Cadmium effects on *Ichthyophthirius*: evidence for metal-sequestration in fish tissues following administration of recombinant vaccines

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SUMMARY

We are developing *Tetrahymena thermophila* as a delivery system for recombinant vaccines against parasitic protozoa, including the common fish parasite, *Ichthyophthirius multifiliis*. *T. thermophila* cell lines expressing *I. multifiliis* genes under the control of a cadmium-inducible metallothionein gene promoter conferred strong protection against a lethal parasite challenge when administered parenterally to naïve fish. Nevertheless, given that heavy metals can be toxic to parasites, a question arose as to whether protection resulted from Cd residues carried over with the vaccine, rather than acquired immunity *per se*. To address this issue, we examined the sensitivity of *I. multifiliis* to Cd *in vitro* and determined Cd concentrations in different host tissues following i.p. injection of juvenile channel catfish with the recombinant vaccine. We found that $CdCl_2$ at concentrations $\geqslant 50$ ppb were lethal to *I. multifiliis* theronts *in vitro*. Furthermore, Cd concentrations were clearly elevated in fish tissues and reached levels equivalent to 74 ng/g wet weight (74 ppb) in the skin within 14 days of injection with recombinant *T. thermophila*. Nevertheless, fish injected with non-transformed *Tetrahymena* grown in the presence or absence of $CdCl_2$ showed no significant difference in either relative survival or parasite load following direct challenge with *I. multifiliis*.

Key words: *Ichthyophthirius multifiliis*, heavy metal, parasite, *Tetrahymena thermophila*, vaccine.

INTRODUCTION

Parasite burdens in fish can be used as indicators of heavy-metal contamination in the environment due to the suppressive effects of heavy metals on the host immune response (MacKenzie *et al*. 1995; Lafferty, 1997). At the same time, heavy metals can be toxic to fish parasites, a notable example being *Ichthyophthirius multifiliis*, a protozoan pathogen responsible for 'white-spot' disease in freshwater species (Matthews, 1994; Dickerson & Dawe, 1995).

In the case of *Ichthyophthirius*, heavy metals kill water-borne stages of the parasite, and copper sulphate is sometimes used to control infections in home aquaria (Ling, Sim & Lam, 1993; Straus, 1993; Schlenk, Golon & Griffin, 1998; Tieman & Goodwin, 2001). However, use of heavy metals on food fish is not recommended and efforts to control outbreaks in commercial facilities have relied more on the use of formalin, which is also hazardous. As an alternative to chemical treatments, the development of immunoprophylactic methods of disease prevention would be highly desirable. In this regard, fish acquire long-term immunity against *Ichthyophthirius* in response to controlled infections and we have identified a class of abundant parasite membrane proteins, known as i-antigens, as having an important role in development of host resistance (Clark, Lin & Dickerson, 1995; Clark & Dickerson, 1997; Wang & Dickerson, 2002). While *Ichthyophthirius* cannot be readily cultured, i-antigens have been expressed as recombinant proteins in the related, free-living ciliate, *Tetrahymena thermophila* (Gaertig *et al*. 1999). *Tetrahymena* can be grown to high-density in inexpensive media, and high-level expression of parasite antigens has recently been achieved in this system using a cadmium-inducible metallothionein gene promoter (Shang *et al*. 2002). Furthermore, as few as 10^6 cells induced with Cd have been shown to elicit protective immunity against 'white-spot' when administered to juvenile channel catfish as live vaccines (Wang, Gaertig, Gorovsky, Dickerson and Clark, unpublished). Nevertheless, treatment with Cd would be expected to stimulate metallothionein synthesis resulting in an accumulation of bound metal ions in transformed *Tetrahymena* (Piccinni *et al*. 1987; Piccinni, Irato & Guidolin, 1990). Thus, the protection afforded by live cells may have been due to toxic effects of residual Cd introduced with the vaccine itself. To test this, we determined the levels of $CdCl_2$ that are toxic to *I. multifiliis in vitro*

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and measured the actual concentrations of Cd in different host tissues following intraperitoneal injection with live recombinant cells. In addition, we tested the effects of non-transformed *T. thermophila* grown in the presence or absence of $CdCl_2$ on both parasite load and fish survival following lethal challenge with *Ichthyophthirius*.

