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Evaluation of the effects of DNA and proteasomes from Ascaris suum in mice infected with A. suum and Toxocara canis

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Summary

Ascaris suum is one of the most prevalent gastrointestinal parasites in animals and its survival in immmunocompetent hosts may be explained by its ability to modulate the host immune system. This experiment tested the ability of proteasomes or DNA derived from A. suum to achieve immunomodulation in mice. Mice were treated with both products 4 times at weekly intervals and were then infected 2 weeks after the last treatment with eggs from A. suum or Toxocara canis. The number of migrating larvae of both parasites was counted in the liver and lungs on day 3 or 5, respectively after infection. The results demonstrated a significant reduction in the number of larvae of A. suum and T. canis in both organs after treating the mice with parasite DNA, thus indicating the immunostimulatory ability of this DNA.

Keywords: DNA, proteasome, immunisation, Ascaris suum, Toxocara sp.

Ascariosis and toxocariosis among people and animals deserve special attention due to their occurrences rate, spread and pathogenicity. The rapid spread of the parasites is due to their high reproductive potential, drug-resistance and the eggs' resistance to environmental factors. Humans have known *Ascaris lumbricoides* since 1683 (4) and it is estimated that 1.4 milliard people are presently infected with this organism (5). A related species, *A. suum*, is also extremely common, especially in young pigs (18). It is therefore particularly surprising that in laboratory conditions, patent infections of *Ascaris spp.* have proven to be very difficult to establish. For example, Stankiewicz et al. (14) infected each ot 10, 4-week-old pigs with a single oral dose of 10 000 eggs. Seven days later numerous milk spots in the liver and many larvae were present in the lungs of 4 animals, indicating that infection was initially successful. However, post-mortem examination of the remaining 6 pigs, 60 days post infection, produced only 2 adult worms from one pig.

Toxocara canis (parasite of dog) is also known to invade humans, especially children, where it causes visceral larva migrans with possible clinical consequences. In addition, the larvae can be transmitted transplacentally and/or lactogenically (5).

The protection of humans and animals against parasitic infections is a major goal of all study on vaccines. We believe that some of the questions related to the life cycle, pathology and infectivity of ascarids can be answered by studying the factors, both present on and produced by parasites, which have an immunomodulatory action on the host. In our experience proteasomes have the factors, both present on and produced by parasites, which have an immunomodulatory action on the host. In our experience proteasomes have proven to be effective immunomodulators (13). In addition, the immunomodulatory activity of DNA from bacteria, protozoa and other various invertebrates (2, 16) indicates that DNA from parasitic nematodes should also merit serious consideration for this effect.

Material and methods

The study was carried out on mature and eggs forms of *Ascaris suum* and *Toxocara canis*. Adult *A. suum* from pig and *T. canis* from dogs were obtained using standard parasitological procedures and embryonation was conducted as described by Jeska et al. (9).

The proteasomes fraction was isolated and purified from frozen (–20°C) adult *A. suum* only, using a procedure that has been described previously (8, 13).

Extraction of genomic DNA from adult *A. suum* muscle and hypodermis was carried out as described by Di Mito and Betschart (7). The purity and similarity of the DNA from different parasites were assessed by 0.7% agarose gel electrophoresis or 10% polyacrylamide gel electrophoresis after digestion with different restriction enzymes.

Tab. 1. Intensity of infection after administered 1500 eggs of *Ascaris lumbricoides* or *Toxocara canis* per mice. Control groups (n = 5)

Tissue/day post infection	*Ascaris lumbricoides*		*Toxocara canis*	
	Mean value ± SD of larvae/mice	% of recovered larvae in tissue ± SD	Mean value ± SD of larvae/mice	% of recovered larvae in tissue ± SD
Liver 3 dpi	1060 ± 63	70.7 ± 4.2%	775 ± 80	51.5 ± 5.3%
Lung 5 dpi	425 ± 50	28.3 ± 3.3%	238 ± 72	15.9 ± 4.8%

One hundred 6- to 8-week-old female BALB/C mice were injected intraperitoneally 4 times at weekly intervals with either the genomic DNA extract (1 or 5 µg) or the proteasomes fraction (50 or 100 µg) per mouse (tab. 1). The control mice were treated with saline solution. Two weeks after the last injection both treated and untreated (control) mice were challenged with infective eggs from *A. suum* or *T. canis* given orally. Three and five days after the challenge, the mice were killed and the numbers of migrating larvae in the liver and lungs were counted, as described by Slotved et al. (12). Histological sections of lungs and liver were stained with H&E using standard methodology. For statistical analysis, Mann Whitney and ANOVA tests were used.

