The degradation rates of cytoplasmic tRNA, rRNA and mRNA in rats are elevated after infection with *Nippostrongylus brasiliensis*

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SUMMARY

The effects of a parasitic infection with the nematode *Nippostrongylus brasiliensis* on the degradation rates of cytoplasmic tRNA, rRNA and mRNA in rats have been investigated by measuring the renal excretion rates of the modified RNA catabolites N^6-threoninocarbonyladenosine, pseudouridine and 7-methylguanine. Between days 9 and 13 post-infection when the expulsion of *N. brasiliensis* is usually the most pronounced, the degradation rates of the different RNA classes were significantly higher than in the control rats ($P < 0{\cdot}05$) by, on average, +24% (tRNA), +34% (rRNA) and +26% (mRNA). We suspect that the elevated degradation rates of RNA are related to an increased production of reactive oxygen species by the host during the expulsion of *N. brasiliensis*.

Key words: *Nippostrongylus brasiliensis*, RNA degradation, modified RNA catabolites, non-invasive methods.

INTRODUCTION

We have developed a non-invasive method for determining the whole-body degradation rates of cytoplasmic t-, r- and mRNA in mammals (Sander *et al.* 1986; Schöch *et al.* 1990*a*, *b*; Topp *et al.* 1991; Topp, Duden & Schöch, 1993). This method is based on measuring the renal excretion rates of special RNA degradation markers in urine, such as the modified nucleosides N^6-threoninocarbonyl-adenosine (t^6A), pseudouridine (ψ) and the nucleobase 7-methylguanine (m^7Gua). These modified RNA components are post-transscriptionally formed in RNA amongst many other modifications carried out by enzymes during the maturation of primarily unmodified RNA precursor molecules. All modifications in RNA are highly specific with regard to position and kind of modification within the RNA molecules. Based on published sequence data of RNA we have calculated the average frequency of occurrence of the modified RNA building blocks in the different RNA classes (Schöch *et al.* 1990*a*, *b*). The modified RNA building blocks released as a consequence of RNA turnover are not re-utilized for *de novo* RNA synthesis. Furthermore, we and others have demonstrated that some special modified RNA catabolites such as t^6A, ψ and m^7Gua are not catabolized but virtually quantitatively excreted in urine (Schöch *et al.* 1990*a*, *b*; Topp *et al.* 1991, 1993). Therefore, these catabolites can be used as RNA degradation markers. The details of the calculation of the degradation rates of t-, r- and mRNA are given in the Materials and Methods section. At metabolic equilibrium, the RNA degradation rates between individuals of the same age within a given species are very similar, e.g. in 90-day-old Wistar rats ($n = 8$; body wt (BW) = 345 ± 23 g) housed in metabolic cages and supplied with a standard diet *ad libitum* the degradation rate of tRNA was $2{\cdot}2 \pm 0{\cdot}19$ μmol/kg BW/day. Furthermore, we found in different mammalian species of different body weights (mouse, hamster, rat, preterm infant, goat, sheep, human adult, pig) that the whole body degradation rates of t-, r- and mRNA at metabolic equilibrium are highly positively correlated with the metabolic rates (Schöch *et al.* 1990*b*; Topp *et al.* 1991, 1993; Schöch & Topp, 1994). Based on the latter findings we have speculated that there is a causal relationship between the degradation rates of the different RNA classes and the metabolic rate, i.e. the oxygen consumption, possibly via damaging effects of reactive oxygen species (ROS) formed dependent on the amount of oxygen consumed (Schöch & Topp, 1994; Topp *et al.* 1995).

The aim of the present study was to investigate, under defined model conditions, the effects of metabolic stress provoked by a parasitic infection of *Nippostrongylus brasiliensis* on the degradation rates of cytoplasmic tRNA, rRNA and mRNA in the host. With the speculation of a relationship between the degradation rates of RNA and the load of ROS this infection model is of special interest because the

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formation of ROS by the host seems to be an important mechanism in the expulsion of *N. brasiliensis* (Smith, 1989; Smith & Bryant, 1989*a*, *b*; Srivastava *et al*. 1992; Batra *et al*. 1993).