MATERIALS AND METHODS

Parasite growth and maintenance

Ichthyophthirius multifiliis (strains G5 and NY1) were maintained on juvenile channel catfish (*Ictalurus punctatus*) as described elsewhere (Noe & Dickerson, 1995; Lin, Clark & Dickerson, 1996). Tomonts were collected by gently rubbing fish. Parasites dislodged from the skin were harvested on wire-mesh filters and incubated overnight at room temperature in carbon-filtered water. After dividing to form theronts, parasites were collected by low speed centrifugation (2 min at $300\times \boldsymbol{g}$) and resuspended in water.

Analysis of cadmium effects in vitro

$CdCl_2$ made up at varying concentrations in carbon-filtered water was added in 50 μl aliquots to individual wells of a flat-bottomed 96-well polystyrene plate. Equal volumes of cell suspensions containing *Ichthyophthirius* theronts were then added. Cells were maintained at room temperature for 18–22 h and, at intervals, examined under a dissecting microscope (Olympus). Cells were photographed using Kodak TMAX-400 film.

Preparation of Tetrahymena *cultures and vaccination of fish*

T. thermophila cell lines were grown at 30 °C with constant shaking in Neff media containing 0·25% proteose peptone, 0·25% yeast extract, 0·55% glucose, and 0·033 mM $FeCl_3$. *Tetrahymena* strains used in this study were a transgenic cell line harbouring parasite i-antigen gene *IAG52B[G5]* (Lin *et al*. 2002; Clark *et al*., unpublished), and CU522, the parental line used in the construction of the vaccine strain. Unless otherwise indicated, cell lines were grown to mid-late log phase ($5–7\times10^5$ cells/ml), and then maintained in the same media either with or without 2 μg/ml $CdCl_2$. After 16–24 h, cells were harvested by centrifugation at $300\times \boldsymbol{g}$ for 3 min, washed twice in 10 mM Tris–HCl (pH 7·5) and resuspended at a concentration of 5×10^6 cells/ml in PBS buffer containing 130 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, pH 7·4. Aliquots containing 200 μl (1×10^6 cells) were injected into the peritoneal cavities (i.p.) of juvenile channel catfish weighing $6·9+2·7$ g using a tuberculin syringe fitted with a 26 gauge needle. Fish were injected twice at two week intervals and either directly challenged with *I. multifiliis* or sacrificed for the collection of tissue samples (below). Animals used in challenge studies were maintained in 15 gallon aquaria with aeration for the duration of experiments.

Sampling of tissues and cells by ICP

Fish were euthanazed by overdose with tricane-methanesulfonate (MS-222; 0·5 g/l) and placed on ice. Kidney, skin and muscle tissues were surgically removed from fish euthanazed at different time points, namely, 1, 5 and 14 days after the last injection with *T. thermophila*. A total of 10 fish were sacrificed at each time point. Tissues from individual fish were combined as follows. For kidney, samples collected from 10 fish were combined for each time point. For muscle, tissues from each of 2 fish were combined yielding 5 separate groups for each time point. For skin, tissues from 3–4 fish were combined yielding 3 separate groups for each time point. Samples were weighed after collection, and individual groups (containing 0·5–3 g tissue) were air-dried. After 14 days, samples were placed in a drying oven at 60 °C for an additional week. Combined tissues were again weighed and sent for analysis using inductively-coupled argon plasma (ICP) emission spectrometry. To determine Cd concentrations in *T. thermophila* and the surrounding growth media, cells were grown to $\sim 8\times10^5$ cells/ml phase in Neff medium containing 2 μg/ml $CdCl_2$. After 16 h, cells were harvested by centrifugation as above and the supernatant fraction collected for ICP analysis. The cell pellet was then re-suspended and washed twice in 40 ml 10 mM Tris–HCl (pH 7·5) buffer. Supernatant, pellet and wash fractions were then dried as for tissue samples. No significant difference was seen in the amount of Cd present in cell pellets before and after washing in Tris buffer (data not shown). For ICP analysis, dried samples (either tissues, cells or media) were digested for 3–5 days in 500 μl 70% perchloric acid, resuspended in 10 ml 0·5% nitric acid and subjected to elemental analysis using a model 51000 ICP spectrometer (Perkin-Elmer/Sciex).

