Pergamon

0020-7519(94)00151-0

*International Journal for Parasitology*, Vol. 25, No. 5, pp. 641–645, 1995

0020–7519/95 $9.50 + 0.00

*RESEARCH NOTE*

# Differential Expression of Glutathione S-transferase (GST) by Adult *Heligmosomoides polygyrus* During Primary Infection in Fast and Slow Responding Hosts

PETER M. BROPHY,*† ANN BEN-SMITH,‡ ALAN BROWN,‡ JERZY M. BEHNKE* and DAVID I. PRITCHARD‡

**Department of Pharmaceutical Sciences, School of Applied Sciences, De Montfort University, Leicester, LE1 9BH, U.K.*
*†Department of Life Science, University of Nottingham, Nottingham NG2 7RD, U.K.*

*(Received 16 February 1994; accepted 18 July 1994)*

**Abstract—Brophy P. M., Ben-Smith A., Brown A., Behnke J. M. & Pritchard D. I. 1995. Differential expression of glutathione S-transferase (GST) by adult *Heligmosomoides polygyrus* during primary infection in fast and slow responding hosts. *International Journal for Parasitology* 25: 641–645. Glutathione S-transferase (GST) specific enzymatic activity, assayed with the model substrate 1-chloro-2,4-dinitrobenzene, was 45% higher in adult *Heligmosomoides polygyrus* passaged through a slow responder mouse strain, C57/BL10 compared to worms passaged through a fast-responder strain (SWR × SJL) $F_1$. Western analysis using polyclonal antisera raised to purified *H. polygyrus* GSTs did not appear to positively correlate the expression of GST protein with functional enzymatic activity. However, western blotting did indicate a sex-linked expression pattern of GST protein, with male worms expressing a higher ratio of the 24 kDa to the 23 kDa GST family than female worms.**

*Key words:* Glutathione S-transferase; nematode; lipid peroxidation; vaccination; *Heligmosomoides polygyrus.*

The expression of glutathione S-transferases (GSTs), E.C. 2.5.1.18, by parasitic helminths may be essential for establishing chronic infections (Brophy & Pritchard, 1992). GST, in the apparent absence of cytochrome P-450 expression (Precious & Barrett, 1989), is one of the major helminth detoxification systems. One proposed function for helminth GSTs is to provide a secondary defence against the toxic products arising from host-immune initiated free radical attack on lipid membranes (Brophy & Pritchard, 1992; Smith 1992).

The mouse–*Heligmosomoides polygyrus* system is an established model for investigating molecular communications between parasitic nematodes and the immune response (Behnke, Barnard & Wakelin, 1992). The enzymes catalase, superoxide dismutase and glutathione peroxidase have previously been implicated as primary immune defence enzymes in *H. polygyrus* due to their ability to neutralise host-derived reactive oxygen species, ROS (Smith & Bryant, 1986). If the primary defences are breached

†To whom all correspondence should be addressed.

ROS can attack lipid membranes causing lipid peroxidation and the release of cytotoxic peroxides and carbonyls (Brophy & Pritchard, 1992). However, in contrast to the primary defences, the secondary lipid peroxidation defences of *H. polygyrus* have not been characterised. Therefore, in this paper we have augmented the primary defence data published by Smith and Bryant (1986) and examined the expression of GST, a potential secondary ROS defence enzyme, in *H. polygyrus*. We have assessed the contribution of GSTs to nematode peroxidation defence using biochemical assay and by vaccination in mice strains termed fast, intermediate or slow responders. (Behnke *et al.*, 1992). The pattern of infection in C57BL/10 (slow-responders, supporting chronic infections) and (SWR × SJL)$F_1$ (fast-responders, supporting acute infections) has been established (Robinson, Wahid, Behnke & Gilbert, 1989; Wahid & Behnke, 1993a). A variation in accompanying immune responses indicate that the relative rates of worm expulsion in the different strains of mice could be an immunologically mediated process. For example, fast responders have a faster and more intense mastocytosis (Wahid, Behnke, Grencis, Else & Ben-Smith, 1994) and faster responders have a faster and more intense IgG1 response (Wahid & Behnke, 1993b). Furthermore, treatment of fast responder strains with cortisone prolongs infection (Wahid & Behnke, unpublished observations).