After the subcutaneous infection of rats with the 3rd-stage larvae (L_3) of the nematode *N. brasiliensis*, the L_3 usually are carried by the bloodstream into the lung where they moult within approximately 2 days post-infection (p.i.) to the 4th-stage larvae (L_4). The L_4 reach the small intestine via the trachea and oesophagus and then mature into the adult worms. About 8 days p.i. the expulsion of adult worms from the small intestine starts and most of the worms are rejected by approximately day 14 p.i. (Ogilvie & Jones, 1971; Kassai, 1982). As mentioned above, an increased generation of ROS by leucocytes in the host intestine seems to play an important role in the expulsion process of the adult worms.

MATERIALS AND METHODS

Animals and design of the study

Sixteen male Wistar rats (HsdCpb:WU; Harlan-Winkelmann, Borchen, Germany), average body weight 335·6 ± 15·2 g, were divided into 2 groups of 8 rats. All animals were housed individually in metabolic cages (model R/M, Altromin, Lage, Germany) and given standard diet (C 1000, Altromin) and drinking water *ad libitum*. Twenty-four h urine samples for the determination of the excretion rates of the RNA catabolites were collected in ice-cooled vessels during the investigation period and stored at −20 °C until analysis.

After 5 days under normal holding conditions 8 rats were infected by subcutaneous injection of 3000 L_3 of *N. brasiliensis* in 1 ml of phosphate-buffered saline (PBS), which had been isolated from coprocultures (König & Bohn, 1986). The other 8 rats (controls) were simultaneously injected with 1 ml of PBS. These control rats were individually pair-fed with respect to the infected rats.

Quantification of t^6A, ψ and m^7Gua from urine

The analytical procedures to determine t^6A, ψ and m^7Gua in urine have been published in detail elsewhere (Schöch *et al*. 1990*a*, *b*; Topp *et al*. 1993) and are only summarized here. Standards of ψ and m^7Gua were obtained from Sigma (Deisenhofen, Germany). t^6A standard was a kind gift from Professor Schlimme (Federal Dairy Research Centre, Kiel, Germany). The ribonucleosides ψ and t^6A were first enriched from urine by affinity chromatography using boronate gel (Affi-Gel 601, Bio-Rad, München, Germany). Then, HPLC analysis of the ribonucleoside fraction was performed using a reversed-phase gel (Nucleosil 120-5 C_{18}, Macherey-Nagel, Düren, Germany) and a ternary elution gradient composed of ammonium dihydrogen phosphate, methanol and acetonitrile. The ribonucleosides were quantified by their absorption at 254 and 280 nm (Topp *et al*. 1993). The nucleobase m^7Gua was first enriched from urine on a cation-exchange column with aromatic sulfonic acid as active groups (SPE system, Baker, Griesheim, Germany). Finally, an aliquot of the m^7Gua-containing fraction was analysed by cation-exchange HPLC (DC-6A, Benson, Reno, USA) using ammonium dihydrogen phosphate with methanol as the eluent. m^7Gua was quantified by its absorption at 254 and 280 nm (Schöch *et al*. 1990*a*).

Calculation procedures for determining the degradation rates of cytoplasmic tRNA, rRNA and mRNA

The background and details for the calculations of the degradation rates of cytoplasmic tRNA, rRNA and mRNA from urinary t^6A, ψ and m^7Gua have been published in detail elsewhere (Schöch *et al*. 1990*a*, *b*). Therefore, only the final formulae used are shown here:

$$\text{mol tRNA}_{\text{degraded}} \hat{=} \text{mol t}^6\text{A}_{\text{measured}}/0{\cdot}22; \quad (1)$$

$$\text{mol rRNA}_{\text{degraded}} \hat{=} (\text{mol }\psi_{\text{measured}} - \text{mol t}^6\text{A}_{\text{measured}} \times 13{\cdot}7)/95; \quad (2)$$

$$\text{mol mRNA}_{\text{degraded}} \hat{=} \text{mol m}^7\text{Gua}_{\text{measured}} - \text{mol t}^6\text{A}_{\text{measured}} \times 2 - (\text{mol }\psi_{\text{measured}} - \text{mol t}^6\text{A}_{\text{measured}} \times 13{\cdot}7)/95. \quad (3)$$

Statistical analysis

A statistical analysis of the degradation rates of tRNA, rRNA and mRNA in the rats between days 9 and 13 p.i. was performed using a Wilcoxon Matched-pairs Signed-ranks Test.