Parasite challenge studies

For whole body infections, channel catfish that had been injected with *T. thermophila* strain CU522 were placed in large beakers containing 100 ml water fish. Theronts of the G5 strain were then added to a final concentration of 10 000 parasites/fish. After 2 h fish were returned to aquaria along with the water containing infective theronts. For tail-fin infections, fish that had been injected with *T. thermophila* were anaesthetized in 100 mg/l MS-222, wrapped in moist paper and placed with their tailfins in water containing NY1 theronts at a concentration of

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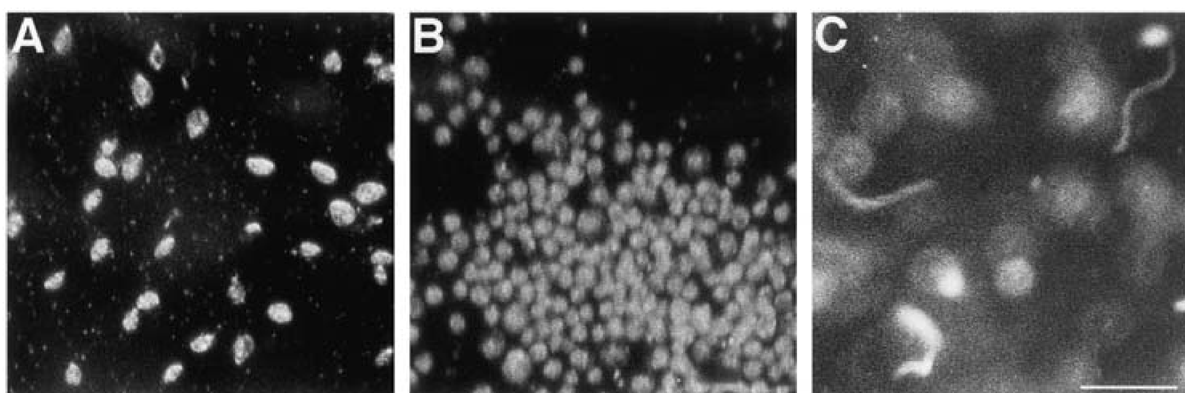

Fig. 1. Effects of Cd on *Ichthyophthirius in vitro*. Theronts were incubated in carbon-filtered H_2O containing 500 ppm $CdCl_2$ (A), 5 ppm $CdCl_2$ (B) and 5 ppb $CdCl_2$ (C). At the highest Cd concentration, cells immediately ceased swimming and were clearly dead within 10 min of exposure. Hours later, cells were still intact and visible at the bottom of the well (A). When exposed to 5 ppm $CdCl_2$, cells rounded-up and formed large aggregates at the bottom of the well. After several hours, cells lost distinct borders (B) and eventually lysed. Exposure to 5 ppb $CdCl_2$ had little apparent affect on theronts. Cells swam rapidly and could not be captured in focus on film (C). Bar = 200 μm.

Table 1. Following incubation in varying concentrations of Cd (ppm) for different lengths of time, theronts were scored for overall changes in cell motility according to an arbitrary scale listed below. Cells that ceased swimming failed to regain movement and were considered dead

(+++ = 100% of cells ceased swimming. ++ = 50–90% of cells ceased swimming. + = 10–20% of cells ceased swimming. +/− = reduction in swimming velocity. − = no obvious change.)

	Time of incubation			
ppm	10 min	1 h	5 h	18–22 h
500	+++			
50	+	+++		
5	+/−	++	+++	
0·5	−	−	++	+++
0·05	−	−	−	++
0·005	−	−	−	−

2000 cells/ml. After 5 min fish were removed, rinsed briefly in carbon-filtered water, and returned to aquaria. Four days later fish were re-anaesthetized and trophonts in host tissue counted under a dissecting microscope. Fish were immediately returned to aquaria where the infection was allowed to cycle.

RESULTS

To determine the concentrations of Cd that are toxic to *I. multifiliis*, swimming theronts were placed in individual wells of a 96-well plate that contained varying concentrations of $CdCl_2$ (ranging from 500 ppm to 5 ppb). Parasites were observed under a dissecting microscope and alterations in their behaviour noted over time. As indicated in Table 1, Cd was toxic to theronts in a concentration and time-dependent manner. At Cd levels of 500 ppm, theronts lost motility almost immediately, and fell to the bottom of the well by 10 min of exposure. In general dead and dying cells remained intact under these conditions and were still visible at the bottom of the container the next day (Fig. 1A). At concentrations between 50 ppm and 0·5 ppm, death occurred more slowly and was accompanied by obvious swelling and eventual cell lysis (Fig. 1B). Below 0·5 ppm, Cd was still toxic and at 50 ppb most theronts appeared dead within 24 h.

