tested on the larval instars of the European corn borer, *Ostrinia nubilalis*, by dipping or topical application. When larvae were treated in instars 2, 3 or 4, the duration of the fifth instar was modified. More permanent and fewer supernumerary larvae were obtained when treatment occurred in the early instars. This non-neurotoxic compound exhibited a strong dose-dependent juvenile hormone type of activity when it was applied to last instar larvae. Fenoxycarb prevented the onset of pupation and produced supernumerary larvae and intermediates. Permanent larvae were obtained if fenoxycarb was applied on day 0 or day 1 of the last instar. The use of such a JH mimetic in the understanding of endocrine control of diapause is discussed.
Key words. European corn borer; *Ostrinia nubilalis*; juvenile hormone mimetic; fenoxycarb; development; insect growth regulator.

Both juvenile hormone and 20-hydroxyecdysone are the key hormones regulating insect molting and metamorphosis [1]. In final instar larvae, the juvenile hormone titer falls to very low levels, allowing ecdysone release to initiate a metamorphic program. Applications of exogenous juvenile hormone during larval development may prolong various instars or delay and even prevent the onset of metamorphosis [2, 3].

Fenoxycarb is a non-terpenoid, non-neurotoxic carbamate exhibiting strong juvenile hormone activity [4]. It is thus classified as an insect growth regulator. It is now commonly used in orchard pest control [5] and tested in the protection of stored products [6]. It has been shown to interact with juvenile hormone esterase activity [7], to act on embryogenesis [8], and on the activity of the corpora allata [9].

Many studies have dealt with fenoxycarb, but few of them have dealt with laboratory experiments on development and molting in Lepidoptera.

The purpose of this study was to determine the effects of fenoxycarb on the larval development of the European corn borer, *Ostrinia nubilalis*, a major pest of maize throughout the world.