*H. polygyrus* was maintained by laboratory infection as described previously (Behnke, Hannah & Pritchard, 1983). The adult worms were removed from the mouse intestine and washed clear of host tissue at week 2 or week 6. We selected week 2 as this is the earliest time at which adult worm burden can be accurately quantified. Week 6 corresponds to a time when we would normally expect fast-responders to have eliminated the majority of their worms (Robinson *et al.*, 1989; Wahid & Behnke, 1993a,b). A summary of the number of worms recovered from C57BL/10 and (SWR × SJL)$F_1$ at week 2 and week 6 post-infection is outlined in Table 1. The C57BL/10 strain lost only 10.4% of the established worm burden whereas (SWR × SJL)$F_1$ lost 71.1% (Table 1). The data was not normally distributed and hence non-parametric statistics (unified analysis by ranks; Meddis, 1984) were used to examine specific predictions with respect to the pattern of our worm burdens. We can predict that there would be fewer worms in weeks 6 versus weeks 2 and this is borne out ($z = 1.84$, $P = 0.033$). We can also predict that C57/BL10 will have more worms than (SWR × SJL)$F_1$ because $F_1$ mice are expected to lose worms faster and again this is borne out ($z = 2.26$, $P = 0.012$).

Table 1—*H. polgyrus* burdens at weeks 2 and 6 post-primary infection in slow-responder (C57/BL10) and fast-responder (SWR × SJL)$F_1$ strains of mice

| Strain | Worms recovered ± S.E.M. (*n*) Week 2 | Week 6 |
|---|---|---|
| C57/BL10 | 142.5 ± 17.6 (6) | 127.7 ± 25.2 (6) |
| (SWR × SJL)$F_1$ | 126.0 ± 22.3 (5) | 36.4 ± 22.3 (8) |

Number of mice used per experiment is shown in parentheses.

Somatic worm extracts were prepared on ice in three volumes of 10 mM sodium phosphate buffer pH 7.4 containing 0.1% v/v Triton X-100 by a hand-driven glass homogeniser. The extracts were centrifuged at 12,000 *g* and the supernatant used for further analysis. 1-Chloro-2,4-dinitrobenzene (CDNB) was used as the standard second substrate for measuring GST activity (Habig, Pabst & Jakoby, 1974). Protein concentration was calculated by a dye-binding procedure using bovine serum albumin as the standard (Sedmak & Grossberg, 1977). The specific activity of GST was measured in somatic extracts of male and female *H. polygyrus* recovered from week 2 or week 6 post-infection following passage via C57/BL10, and (SWR × SJL)$F_1$ strains. Statistical analysis of the data on GST activity indicated that there was a strain effect and a time effect but no sex effect (Table 2). Thus, multiple analysis of

Table 2—Glutathione/1-chloro-2,4-dinitrobenzene transferase activity in somatic extracts of adult *H. polygyrus* following passage through slow-responder, C57/BL10, and fast-responder (SWR × SJL)$F_1$, hosts

| Strain used for passage | *H. polygyrus* GST activity | | | |
|---|---|---|---|---|
| | Week 2 post-infection | | Week 6 post-infection | |
| | Male | Female | Male | Female |
| Slow-responder (C57/BL10) | 145.1 ± 14.7 | 162.0 ± 13.5 | 180.7 ± 17.6 | 174.0 ± 19.9 |
| Fast-responder (SWR × SJL)$F_1$ | 111.1 ± 4.4 | 106.4 ± 15.6 | 133.9 ± 11.2 | 101.6 ± 6.9 |

GST activity was expressed as nmol $min^{-1}$ $mg^{-1}$ somatic extract protein. The results were shown as ± S.D. using at least 3 replicate assays.