We had previously shown that *T. thermophila* expressing parasite antigens, under the control of a Cd-inducible promoter, protect juvenile channel catfish against a lethal challenge with *Ichthyophthirius* following i.p. administration of the recombinant vaccine (Wang, Gaertig, Gorovsky, Dickerson and Clark, unpublished). To determine whether Cd concentrations in host tissues become elevated following vaccination, juvenile channel catfish were injected twice with 10^6 transformed *T. thermophila* and metal concentrations in different host tissues determined at varying times thereafter. As shown in Fig. 2C, Cd levels in the kidney were elevated within 1 day, and accumulated to levels >60 ppm (dry weight) within 5 days of injection. By 14 days, Cd levels were somewhat diminished but were still high in the kidney (>40 ppm dry weight). Cadmium concentrations in the skin and muscle were also elevated, although to a lesser extent. In the skin, levels were 0·8 ppm after 24 h and declined to $\sim 0{\cdot}4$ ppm (dry weight) by 14 days post-vaccination (Fig. 2B). Cadmium concentrations in muscle (Fig. 2A) were roughly one-third of that in skin over the same time period, although skin was considered more relevant since it is the primary site of *Ichthyophthirius* infection. Based on fresh weight, Cd concentrations in the skin and muscle were determined to be 0·152 and 0·037 ppm respectively, at 1 day and 0·074 and 0·023 ppm at 14 days post-injection.

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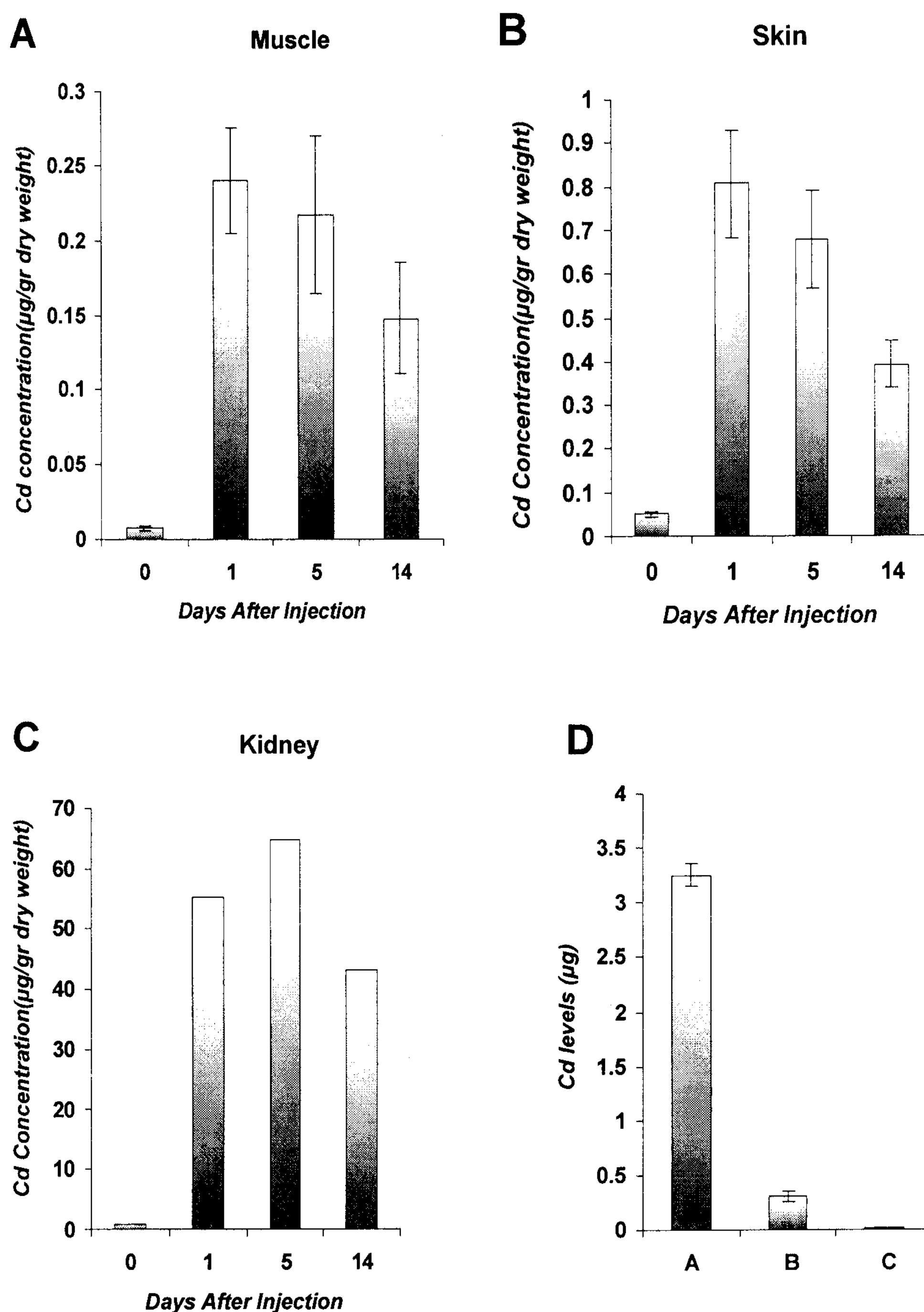