Results and Discussion

The electrophoretic tests showed that proteasome and the DNA of *Ascaris suum* and *Toxocara canis* have similar electrophoretic patterns. Of the two nematode products tested, only the DNA extract gave statistically significant treatment effects (tab. 2 and 3). Injections of DNA obtained from *A. suum* decreased the number of migrating larvae in the liver and lungs of mice challenged with homologous *A. suum* as well as when eggs from the non related *T. canis* were used. Also, the inflammatory response in lungs of the nematode challenged mice was greater in those treated with DNA (fig. 1) but not with the proteasomes fraction. No increased inflammation was seen in DNA treated, but not challenged, mice. Similar results as observed in the lungs were seen in the liver (fig. 2).

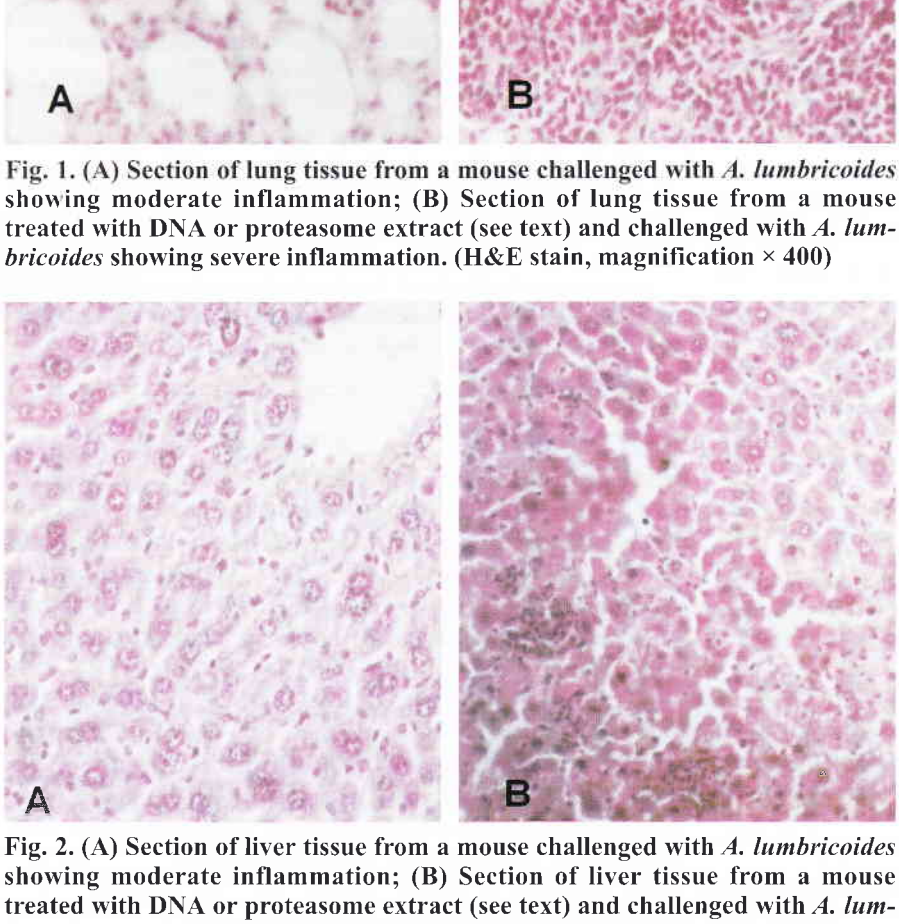

Fig. 1. (A) Section of lung tissue from a mouse challenged with *A. lumbricoides* showing moderate inflammation; (B) Section of lung tissue from a mouse treated with DNA or proteasome extract (see text) and challenged with *A. lumbricoides* showing severe inflammation. (H&E stain, magnification × 400)