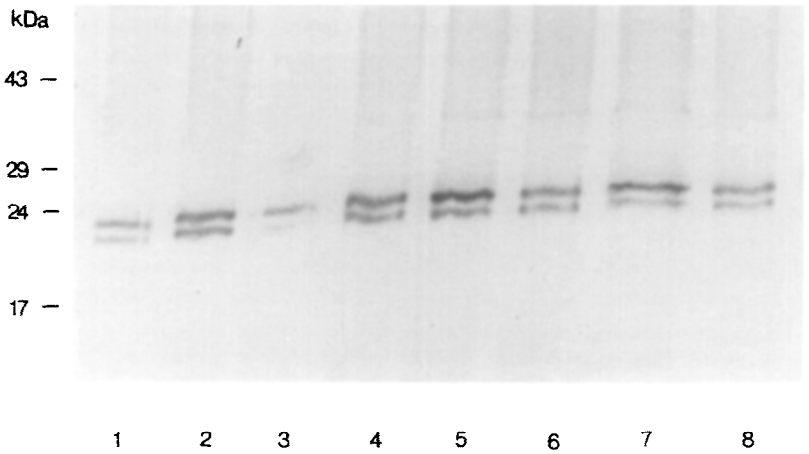


Fig. 1. Western-blot following 15% SDS-Page to show expression of *H. polygyrus* 23 and 24 kDa GsT subunits in somatic extracts of male and female worms at weeks 2 and 6. Worms were passaged in both slow (C57/BL10) and fast responder (SWR × SJL)$F_1$, strains. Ten microgrammes of extract protein was loaded per lane. Lane 1, extract of male, week 2 worms from slow-responder. Lane 2, female week 2 worms from slow-responder. Lane 3, male week 6 worms from slow-responder. Lane 4, female week 6 worms from slow-responder. Lane 5, male week 2 worms from fast-responder. Lane 6, female week 2 worms from fast-responder. Lane 7, male week 6 worms from fast-responder. Lane 8, female week 6 worms from fast-responder. Blots were developed by alkaline phosphatase linked secondary antibodies (Brophy *et al.* 1994; Harlo & Lane, 1988).

variance suggested that the strain effect was significant ($F_{1,16}$ = 49.8, $P$ < 0.0001). The worms in C57BL/10 mice had higher GST activity levels. Overall these levels changed with time (2 – 6 weeks), i.e. increased ($F_{1,16}$ = 8.8, $P$ < 0.01). There was no GST activity sex effect. However, there was a significant interaction between strain and sex indicating that the difference between sexes was dependent on sex to some extent (interaction $F_{1,16}$ = 8.0, $P$ = 0.01). The data indicated that in the (SWR × SJL)$F_1$ mice the male worms tended to have higher GST activity levels, whereas in C57BL/10 mice this was not the case. We found no other interactions.

Adult *H. polygyrus* has two major families of GST, the 24 and 23 kDa subunit families and at least 4 forms of dimeric GST can be purified from the nematodes by affinity chromatography (Brophy, Ben-Smith, Brown, Behnke & Pritchard, 1994). Polyclonal antisera, previously raised to the purified adult *H. polygryus* GST enzymes (Brophy *et al.*, 1994), was also employed to follow the protein expression pattern of *H. polygyrus* GST in fast- and slow-responders. Although protein levels were not formally quantified there did not appear to be a positive correlation between the amount of GST activity and GST protein present in the worms following passage through fast or slow responders (Fig. 1), In contrast to GST activity levels there was evidence of GST sex-linked protein expression patterns from *H. polygyrus* passaged through both fast- and slow-responders. Male worms had a higher ratio of the 24 kDa GST subunit family to the 23 kDa family compared with females (Fig. 1). Furthermore, male worms in slow-responders appeared to contain less GST protein than the respective females. The failure to positively correlate the GST protein expression pattern with GST activity levels in *H. polygyrus* may be the result of post-translational activation of one or more of the GST forms. We are currently examining the expression of individual GST forms in *H. polygyrus*.

We have demonstrated a significant reduction (overall, about 45%) in the expression of GST activity by *H. polygyrus* passaged through fast-responder compared to slow-responder mice. It may have been predicted that GST activity would have been up-regulated under the proposed increased immune stress in fast responder strains. However, there is a precedence for GST activity being reduced in parasites infecting resistant hosts. Thus, in common with the mouse–*H. polygyrus* model, GST activity was generally higher in *Fasciola hepatica* recovered from susceptible hosts (sheep and mice) compared to fluke recovered from resistant hosts (cattle and rats)

(Miller, Howell & Boray, 1993). These data suggest that resistant hosts could be suppressing helminth GST activity. Alternatively, the two sets of data may indicate that over-expression of GST by helminths is a prerequisite for the establishment of chronic infections and this capability is targeted by fast responder hosts leading to selection of parasites expressing relatively low levels of GST activity. In either scenario, GST is implicated as an important functional enzyme in parasite metabolism.