Fig. 2. [Cd] in fish tissues following vaccination with recombinant *T. thermophila*. Cd concentrations in skin (panel B), muscle (panel A) and kidney (panel C) were measured by ICP spectrometry. Bars represent the average concentration (in dry weight) for groups of samples taken from fish at different time points after vaccination. Bracketed lines represent the standard error between individual groups (note that kidney samples show no lines since tissue from all fish at a given time point were combined and measured as a single group). Bars at the zero time point represent the values for control fish injected with recombinant *T. thermophila* that had not been induced with $CdCl_2$. Panel D shows the level of Cd in cell pellets containing 2×10^6 recombinant *T. thermophila* (A), and in the surrounding growth medium (B) 16 h after incubation in 2 μg/ml $CdCl_2$. The basal level of Cd associated with the CU522 host strain grown in the absence of heavy metals (C) is also shown.

The fact that Cd could easily be measured in host tissues following vaccination with *T. thermophila* clearly suggested that the cells themselves had taken up Cd from the growth medium following induction with $CdCl_2$. To determine the extent to which these cells accumulate Cd, we conducted ICP analysis on *T. thermophila* cell pellets as well as the surrounding medium. As shown in Figure 2D, ~92% of the Cd in the original growth medium was associated with the cell pellets 16 h after induction with 2 μg/ml $CdCl_2$.

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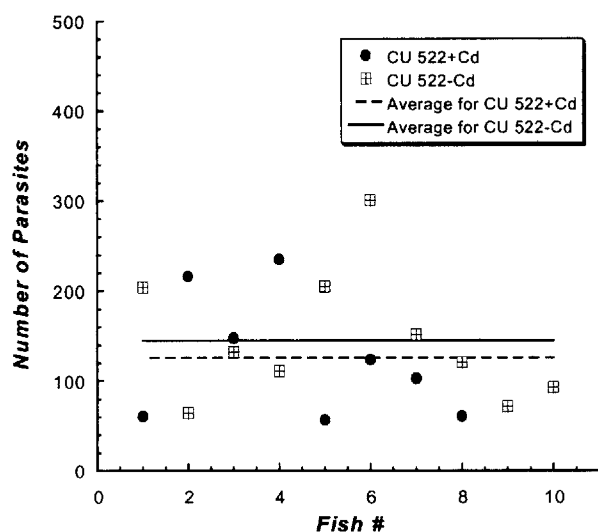

Fig. 3. Effects of vaccine-associated Cd on parasite establishment *in vivo*. Groups of fish were vaccinated with *T. thermophila* strain CU522 that had been incubated in the presence (dashed line) or absence (solid line) of 2 μg/ml $CdCl_2$. Two weeks later, fish were exposed to *I. multifiliis* and parasites on the tailfins were counted 4 days later. Horizontal lines represent the average numbers of trophonts on fish in each group. Symbols (closed circles, or hatched squares) are the actual parasite numbers in individual fish. The average number for the group containing Cd was 122·1 + 20 ($n = 8$), and for the group lacking Cd was 145·8 + 72 ($n = 10$). Based on one-way analysis of variance (ANOVA), the difference in the average numbers of parasites in the two groups was not significant.

In standard vaccine trials with channel catfish, animals are challenged 14–28 days post-vaccination. Since Cd concentrations in skin at day 14 were above those that killed *Ichthyophthirius in vitro*, we sought to determine the potential effects of Cd on parasites *in vivo*. Two groups of fish were injected with non-transformed *T. thermophila* strain CU522 that were grown either in the presence or absence of $CdCl_2$. Fourteen days after injection, animals were subjected to tailfin infections with the virulent NY1 strain of parasites and the number of trophonts that established in the skin by 4 days of infection was determined. The results of this experiment are shown in Fig. 3. While the variation in parasite numbers among individual fish was relatively large, no significant difference was seen in the average number of parasites in the two groups (ANOVA). Rather than terminate the experiment, we allowed the infection to cycle and determined per cent mortality in each group following secondary re-infection. No protection was seen in either group, with all fish dying by 14 days of the initial challenge (Fig. 4A). We repeated this experiment using a slightly less virulent strain of parasite (namely, G5), and fish exposed to whole-body infections. Again, all fish died in both groups (Fig. 4B).