RESULTS AND DISCUSSION

The weight development of the rats infected by *N. brasiliensis* and of the pair-fed control rats was similar (Fig. 1). A clear weight loss of, on average, 15 g occurred on day 2 p.i. and correspondingly on day 3 in the pair-fed control rats caused by a drastically reduced food intake on these days. Furthermore, a very slight weight loss of, on average, 3 g occurred between days 6 and 9 p.i. Between days 9 and 15 p.i. the average body weights of the infected rats were slightly lower (on average −2·4 %) than those of the control rats.

In Fig. 2 the molar degradation rates of tRNA, rRNA and mRNA/kg body weight/day in the infected rats are presented in comparison with respective values of the control rats. The slight drop in the degradation rates of the different RNA classes in the control rats during the investigation period

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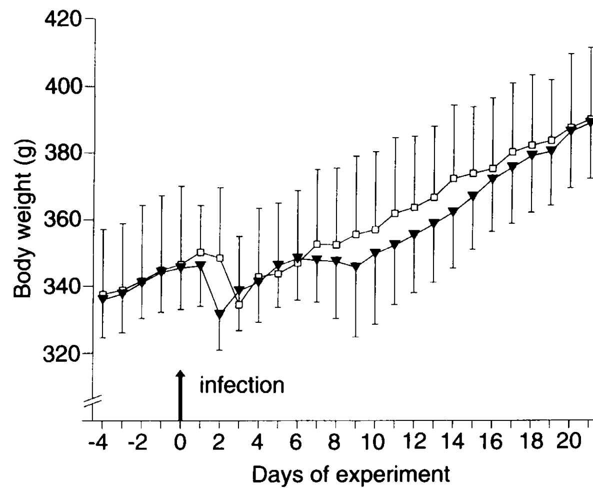

Fig. 1. Average body weights of 8 rats infected with 3000 L_3 of *Nippostrongylus brasiliensis* (▼) and of 8 control rats (□) given standard diet by pair-feeding. The error bars represent 1 S.D.

probably was caused by a decrease in the metabolic activity/unit body weight with increasing body size (Schöch *et al.* 1990*b*; Schöch & Topp, 1994) and by an increase in body fat relative to the metabolically active body mass. No elevated RNA degradation rates were noticeable during the conversion of the L_3 to the L_4 in the lung which usually occurs approximately within 2 days p.i. (Ogilvie & Jones, 1971; Kassai, 1982). Slightly elevated RNA degradation rates were observed from day 5 p.i. onwards. Between days 9 and 13 p.i. the expulsion of *N. brasiliensis* by the host is usually the most pronounced (Ogilvie & Jones, 1971; Smith & Bryant, 1989*a*). During this period the RNA degradation rates in the infected rats were significantly higher than in the non-infected rats ($P < 0{\cdot}05$); on average + 24 % (tRNA), + 34 % (rRNA) and + 26 % (mRNA). Peak values occurred around day 10.

The inflammatory response of the host involved in the expulsion process of *N. brasiliensis* is complex, it includes proliferation of mucosal mast cells releasing several mediators, synthesis of IgE antibodies and acute-phase proteins (Smith, 1989; Stadnyk, Baumann & Gauldie, 1990; Ramaswamy & Befus, 1993; Kasugai *et al.* 1995; McKay *et al.* 1995; Chen & Enerbäck, 1996). From several studies it can be concluded that ROS generated by the host leucocytes are an important mechanism in the expulsion process of *N. brasiliensis* (Smith, 1989; Smith & Bryant, 1989*a*, *b*; Srivastava *et al.* 1992; Batra *et al.* 1993). It has been described that the number of total peritoneal leucocytes increased after day 5 p.i., reached peak levels on day 9 p.i. and was still elevated above control levels on day 15 p.i. (Smith & Bryant, 1989*a*). In the same study the generation of ROS by peritoneal leucocytes, determined by luminol-dependent chemiluminescence, increased after day 5 p.i., was maximal between days 9 and 13 p.i. and was still elevated on day 15 p.i. On the other hand,

