Variability Between Infrapopulations of Infective and Non-Infective Taenia Crassiceps WFU Cysticerci

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Variability Between Infrapopulations of Infective and Non-Infective Taenia Crassiceps WFU Cysticerci

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Abstract

Taenia crassiceps WFU strain metacestodes obtained from continuous mouse passages and dog-derived T. crassiceps metacestodes were used to produce infrapopulations. Individual mice were infected with a single intraperitoneal cysticercus and examined 4 months post-infection. Three cloned infrapopulations were obtained from the WFU strain (WS), which had lost the ability to grow into tapeworms in experimental hamsters after being propagated in mice for four years. Twelve cloned isolates were obtained from mouse cysticerci recovered after intraperitoneal inoculation of whole untreated eggs obtained from a dog tapeworm which had been fed WS cysticerci. The metacestodes from the dog-derived T. crassiceps strain (DS) recovered the ability to grow adult tapeworms in experimental hamsters. Analysis of randomly amplified polymorphic DNA (RAPD) from each infrapopulation used to construct a Neighbor-joining tree showed that the WS clones that had been propagated for long periods in mice only were closely related in amplified DNA analysis, while DS clones exhibited differences in DNA fragments between each other and the mouse-propagated WFU strain clones.

Background

Taenia crassiceps is a cestode parasite whose larvae multiply by asexual budding in an intermediate murine host. Formation of new strains due to both, interspecific genetic variations or intraspecific competition linked to lost reproductive success in the definitive host can be promoted by asexual reproduction in cestodes (9,13,15). A genetic mutation was found in the T. crassiceps ORF strain, in which the loss of two chromosomes due to aneuploidy, has promoted the appearance of cysticerci without a scolex and therefore, with no ability to become an adult tapeworms in a definitive host (5,10,12). Diminished infectivity for experimental hamsters has been observed in the morphologically normal WFU strain of T. crassiceps when maintained in laboratory mice by periodic peritoneal passages for several years. We have, however, reported a significant rise in infectivity for hamsters after the WFU strain was passed through a dog, in which the infection produced an adult tapeworm with gravid proglottids, the eggs of which were used to infect naïve BALB/c mice (17). We analyzed cloned infrapopulations by randomly amplified polymorphic DNA (RAPD) in order to test for differences in amplified fragment patterns between the cysticerci after 11 passages carried out in mice, as well as cysticerci obtained from dog-derived isolates. To our knowledge, this is the first report to show differences in RAPD analysis of cloned infrapopulations derived from aged mouse metacestodes, which have lost the ability to infect the experimental hamster, when compared to recent dog-derived metacestodes that were capable of producing viable tapeworms in hamsters.

Methods

Animals

Inbred 6 week old females BALB/c mice (Mus musculus) were donated by the animal care house from the Medical School (Facultad de Medicina, Universidad Nacional Autónoma de México) and used as hosts for asexual intraperitoneal (i.p.) proliferation of the WFU strain of T. crassiceps (17). Infected mice were kept for four months and killed by cervical dislocation. The larvae were recovered from the peritoneal cavity, washed in phosphate-buffered saline (PBS, pH 7.4) supplemented with 50 g/ml gentamicin (Genkova, Laboratorios Química SON’S, México) and used to feed to hamsters or frozen at -70°C until processed for RAPD. Outbred 6-8 month old male golden hamsters (Mesocricetus auratus) were used as definitive hosts. Animals were pre-treated with 40 mg/kg of albendazole (Vermisen, Novag, México) given orally 8 days before they were administered with 10 viable cysticerci per os and injected with 20 mg/kg of methyl-prednisolone acetate (Depomedrol, Upjohn, México) on the day of infection and every 2 weeks for the duration of the experiment. Animals were killed 6-8 week post-infection by injection of 440 mg/kg of sodium pentobarbital (Sedalphorte, México), the small intestine excised and contents examined for strobilae as previously described (17). Animals were handled under technical recommendations established by Mexican Official Regulation NOM-062-ZOO-1999.