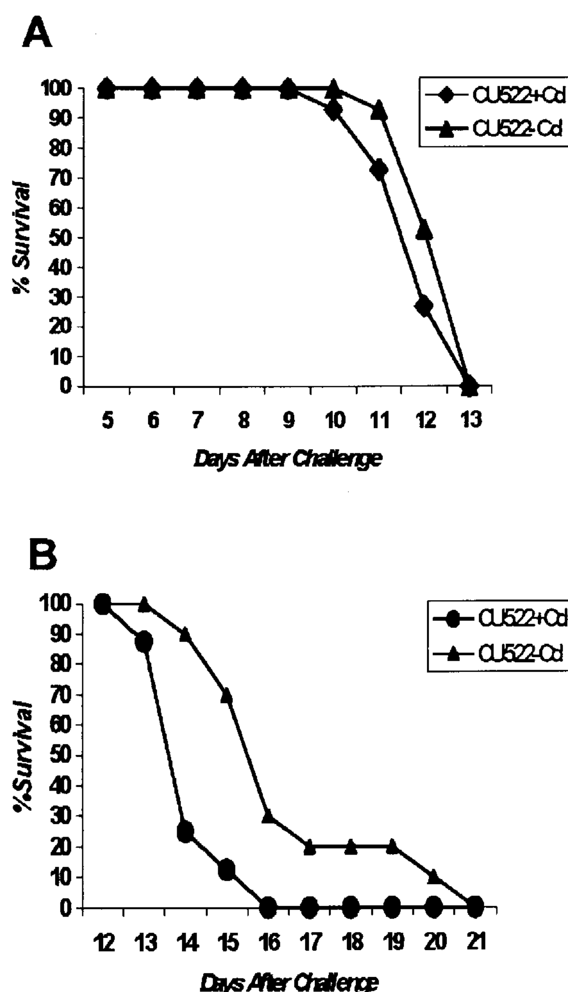

Fig. 4. Survival rates of fish vaccinated with non-recombinant *T. thermophila*. In (A), groups of fish that had been vaccinated with *T. thermophila* grown in the presence (diamonds) or absence (triangles) of $CdCl_2$ were subjected to tailfin infections with *I. multifiliis* strain NY1. Within 13 days of the initial exposure, all fish in both groups had died. In (B), groups of fish (15 animals/group) vaccinated as in (A) were subjected to whole-body infections with *I. multifiliis* strain G5, and % survival measured over time. By 21 days, all fish in both groups had died.

DISCUSSION

While the recombinant cells used in this study were washed extensively in Tris buffer following induction with Cd, metal concentrations in fish tissues were initially quite high. Consistent with previous reports on kidney as being the primary site of heavy metal accumulation in fish from contaminated streams (de Conto Cinier *et al.* 1999; Hollis, Hogstrand & Wood, 2001; Woodling, Brinkman & Horn, 2001), Cd levels in the kidneys of vaccinated fish were > 60 times that in skin and muscle. Indeed, while kidney represents less than 1% of the animal's mass, its overall contribution to Cd was roughly one-third the total, or ~410 ng per fish one day post-injection.

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Cadmium concentrations in the skin and muscle were 152 and 37 ng/g of fresh tissue, respectively. Assuming these values were representative of the average Cd concentrations in all tissues, then the total amount of Cd in vaccinated fish (including kidney) 1 day post-vaccination was estimated to be $\sim 1{\cdot}4\ \mu g$. The maximum amount that could be delivered to fish with the vaccine if all the Cd in the growth medium was associated with *Tetrahymena* would be $\sim 4{\cdot}9\ \mu g$. Thus, the actual amount of Cd present in fish was a substantial fraction of the theoretical maximum (roughly 37%, assuming a half-life in the animal of 14 days), which in turn suggested that *Tetrahymena* took up large amounts of Cd from the growth medium. This inference was in fact borne-out by ICP measurements on *T. thermophila*. Based on these measurements, ~92% of the Cd in the original medium was associated with the cell pellet after 16 h. The ability of *Tetrahymena* to concentrate Cd from the growth medium is likely due to the up-regulation of endogenous metallothionein synthesis in response to heavy-metal treatment (Piccinni *et al*. 1987, 1990). Much of this Cd must then become available to fish following intraperitoneal injection with live cells.

