THE EXPULSION OF *ECHINOSTOMA TRIVOLVIS* CAUSED BY GOBLET CELL HYPERPLASIA IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE

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Summary :

Mice with severe combined immunodeficiency (SCID), lacking functional T and B lymphocytes, were each infected with 40 *Echinostoma trivolvis* metacercarial cysts on day 0. The mice of the test group were given intramuscular injections of dexamethasone (DEX) daily for 2 weeks and necropsied on days 5, 8, 12, 15, 20 and 30 post-infection (p. i.). The control mice, not treated with DEX, were each infected with 40 echinostome cysts on day 0 and necropsied on the same days as the DEX-treated mice. In the control mice, worm rejection began about day 8 p. i. and the worms were completely rejected by day 15 p. i., corresponding to the peak in goblet cell hyperplasia, about day 12 p. i. In the DEX-treated mice, goblet cell hyperplasia was significantly suppressed and the worms were retained until day 15 p. i., and then rejected after the last treatment with DEX. The number of mucosal mast cells, that increased with worm infection and peaked about day 15 p. i., was apparently suppressed by treatment with DEX. The eosinophil number in the controls increased on day 15 p. i. approximately and then decreased. The eosinophil number in the DEX-treated mice increased as in the controls, but was significantly suppressed compared to that of the controls during the period of the experiment. Enzyme-linked immunosorbent assay (ELISA) showed no marked rise in titres of the sera IgM, IgA and IgG throughout the experiment in both groups. These results indicate that DEX-treatment delayed the rejection of *E. trivolvis* from the small intestine of SCID mice in association with the suppression of goblet cell hyperplasia. It is concluded that the host immune system is not involved in the rejection of *E. trivolvis* and the effector cells for worm rejection are goblet cells that markedly increase in numbers by infection with *E. trivolvis*.

KEY WORDS : *Echinostoma trivolvis*, goblet cell hyperplasia, worm rejection, SCID mice.

Résumé : EXPULSION D'*ECHINOSTOMA TRIVOLVIS* PROVOQUÉE PAR L'HYPERPLASIE DES CELLULES CALCIFORMES CHEZ LA SOURIS PORTEUSE D'UN DÉFICIT IMMUNITAIRE SÉVÈRE (SCID)

Des souris porteuses d'un déficit immunitaire (SCID), manquant de lymphocytes T et B, ont été infectées chacune à J0 avec 40 métacercaires d'*Echinostoma trivolvis*. Les souris du groupe testé ont reçu une injection quotidienne de dexaméthasone pendant deux semaines et ont été nécropsiées à J5, J8, J12, J15, J20 et J30 après infection (p. i.). Les souris témoins, non traitées par dexaméthasone, ont été chacune infectées avec 40 métacercaires d'échinostome à J0 et nécropsiées les mêmes jours que les souris traitées. Chez les souris témoins, l'élimination des vers commence vers J8 p. i. et les vers sont complètement éliminés à J15 p. i., ce qui correspond au pic d'hyperplasie des cellules calciformes vers J12 p. i. Chez les souris traitées, l'hyperplasie des cellules calciformes est supprimée de manière significative et les vers sont conservés jusqu'à J15 p. i. avant d'être éliminés après la dernière injection de dexaméthasone. Les mastocytes de la muqueuse, dont le nombre augmente avec l'infection par l'helminthe pour atteindre un pic vers J15 p. i., est apparemment supprimé par le traitement à la dexaméthasone. Chez les témoins, le nombre d'éosinophiles augmente vers J15 p. i. puis diminue. Chez les souris traitées, le nombre d'éosinophiles augmente comme chez les souris témoins, mais est supprimé de manière significative en comparaison aux témoins pendant la période expérimentale. Dans aucun des deux groupes il n'a été observé d'élévation notable des taux sériques d'IgM, IgA et IgG déterminés par ELISA au cours de l'expérimentation. Ces résultats indiquent que le traitement par dexaméthasone retarde l'élimination d'*E. trivolvis* par l'intestin des souris SCID, simultanément à la suppression de l'hyperplasie des cellules calciformes. Les auteurs en concluent que le système immunitaire de l'hôte n'est pas impliqué dans l'élimination des vers, et que les cellules effectrices de l'élimination des vers sont les cellules calciformes, dont le nombre augmente notablement lors de l'infection par *E. trivolvis*.

MOTS CLÉS : *Echinostoma trivolvis*, hyperplasie des cellules calciformes, élimination des vers, souris SCID.