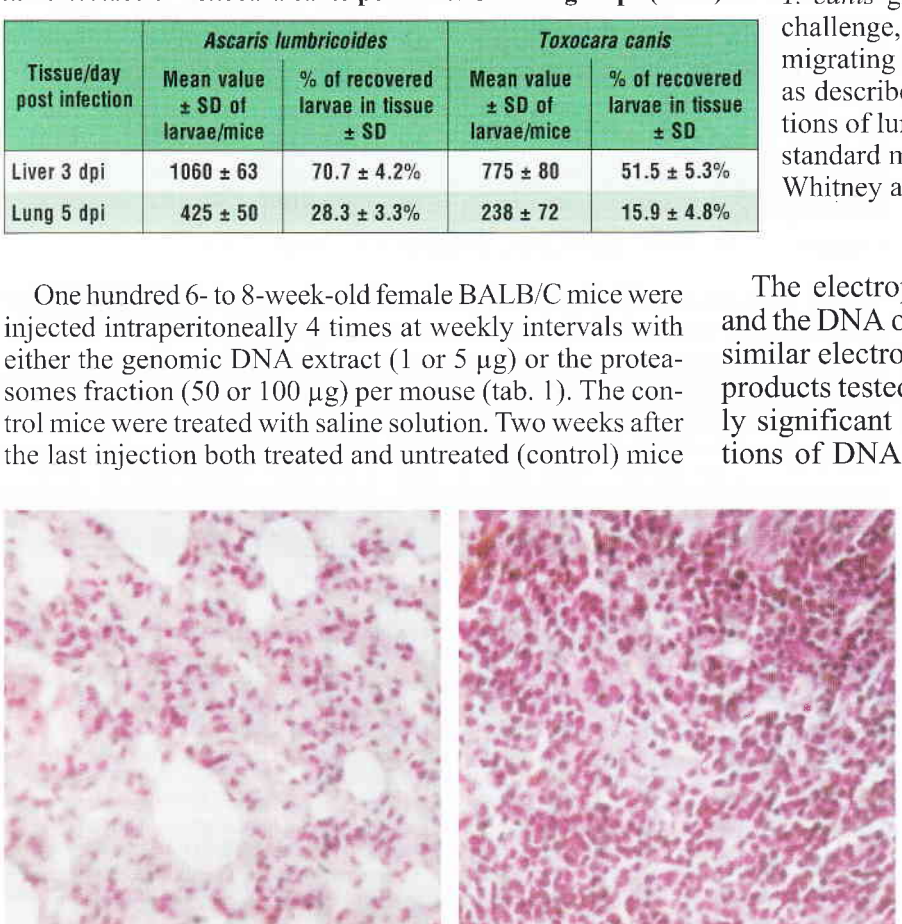

Fig. 2. (A) Section of liver tissue from a mouse challenged with *A. lumbricoides* showing moderate inflammation; (B) Section of liver tissue from a mouse treated with DNA or proteasome extract (see text) and challenged with *A. lumbricoides* showing severe inflammation. (H&E stain, magnification × 400)

If cDNA had been used this observation could be interpreted as showing that, as a result of injection of DNA, some protective antigens, which gave specific immunity against migrating larvae, were expressed. However, because genomic DNA was used it is most likely that non-specific immunostimulation had occurred, as discussed by Brown et al. (2) and Sun et al. (16). This immunostimulation would explain the increased inflammation in the lungs and liver, thus leading to the destruction and/or immobilisation of migrating larvae.

To our knowledge this is the first demonstration of immunomodulatory effect(s) of DNA from parasitic nematode larvae. Immunostimulatory effects of DNA are difficult to interpret considering the survival strategy of the parasite. However, this provides a plausible explanation of why larger doses of *Ascaris* eggs are not as good at producing patent infections as very small doses, as reported by Stewart (15), Anderson et al. (1) and Stankiewicz et al. (14). It can be argued that following a large dose infection, the larvae that