Cloning of larvae in Balb/c mice
WFU metacestodes (WS) were obtained from a wild field mouse and maintained for 4 years by intraperitoneal (i.p.) passages in female BALB/c mice as described previously (17). In order to obtain clones derived from a single metacestode, each mouse was inoculated i.p. with a single cysticercus. Three infrapopulations were obtained (WS1, WS2 and WS3) (Illustration 1). Cysticerci recovered from the peritoneal cavity of mice four month later were used to infect hamsters and perform RAPD assays. Dog metacestodes (DS) were obtained from mice fed T. crassiceps WFU eggs or proglottids recovered from a dog tapeworm fed with 11th passage WS strain cysticerci as described by Zurabian et al. (17). To obtain cloned infrapopulations, metacestodes obtained after dog infection and subsequent mouse infections (1st mice passage) were used for implanting single cysticerci (2nd mouse passage) in the peritoneal cavity of BALB/c mice and maintained for four months (DS1-DS12). Single metacestodes harvested from mice infections were frozen at -70°C until processed. Primary and second passage DS infrapopulations were used to infect hamsters. The aneuploid larvae of a T. crassiceps ORF strain infrapopulation were used to prepare a mouse clone from a single ORF metacestode.

**RAPD assays**

**DNA template:** DNA was purified using the GenomicPrep Cells & Tissue DNA Isolation Kit specifications (Amersham Biosciences). Ten metacestodes from three WS and thirteen DS infrapopulations were used to obtain DNA templates. Concentration of DNA in the samples was measured in a spectrophotometer (Ultraspex 1000, Pharmacia Biotech). RAPD analysis: Twenty-two (Operon Technologies Inc., RAPD Kits A and B) and 6 (RAPD primers; Amersham Biosciences) primers of 10 bp and 60 to 80% GC content were screened for their ability to detect differences between amplified fragments from cloned isolates of DNA. Reaction parameters such as the concentration of MgCl2, primer, and DNA template were sequentially varied until optimal conditions were found to obtain reproducible RAPD patterns. All the primers were then tested under the following conditions: DNA 25 ng, and 3 mM MgCl2 reactions were set to a final volume of 25 l. Amplifications were performed in a Peltier effect thermal cycler (AB Prism 2400) programmed as follows: 1 cycle of 5 min at 95 C, 45 cycles of 1 min at 95 C, 1 min at 36 C, and 1 min at 72C. PCR products were run on a 2 % agarose gel and stained with ethidium bromide at a concentration of 1 pg/ml. Gels were observed using a UV-trans illuminator at 302 nm, and the banding profiles were recorded using a digital camera (Konica-Minolta Maxxum 7D, Japan).

**Scoring and statistical analysis of the banding patterns**

Five readers independently scored clearly visible and reproducible RAPD products from the digitised photographs. Only those bands that were identified by 80% of the readers were used in the analysis. The presence or absence of bands was recorded, and the scores for each primer were recorded in a data matrix using the computer program MacClade v.4.07 (8). The data matrix was analyzed with the aid of the PAUP* v.4.0b10 program (14). A distance analysis was performed using a Neighbor-joining algorithm. The search parameters included a minimum evolution model as the optimal criterion = distance, negative branch lengths were allowed, total character differences were plotted as distance measurements between clones, random addition of units, zero-length branches were collapsed when ties were encountered they were broken randomly, and topological constraints were not enforced. To evaluate node support we used a bootstrap test with 1,000 replications.