Perhaps not surprisingly, *Ichthyophthirius* theronts were found to be highly sensitive to Cd in water. Heavy metals (including copper sulphate and silver nitrate) have been previously shown to kill theronts (Farley & Heckmann, 1980; Strauss, 1993; Ling *et al*. 1993; Schlenk *et al*. 1998; Tieman & Goodwin, 2001), with copper being toxic at concentrations >50 ppb. As shown here, Cd levels in the skin of vaccinated fish were 74 ppb at the time of challenge, a concentration higher than that which killed theronts in water. Despite this, there was no evidence that parasites in host tissues were sensitive to Cd residues delivered with the vaccine. Indeed, in CU522 vaccinated fish challenged by whole-body exposure to *Ichthyophthirius*, fish died more rapidly in the group injected with Cd-treated *T. thermophila* (Fig. 4B). To determine parasite sensitivities to potential Cd residues introduced with the vaccine, we had to inject fish with the non-transformed *Tetrahymena* strain CU522 since an immune response to recombinant cells would otherwise compromise the results. Although we have no reason to believe that Cd levels associated with the vaccine strain are different from those associated with CU522, we are in the process of comparing Cd concentrations in *Tetrahymena* cell lines that either do or do not contain foreign genes. The absence of an effect of elevated Cd on parasites *in vivo* is most likely attributable to the fact that metal ions are sequestered in the tissue and not available to interact with parasites. As in the kidney, heavy metals in skin are almost certainly complexed with metallothioneins or other metal-chelating proteins, and not free in the intersitial fluids (Hollis *et al*. 2001; Olsvik *et al*. 2001).

Finally, while the presence of Cd residues in fish vaccinated with recombinant *Tetrahymena* is clearly relevant to food safety, final concentrations in muscle were 23 ppb 14 days after vaccine administration. In production facilities, these concentrations would be expected to further decline due to excretion and continued growth of the fish. Furthermore, 23 ppb is below the Cd limit proposed for fish by European Commission Regulation (III/5125/95 Rev. 3), and is only slightly higher than the normal range for human body Cd according to EBI, a Canadian environmental watchdog group (http://www.e-b-i.net/ebi/contaminants/cadmimum.html). Still, efforts to reduce Cd associated with recombinant *T. thermophila* vaccines are currently being considered, including the possible use of zinc as an alternative for regulating foreign gene expression.

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REFERENCES

CLARK, T. G. & DICKERSON, H. W. (1997). Antibody-mediated effects on parasite behavior: evidence of a novel mechanism of immunity against a parasitic protist. *Parasitology Today* **13**, 477–480.

CLARK, T. G., LIN, T. L. & DICKERSON, H. W. (1995). Surface immobilization antigens of *Ichthyophthirius multifiliis*: their role in protective immunity. *Annual Review of Fish Diseases* **5**, 113–131.

DE CONTO CINIER, C., PETIT-RAMEL, M., FAURE, R., GARIN, D. & BOUVET, Y. (1999). Kinetics of cadmium accumulation and elimination in carp *Cyprinus carpio* tissues. *Comparative Biochemistry and Physiology Part C* **122**, 345–352.

DICKERSON, H. W. & DAWE, D. L. (1995). *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (Phylum Ciliophora). In *Fish Diseases and Disorders* (ed. Woo, P. T. K.), pp. 181–227. Wallingford Oxon, CAB International.

FARLEY, D. G. & HECKMANN, R. (1980). Attempts to control *Ichthyophthirius multifiliis* Fouquet (Ciliophora: Ophryoglenidae) by chemotherapy and electrotherapy. *Journal of Fish Diseases* **3**, 203–212.

GAERTIG, J., GAO, Y., TISHGARTEN, T., CLARK, T. G. & DICKERSON, H. W. (1999). Surface display of a parasite antigen in the ciliate *Tetrahymena thermophila*. *Nature Biotechnology* **17**, 462–465.

HOLLIS, L., HOGSTRAND, C. & WOOD, C. M. (2001). Tissue-specific cadmium accumulation, metallothionein induction, and tissue zinc and copper levels during chronic sublethal cadmium exposure in juvenile rainbow trout. *Archives of Environmental Contamination and Toxicology* **41**, 468–474.