INTRODUCTION

The mouse can be infected with *Echinostoma trivolvis*, but the worm does not reach sexual maturity in this host and is rejected within two

to three weeks p. i. (Hosier & Fried, 1986; Weinstein & Fried, 1991; Fujino *et al.*, 1993, 1996a). Fujino *et al.* (1993) investigated worm kinetics and intestinal cytopathology of *Echinostoma trivolvis* adults that were rejected from both conventional and athymic BALB/c mice. They proposed that the expulsion of *E. trivolvis* was associated mainly with an increased number of goblet cells, but not mast cells. It is questionable whether factors in the immune system other than goblet cells are involved in worm expulsion. Fujino *et al.* (1998) examined the possible involvement of T-cell mediated immunity in the rejection of *E. trivolvis* from C3H/HeN mice, by using the well-defined immuno-

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suppressant, FK 506 (Tacrolimus). The treatment with FK506 did not delay worm expulsion, probably because the drug did not suppress goblet cell hyperplasia, thus negating the involvement of T lymphocytes in worm rejection. In C. B. SCID/SCID mice (congenic partners of BALB/c), functional T and B lymphocytes are deficient (Bosma *et al.*, 1983), therefore in this system it is possible to discount the effect of antibody and T cell function from host responses. The present investigation was carried out to confirm in SCID/SCID mice that the expulsion of *E. trivolvis* is associated with goblet cell hyperplasia without involving the immune system.

MATERIALS AND METHODS

Metacercarial cysts of *E. trivolvis* were obtained from the kidney and pericardial sac of laboratory-infected *Biomphalaria glabrata* snails. The worm strain was previously described by Fujino & Fried (1993). A total of 49 severe combined immunodeficient male c.B-17/Icr-scid Jcl mice (SCID mice) aged 6-8 weeks were used. Animals were maintained in filtered-barrier cages. Forty cysts in half strength Locke's saline were fed via a stomach tube to each mouse, and seven mice each were killed by light anaesthesia with ether and cervical dislocation on days 5, 8, 12, 15, 20 and 30 post-exposure (Fujino *et al.*, 1996b). The same number of mice as in the untreated controls were given daily intramuscular injections of dexamethasone, DEX (D1756, Sigma) at 2 mg/kg per day for 14 days (Fujino *et al.*, 1996b). Six groups of treated mice, seven per group, were killed on the same days as the untreated control mice. For histological examination, paraffin sections fixed with Carnoy's fixative or

10 % neutral buffered formalin were stained with acid Schiff (PAS) for goblet cell mucins, alcian blue (pH 0.3) and safranin O for mucosal mast cells, and Biebl's scarlet (Konwalinka *et al.*, 1980) or hematoxylin and eosin for eosinophils (Fujino *et al.*, 1996b). All counts were expressed as the number of cells per villus-crypt unit (VCU) (Miller & Jarrett, 1971). For enzyme-linked immunosorbent assay (ELISA) studies, blood was obtained by heart puncture from seven mice for both the control and DEX-treated groups just prior to necropsy. ELISA protocol followed the methods of Engvall & Perimann (1971) and Van Weekmen & Schuurs (1971). Where applicable Student's t-test was used to analyze differences between means with $P < 0.05$ being considered significant.

RESULTS

INFECTIVITY AND DISTRIBUTION

Infectivity and worm distribution data are presented in Table I. All untreated control mice were infected on day 5 p. i., and the worm recovery was less than 31 %. The worm recovery dropped to 7.5 % on day 8 p. i., and then only to 0.4 % on day 12 p. i. Worm recoveries in the DEX-treated mice were greater than 40 % from day 5 p. i. until day 15 p. i., fell rapidly to 7.1 % on day 20 p. i., and then to zero by day 30 p. i. Distribution data showed that most worms in the control mice were located in the middle or posterior part of the small intestine on day 5 p. i. Most worms in the DEX-treated mice were located in the middle or posterior part of the small intestine from day 5 to 15 p. i., and then were found in the posterior part of

Group	Day post-infection	No. of exposed mice (infected)	Means ($\pm$ SE) no. of worms recovered (%)	No. of worms located in the				CR	
				Small intestine Caecum					
Control									
A	5	7 (7)	12.3 $\pm$ 2.8 (30.7)	86	(0	44	42)	0	0
B	8	7 (2)	3.0 $\pm$ 2.6 (7.5)	21	(3	1	17)	0	0
C	12	7 (1)	0.1 $\pm$ 0.1 (0.4)	1	(0	0	1)	0	0
D	15	7 (0)	0	0				0	0
E	20	7 (0)	0	0				0	0
F	30	7 (0)	0	0				0	0
Treated									
G	5	7 (7)	23.0 $\pm$ 1.7 (57.5)**	161	(2	117	42)	0	0
H	10	7 (7)	19.3 $\pm$ 1.4 (48.2)**	135	(0	16	119)	0	0
I	12	7 (7)	18.1 $\pm$ 1.1 (45.4)**	121	(3	42	76)	0	6
J	15	7 (7)	16.4 $\pm$ 3.1 (41.1)	91	(0	29	62)	10	14
K	20	7 (2)	2.9 $\pm$ 2.3 (7.1)	2	(0	0	2)	7	11
L	30	7 (0)	0	0				0	0

Groups A-F of upper table are untreated controls, and groups G-L of lower table are dexamethasone-treated mice. CR, colon + rectum. * I, anterior; II, middle; III, posterior. ** significant against untreated control (Student's t-test; $p < 0.005$).

Table I. – Infectivity and distribution of *Echinostoma trivolvis* in SCID mice untreated or treated daily for 14 days with dexamethasone; each mouse exposed to 40 metacercarial cysts.

the small intestine, caecum and colon plus rectum on days 15 and 20 p. i.