**Results**

**Cloned infrapopulations and hamster tapeworms recovered after infections with WS and DS metacestodes**

Three cloned infrapopulations WS1–WS3 were obtained after 4 month infection. Ten cysticerci from each infrapopulation were used to infect hamsters (n=22) but none developed intestinal strobilae (Table 1). Cysticerci obtained from 3 different mice after oral infection with proglottids or eggs from a dog tapeworm, did not develop intestinal worms in hamsters (n=19). Twelve out of 22 mice infected with a single larva from DS developed twelve infrapopulations labelled as DS1-DS12, each generating approximately 50 metacestodes after 4 month of i.p. budding. Nine mice had no intraperitoneal larvae, and 1 mouse had a single calcified cyst. Cysticerci from dog-derived infrapopulations DS1, DS3-DS12 were infective for hamsters (n=31) and developed 86 adult tapeworms.

**Metacestode clones analyzed by RAPD**

A total of 476 PCR reactions were used to examine differences between three WS, twelve DS infrapopulations, one dog-derived primary (DS13) and ORF cysts. The electrophoretic pattern of amplified fragments was analyzed (Illustration 2) and results shown in a Neighbor-joining tree (Illustration 3). DS13
cysticerci, recovered from a mouse orally infected with T. crassiceps eggs, were not infective for hamsters (n=16) and closely related to the WS1–WS3 (Illustration 2). Twelve dog-derived clones DS1, DS3–DS12 showed different RAPD patterns, and were obtained from cysticerci that were also infective for the hamster. An aneuploid ORF strain which lacks a scolex and therefore cannot develop into a tapeworm, was genetically close to the DS2 clone, which for unknown reasons was not infective for hamsters (n=3).

Discussion

In the present study, we have considered our previous observations on the fitness of T. crassiceps WFU metacestodes to infect experimental hamsters, experiments in which we showed that parasites reared in the mouse peritoneal cavity for 4 years lost the ability to produce adult tapeworms in experimental hamsters (17). In contrast, second passage metacestodes from mice cysticerci produced from the eggs released by a canine tapeworm, were highly infectious for experimental hamsters (17). We also found that only 50% of mice implanted with a single cysticercus in the peritoneal cavity, developed infapopulations, whether due to rejection by the immune response or loss of infective capacity, remains to be established. Every cloned infrapopulation of the DS metacestodes exhibited a unique amplified DNA bands by RAPD analysis. Mean RAPD locus diversity for individuals within DS infrapopulations ranged from 5.6% to 54.6%, with an average of 30.1% for all paired comparisons. The results suggest that each cysticercus is derived from one tapeworm oncosphere. In the natural course of events, one egg produces a single metacestode and a single tapeworm derived from genetic recombination during the meiotic cycle of oocytes and spermatids, so that oncospheres with a new genetic background are expected. Genetic differences between isolates of T. solium metacestodes have also been reported by Vega et al. (16) who suggested that differences were related to variability in parasite infectivity and pathogenicity; similarly we suggest that within the genetic variability some cysticerci might lose the ability to infect a definitive host, as also observed in the case of dog-derived cysts. A 33.81% diversity within the range expected from a recombination process due to sexual reproduction of T. solium cysticerci dissected from naturally infected pigs, was reported by Bobes et al (3). On the other hand, André and Godell (2) suggested that long periods of continuous asexual reproduction, which probably do not occur in the natural environment, possibly promote the accumulation of mutations; continuous asexual reproduction may increase the fitness of cysticerci to propagate in mice, but diminish their ability to infect a definitive host, which in the present experiment is expressed by the differences in RAPD patterns observed between the DS and WS clones. Long periods of asexual proliferation of T. crassiceps WFU strain in mice may represent an adaptation process of this parasite to the mouse environment in controlled laboratory conditions, similar to what was suggested by Andersson and Kurland (1) who proposed a pattern of genome decay with increased adaptation to the host, during which some genes are lost or shut off in certain environments. Most theoretical models on evolution of sexual and asexual reproduction, predict a high beneficial mutation rate during asexual replication that allows for better adaptation of the organisms (11), by saving energy and time when not required to carry out accurate DNA replication (2). We assume, that the inability of some metacestodes to infect hamsters suggests that T. crassiceps strains probably possess DNA plasticity, from an age-related aneuploidy due to damaged DNA (6), which originated and remained within metacestodes after long clonal reproduction in mice. The Neighbor-joining tree shows that infective and non-infective clones appear in separate groups. When we compare infectivity for hamsters with genetic differences using the RAPD analysis, there is a remarkable coincidence between dog-derived cysts and infective capability, whereas cysticerci derived from long asexual replication periods in mice are not infective for hamsters. Passage of non-infective cysticerci through a dog renews reproductive ‘vigor’ for mouse cysticerci in hamsters, possibly because a renewed cycle in a natural candid host, promotes the production of tapeworms producing eggs and eventually larvae with intact biological traits. It is of interest, that metacestodes obtained from a primary infection with dog-derived cysticerci probably require a second passage or adaptation period in the mouse peritoneal cavity to become infective for the hamster. For example, nematode eggs that require a maturation period in the soil before becoming infective for the host (4), and delayed maturation may be a parasite strategy to survive and reach a susceptible definitive host (7). The Neighbor-joining tree shows branches corresponding to the infective and non infective cysticerci for hamsters. The branch corresponding to DS13 cysticercus, obtained from a primary mouse infection, was situated within the non infective WS clade. Except for DS2, all other DS characters started at the branch next to DS2 sample, were maintained
together within tree and were infective. Although DS2 was derived from the dog infection and then cloned in mice, it was not infective for hamsters. Lack of DS2 infectivity was unexpected and might be explained by impaired infectivity genes or some other unknown reason. Genomic and proteomic data are essential for answering some fundamental questions concerning the genetic plasticity of asexually reproducing Taeniids.