LAFFERTY, K. D. (1997). Environmental parasitology: what can parasites tell us about human impacts on the environment? *Parasitology Today* **13**, 251–255.

https://doi.org/10.1017/S0031182003003731 Published online by Cambridge University Press

LIN, T. L., CLARK, T. G. & DICKERSON, H. W. (1996). Passive immunization of channel catfish (*Ictalurus punctatus*) against the ciliated protozoan parasite *Ichthyophthirius multifiliis* by use of murine monoclonal antibodies. *Infection and Immunity* **6**, 4085–4090.

LIN, Y., LIN, T. L., WANG, C. C., WANG, X., KLOBFLEISCH, R., STIEGER, K. & CLARK, T. G. (2002). Variation in primary sequence and tandem repeat copy number among i-antigens of *Ichthyophthirius multifiliis*. *Molecular and Biochemical Parasitology* **120**, 93–106.

LING, K. H., SIN, Y. M. & LAM, T. J. (1993). Effect of copper sulfate on ichthyophthiriasis (white spot disease) in goldfish (*Carassius auratus*). *Aquaculture* **118**, 23–35.

MACKENZIE, K., WILLIAMS, H. H., WILLIAMS, B., McVICAR, A. H. & SIDDALL, R. (1995). Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *Advances in Parasitology* **35**, 85–144.

MATTHEWS, R. A. (1994). *Ichthyophthirius multifiliis* Fouquet, 1876: infection and protective response within the fish host. In *Parasitic Diseases of Fish* (ed. Pike, A. W. & Lewis, J. W.), pp. 17–42. Dyfed, Samara.

NOE, J. G. & DICKERSON, H. W. (1995). Sustained growth of *Ichthyophthirius multifiliis* at low temperature in the laboratory. *Journal of Parasitology* **81**, 1022–1024.

OLSVIK, P. A., GUNDERSEN, P., ANDERSEN, R. A. & ZACHARIASSEN, K. E. (2001). Metal accumulation and metallothionein in brown trout, *Salmo trutta*, from two Normwegian rivers differently contaminated with Cd, Cu and Zn. *Comparative Biochemistry and Physiology Part C* **128**, 189–201.

PICCINNI, E., IRATO, P., COPPELLOTTI, O. & GUIDOLIN, L. (1987). Biochemical and ultrastructural data on *Tetrahymena pyriformis* treated with copper and cadmium. *Journal of Cell Science* **88**, 283–293.

PICCINNI, E., IRATO, P. & GUIDOLIN, L. (1990). Cadmium-thionein in *Tetrahymena thermophila* and *Tetrahymena pyriformis*. *European Journal of Protistology* **26**, 176–181.

SCHLENK, D., GOLLON, J. L. & GRIFFIN, B. R. (1998). Efficacy of copper sulfate for the treatment of ichthyophthiriasis in channel catfish. *Journal of Aquatic Animal Health* **10**, 390–396.

SHANG, Y., SONG, X., BOWEN, J., CORSTANJE, R., GAO, Y., GAERTIG, J. & GOROVSKY, M. A. (2002). A robust inducible-repressible promoter greatly facilitates gene knockouts, conditional expression, and overexpression of homologous and heterologous genes *in Tetrahymena thermophila*. *Proceedings of the National Academy of Science, USA* **99**, 3734–3739.

STRAUS, D. L. (1993). Prevention of *Ichthyophthirius multifiliis* infestation in channel catfish fingerlings by copper sulfate treatment. *Journal of Aquatic Animal-Health* **5**, 152–154.

TIEMAN, D. M. & GOODWIN, A. E. (2001). Treatments for *Ichthyophthirius multifiliis* infestations in channel catfish evaluated under static and flow-through water conditions. *North American Journal of Aquaculture* **63**, 293–299.

WANG, X. & DICKERSON, H. W. (2002). Surface immobilization antigen of the parasitic ciliate *Ichthyophthirius multifiliis* elicits protective immunity in channel catfish (*Ictalurus punctatus*). *Clinical and Diagnostic Laboratory Immunology* **9**, 176–181.

WOODLING, J. D., BRINKMAN, S. F. & HORN, B. J. (2001). Nonuniform accumulation of cadmium and copper in kidneys of wild brown trout (*Salmo trutta*) populations. *Archives of Environmental Contamination and Toxicology* **40**, 381–385.

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