Therefore, the importance of the functional expression of GST to parasitic nematode physiology was further assessed by vaccination. The "Achilles heel" hypothesis of Smith (1992) has suggested that helminth GSTs are legitimate targets for the host protective immune response and the protective properties of digenean GSTs are well documented (Mitchell, 1989). There is no information on the protective properties of GSTs from adult gastrointestinal nematodes, although GSTs derived from the L3 stage of *Haemonchus contortus* failed to provide protection in a guinea pig host (Sharp, Smith, Bach, Wagland & Cobon, 1991). Slow-responder (C57/BL10) and intermediate-responder (NIH) strains were vaccinated with *H. polygyrus* GSTs prepared by glutathione-affinity chromatography purification (Brophy *et al.*, 1994). Fast-responder strains were not used for vaccination due to the difficulty in analysing worm data caused by the relatively high rate of natural expulsion. For the vaccinations 2 μg of purified GST were given per mouse in Freund's Complete Adjuvant, followed by three weekly booster injections of 2 μg in Incomplete Freund's Adjuvant. ELISA was routinely used to assess the mouse antibody response to *H. polygyrus* GST. Anti-IgGAM antibodies to GST could be detected in sera from the vaccinees and not in sera from saline only controls. However, no significant protection against *H. polygyrus* was obtained in the vaccinated NIH of C57/BL10 compared to non-vaccinated controls following the infection of mice with a 100 L3 larvae 10 days after the third boost (results not shown). There was also no significant variation in egg production or worm length of *H. polygyrus* passaged via vaccinated animals and non-vaccinated controls. Unfortunately, therefore, our, and other (Sharp *et al.*, 1991) vaccination trials suggest that nematode GST may not be a good vaccine candidate.

*Acknowledgements*—P. M. B. is a Wellcome Trust Research Fellow. A. B. S. is supported by a Medical Research Council project grant (G3923735/1) to J. M. B.

## REFERENCES

Behnke J. M., Barnard C. J. and Wakelin D. 1992. Understanding chronic nematode infections: evolutionary considerations, current hypotheses and the way forward. *International Journal for Parasitology* **22,** 861–907.

Behnke J. M., Hannah J. and Pritchard D. I. 1983. *Nematospiroides dubius* in the mouse: evidence that worms depress the expression of homologous immunity. *Parasite Immunology* **5,** 397–408.

Brophy P. M., Ben-Smith A., Brown A., Behnke J. M. and Pritchard D. I. 1994. Glutathione S-transferases from the gastro-intestinal nematode *Heligmosomoides polygyrus* and mammalian liver compared. *Comparative Biochemistry and Physiology,* **109B,** 585–592.

Brophy P. M. and Pritchard D. I. 1992. Immunity to helminths: ready to tip the biochemical balance? *Parasitology Today* **8,** 419–422.

Habig W. H., Pabst M. J. & Jakoby W. B. 1974. Glutathione S-transferase: first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* **249,** 7130–7139.

Harlo E. and Lane D. 1988. *Antibodies: a Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor.

Meddis R. 1984. In: *Statistics Using Ranks. A Unified Approach.* Blackwell Ltd, Oxford.

Miller C. M. D., Howell M. J. and Boray J. C. 1993. Host effects on glutathione S-transferase activity in *Fasciola hepatica. International Journal for Parasitology* **23,** 1073–1076.

Mitchell G. F. 1989. Glutathione S-transferases–potential components of anti-schistosome vaccines. *Parasitology Today* **5,** 34–37.

Precious W. Y. and Barrett J. 1989. The possible absence of cytochrome P-450 in helminths. *Biochemica et Biophysica Acta* **992,** 215–222.

Robinson M., Wahid F. N., Behnke J. M. and Gilbert F. S. 1989. Immunological relationships during primary infection with *Heligmosomoides polygyrus:* dose dependent expulsion of adult worms. *Parasitology* **98,** 115.

Sedmak J. J. and Grossberg S. E. 1977. A rapid sensitive and versatile assay for protein using Coomassie Blue G-250. *Analytical Biochemistry* **79,** 544–552.

Sharp P. J., Smith D. R. J., Bach W., Wagland B. M. and Cobon G. S. 1991. Purified glutathione S-transferases from parasites as candidate protective antigens. *International Journal for Parasitology* **21,** 839–846.

Smith N. C. and Bryant C. 1986. The role of host generated free radicals in helminth infections: *Nippostronglus brasiliensis* and *Nematospiroides dubius. International Journal for Parasitology* **16,** 617–622.

Smith N. C. 1992. Concepts and strategies for anti-parasite immunoprophylaxis and therapy. *International Journal for Parasitology* **22,** 1047–1082.

Wahid F. N. and Behnke J. M. 1993a. Immunological relationships during primary infection with *Heligmosomoides polygyrus:* regulation of fast response phenotypes by H-2 and non-H-2 genes. *Parasitology* **107,** 343–350.

Wahid F. N. and Behnke J. M. 1993b. Immological relationships during primary infection with *Heligmosomoides*

*polygyrus:* parasite specific IgG1 antibody responses and primary response phenotypes. *Parasite Immunology* **15,** 401–413.

Wahid F. N., Behnke J. M., Grencis R. K., Else K. J. and Ben-Smith A. (in press). Immunologial relationships during primary infection with *Heligmosomoides polgyrus:* Th2 cytokines and primary response phenotype. *Parasitology.*