Abbreviation(s)

Taenia crassiceps WFU, infectivity, RAPD

Acknowledgement(s)

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References

Illustrations

Illustration 1

Table


taenia crassiceps\n
strain WFU infrapopulations. Not infective for hamsters and maintained for 4 year period T. crassiceps cysts, were used to produce cloned populations. Three (WS1-WS3) infrapopulations were obtained after intraperitoneal inoculation of a single cyst. Dog-derived cysticercae (1st passage; DS13) were found in mice after infection with T. crassiceps eggs or proglottids, obtained after tapeworm sexual reproduction in dog. Twelve DS1-DS12 infrapopulations were grown after propagation of single cyst from 1st dog-derived passage. WS, WFU strain; DS, dog-derived strain.

<table>
<thead>
<tr>
<th>Taenia crassiceps life cycle</th>
<th>Cyst passage #</th>
<th>Infrapopulations in mice</th>
<th>Adult tapeworms in hamsters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asexual proliferation in murine host</td>
<td>11th (cyst → cyst)</td>
<td>Wild-derived (WS1–WS3)</td>
<td>Absent</td>
</tr>
<tr>
<td>Asexual proliferation after complete sexual reproduction in canid host</td>
<td>1st (egg → cyst)</td>
<td>Dog-derived (DS13)</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>2nd (cyst → cyst)</td>
<td>Dog-derived strain (DS2)</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dog-derived strain (DS1, DS3 - DS12)</td>
<td>Present</td>
</tr>
</tbody>
</table>
Illustration 2

Figure-1

Example of a 2% agarose gel with amplified PCR products using OPA-20 primer and DNA templates obtained from WFU (WS1–WS3), dog-derived (DS1-DS13) and ORF strain metacestodes of *T. crassiceps*. bp, molecular weights.
Neighbor-joining tree inferred from RAPD assays for *Taenia crassiceps* WFU (WS) and dog–derived strain (DS) metacestodes. Numbers above branches indicate branch length, whereas percentage below branch indicates bootstrap support. Infective DS clones are enclosed by bracket and represented by black branches. Grey branches correspond to non-infective WS1-WS3 and DS13 strains.
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