are killed during migration serve as donors of DNA, which in turn increases the inflammatory response due to its immunostimulatory action. This hypothesis can be supported by our results showing that higher doses of DNA were effective but smaller were not (tab. 1). However, further studies are needed to find out how useful DNA might be when used simply as an adjuvant in order to increase specific responsiveness against protective antigens obtained from *Ascaris*. Although considerable progress has been made in the isolation, characterisation and cloning of protective parasitic antigens (3, 6, 10, 11, 17), the development of effective vaccines against *Ascaris* and *Toxocara spp.* has not been very successful. This could be explained by differences in responsiveness of individual animals in outbreed populations. Because the nature of the immunostimulation produced here seems to be non-specific, its use in outbreed populations could contribute to protection against nematode infections. We were surprised that the proteasome fraction did not produce any effects in this experiment although it was immunosuppressive in sheep (13). At this stage we cannot offer any firm explanation for the differences observed but we speculate that this might be due to differences in immune responsiveness of rodents and ruminants. The higher decrease in numbers of *T. canis* larvae (tab. 2) as compared to *A. suum* (tab. 3) in the immunized animals was unexpected. However, antigenic and DNA similarity of both parasites shown by electrophoretic tests can mediate cross immunity. It is algo possible that *T. canis* larvae are more susceptible to the host response as indicated by the higher reduction in number of larvae in non-immunized animals (tab. 1).

Tab. 2. Reduction of infection *Ascaris lumbricoides* after immunisation by proteasome or DNA *Ascaris sp.* to the control group (n = 5)

Tissue/day post infection	Dose of proteasome	Medium value larvae/mice	% of reduction	Dose of DNA	Medium value larvae/mice	% of reduction
Liver 3 dpi	4 × 50 µg 4 × 100 µg	1035 ± 12.9 1022 ± 12.6	2.4 3.6	4 × 1 µg 4 × 5 µg	1010 ± 112 780 ± 189*	4.7 26.4*
Lung 5 dpi	4 × 50 µg 4 × 100 µg	410 ± 8.2 402 ± 8.6	3.5 5.3	4 × 1 µg 4 × 5 µg	388 ± 124 369 ± 21*	8.7 13.2*

Explanation: *p < 0.05, significantly different from control value

Tab. 3. Reduction of infection *Toxocara canis* after immunisation by DNA or proteasome of *Ascaris lumbricoides* to the control group (n = 5)

Tissue/day post infection	Dose of proteasome	Medium value larvae/mice	% of reduction	Dose of DNA	Medium value larvae/mice	% of reduction
Liver 3 dpi	4 × 50 µg 4 × 100 µg	647 ± 25.0 612 ± 15.0	16.5 21.0	– 4 × 5 µg	– 612.5 ± 85.4*	– 21.0*
Lung 5 dpi	4 × 50 µg 4 × 100 µg	195 ± 12.9 162 ± 12.6	18.1 31.7	– 4 × 5 µg	– 145.0 ± 42.0*	– 39.0*

Explanation: as in Tab. 2.

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SMITH-PALMER A., STEWART W. C., MATHER H., GREIG A., COWDEN J. M., REILLY W. J.: Epidemiologia *Salmonella enterica* serowar *Enteritidis* i *Typhimurium* u zwierząt i u ludzi w Szkocji w okresie 1990-2001. (Epidemiology of *Salmonella enterica* serovars *Enteritidis* and *Typhimurium* in animals and people in Scotland between 1990-2001). Vet. Rec. 153, 517-520, 2003 (17)

Najważniejsze znaczenie w patologii człowieka i zwierząt odgrywa *Salmonella enterica* serowar *Enteritidis*, typ fagowy 4 (PT4) i serowar *Typhimurium*, typ fagowy 104 (DT104) W 1996 r. liczba ognisk zachorowań u ludzi wywołana przez DT104 wynosiła 96. Zachorowania bydła wywołane również przez ten serotyp osiągnęły maksymalny poziom wynoszący 138 ognisk w 1996 r. W 2001 r. wystąpił spadek incydentów salmonellozy do 10. W tym czasie obniżyła się liczba zachorowań wywołanych przez salmonelle u owiec i kóz. W 1997 r. przypadł szczyt infekcji (684 ogniska) wywołanych u ludzi przez PT4. Następnie wystąpił wyraźny spadek i w 2001 r. stwierdzono 457 ogniska. W 1998 r. u drobiu stwierdzono 34 ogniska zachorowań wywołane przez PT4.

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