GOBLET CELL NUMBER

Kinetic changes in the number of goblet cells/VCU in the anterior section of the ileum are shown in Fig. 1. The number of goblet cells in the untreated control mice increased rapidly from day 5 p. i., reached a peak of 14.1 ± 0.3 /VCU on day 8 p. i., and then declined gradually. The number of goblet cells of the mice treated with DEX for 14 days was between 8 and 12/VCU until day 15 p. i., increased to reach a peak on day 20 p. i., and then declined on day 30 p. i. There was a significant difference in goblet cell numbers on days 5, 8, 20 and 30 p. i. between the control and treated groups.

MAST CELL NUMBER

Kinetic changes in the number of mucosal mast cells/VCU are shown in Fig. 2. The number of mast cells in the untreated control mice increased rapidly to reach a peak of 2.67 ± 0.2 /VCU on day 15 p. i., and then declined markedly afterward. The number of mast cells of the mice treated with DEX increased from day 5 p. i., peaked on day 8 p. i., and then decreased to the level of the control on day 20 p. i. It slightly increased again from day 20 p. i. There was a significant difference in mast cell numbers on days 12, 15 and 20 p. i. between the control and treated groups.

EOSINOPHIL NUMBER

The number of eosinophils in the untreated control mice increased from day 8 p. i. to reach a peak on day 15 p. i., twice as high as on day 5 p. i., and then declined (not shown). The eosinophil number in the treated mice increased following the fluctuation pat-

tern in the control but was slightly but not significantly suppressed throughout the experiments compared to that of the controls.

ELISA STUDIES

The ELISA showed no marked increase in titration of sera IgG, IgM and IgA in the untreated control as well as the mice treated with DEX throughout the experiments (not shown).

DISCUSSION

Elimination of *Echinostoma trivolvis* from mouse hosts varies in time with differences in the mouse strains used in the experiments: within three weeks in ICR mice (Hosier & Fried, 1986), within 17 days in BALB/c mice (Fujino *et al.*, 1993) and C3H/HeN mice (Fujino *et al.*, 1996a). In the present study with SCID mice, the worms were completely rejected within 15 days.

DEX-treatment daily for 14 days in SCID mice delayed the rejection of *E. trivolvis* until day 20 p. i. Similar delayed rejection of the worms occurred in C3H/HeN mice treated for five or seven days with DEX (Fujino *et al.*, 1996b). Goblet cell hyperplasia was suppressed with DEX during the period of its injection (Fujino *et al.*, 1996b, 1997). The above-mentioned delayed rejection corresponds with the suppression of goblet cell hyperplasia with DEX in the small intestine in C3H/HeN mice (Fujino *et al.*, 1996b, 1997). Increased number of mucosal mast cells in the intestinal epithelium of the DEX-treated mice was suppressed during the experiment compared with that in the control as seen in C3H/HeN mice (Fujino *et al.*, 1996b). However, the increased number

Fig. 1. – Goblet cell number ($x \pm SE$)/VCU of the anterior section of ileum of the intestine of the control (—○—) and dexamethasone-treated (—●—) mice, which were both infected with 40 *Echinostoma trivolvis* metacercarial cysts.

Fig. 2. – Mast cell number ($x \pm SE$)/VCU of the anterior section of ileum of the intestine of the control (—○—) and dexamethasone-treated (—●—) mice, which were both infected with 40 *Echinostoma trivolvis* metacercarial cysts.

of mucosal mast cells was not considered responsible for the rejection of *E. trivolvis* (Fujino *et al.*, 1993). The inhibition of the increase in mast cell numbers by treatment with the well-defined immunosuppressant, FK506 (Tacrolimus), may not be responsible for suppressing the worm rejection (Fujino *et al.*, 1998).

In the present study, ELISA showed that no distinct increase in titration of the sera IgG, IgM and IgA occurred in both groups of SCID mice during the period of this experiment. It is considered that this may be due to the lack of functional B-lymphocytes. This deficiency is restricted to lymphoid cells, while monocytes, granulocytes, erythrocytes and natural killer cells were found to be normal (Custer *et al.*, 1985; Czitrom *et al.*, 1985; Dorshkind *et al.*, 1984, 1985). A marked increase in titration of serum IgM was observed after 12 days p. i. in both C3H/HeN mice treated for 30 days after the worm infection and in the controls (Fujino *et al.*, 1997). Rotman *et al.* (1995) infected BALB/cByJ and C. B-17-SCID mice with the third stage larvae of the nematode *Strongyloides stercoralis* and found that SCID mice were vulnerable to the parasites that did not infect the immunocompetent mice BALB/cByJ. They noted that « As nude mice infected with *S. stercoralis* infective larvae are not permissive for the development of adult worms (Dawkins & Grove, 1982), B-cells seem to be the likely cause of the refractory nature of immunocompetent mice to *S. stercoralis* infection ». In the present experiment using SCID mice, *E. trivolvis* was rejected in two weeks after infection, and SCID mice were not permissive hosts to the worms. It is concluded that the effector cells to reject these worms are goblet cells and that the immune system other than goblet cells is not involved in the worm rejection.

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