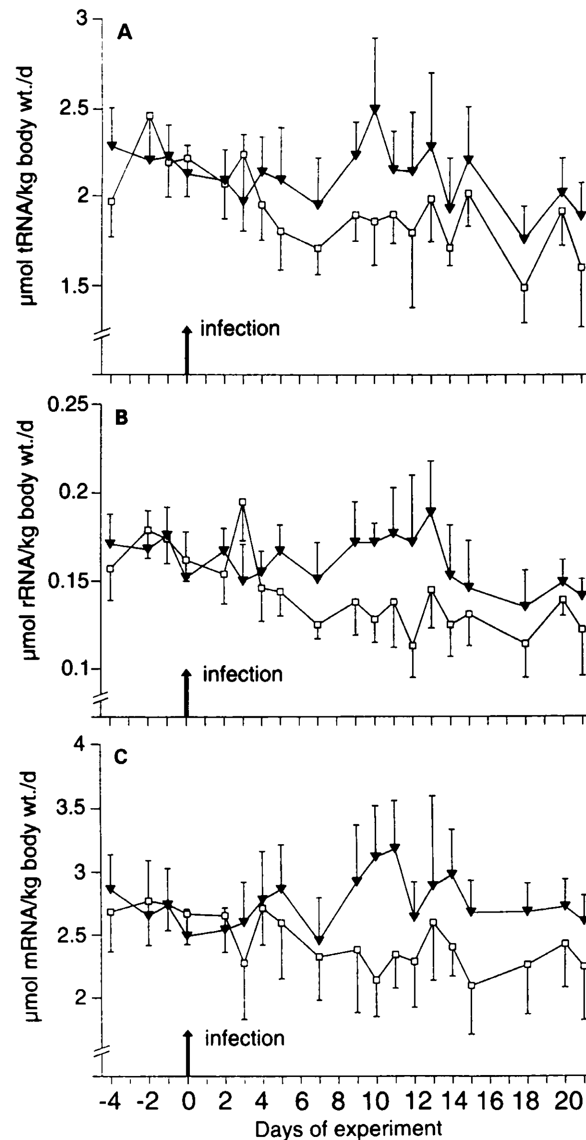

Fig. 2. Average degradation rates of cytoplasmic tRNA (A), rRNA (B) and mRNA (C) in 8 rats infected with 3000 L_3 of *Nippostrongylus brasiliensis* (▼) and in 8 control rats (□) given standard diet by pair-feeding. The error bars represent 1 S.D.

superoxide dismutase, reduced glutathione and vitamin E in *N. brasiliensis* gradually declined from day 7 p.i. onwards, indicating a reduced antioxidant defence of the parasite making the parasite highly susceptible to ROS (Batra *et al.* 1993). However, ROS-induced damage caused by the respiratory burst of leucocytes of the host is probably not restricted to the parasite but cells or cell components of the host may also be damaged. There is a large body of evidence showing the damaging effects of ROS on cell components such as lipids, proteins and nucleic acids (e.g. Adelman, Saul & Ames, 1988; Halliwell & Gutteridge, 1989; Halliwell & Aruoma, 1991; Sies, 1991; Park *et al.* 1992; Ames, Shigenaga & Hagen, 1993; Loft *et al.* 1994; Pryor, 1994; Simic, 1994; Grune *et al.* 1995; Topp *et al.* 1995). The time-courses of the increased RNA degradation rates

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and that described for the production of ROS (Smith & Bryant, 1989*b*) are very similar. Therefore, it is tempting to speculate that there is a causal relationship between both phenomena. The increase in the RNA degradation rates of approximately 25–35% between days 9 and 13 p.i. is moderate. However, with our non-invasive method for determining the degradation rates of t-, r- and mRNA by measuring the renal excretion rates of special RNA catabolites we can only determine the sum of the RNA degradation in the whole body. Therefore, a highly elevated RNA-degradation in a given tissue area is always partly disguised by the RNA degradation in the rest of the body. Furthermore, from our results we cannot conclude whether the increased RNA degradation is the result of an elevated turnover of cells or of an increased turnover of the RNA within intact cells. However, in the present investigation we have demonstrated for the first time that a parasitic infection with *N. brasiliensis* results in elevated degradation rates of t-, r- and mRNA in the host during the expulsion process. With regard to the possible role of increased formation of ROS in the elevated RNA degradation rates it would be of interest to investigate the reaction of further non-invasive measurable indicators of oxidative stress in the same model, e.g. the renal excretion of markers of oxidative DNA damage such as thymine glycol, thymidine glycol and 8-oxo-7,8-dihydro-2′-deoxyguanosine (Adelman *et al*. 1988; Park *et al*. 1992; Loft *et al*. 1994; Simic, 1994).

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