Contamination of Transplantable Human Tumor-Bearing Lines by Helicobacter hepaticus and Its Elimination

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Helicobacter hepaticus contaminating nonfrozen tumors was transmissible to severe combined immunodeficient (SCID) mice, but the organism in cryopreserved samples was not. This suggests that H. hepaticus has the ability to spread via biomaterials and that freezing-thawing is able to reduce the numbers of organisms to levels insufficient for subcutaneous infection of SCID mice.

Human tumor xenografts transplanted into immunodeficient mice, such as nude and severe combined immunodeficient (SCID) mice, have shown increasing usefulness in the evaluation of new antitumor drugs (9). At our institute, transplantable human tumors have been maintained in immunodeficient mice under strict microbiological control since 1973.

After Fox et al. reported in 1994 that Helicobacter hepaticus has the pathogenic potential to elicit persistent hepatitis in mice (2), we detected H. hepaticus in feces of human tumor-bearing SCID mice with hepatic lesions in our facility. In this study, transplantable human tumors—tumors implanted in SCID mice and tumors cryopreserved in liquid nitrogen (LN2)—were examined for contamination by H. hepaticus, and the transmissibility of the H. hepaticus organisms contaminating the tumors was evaluated.

Mice. Six- to eight-week-old C.B-17 SCID mice were maintained in vinyl isolators by ordinary techniques in accordance with the Animal Care Guidelines of the Central Institute for Experimental Animals (Kanagawa, Japan). The mice were consistently found to be free of ectromelia virus, lymphocytic choriomeningitis virus, mouse adenovirus, minute virus of mice, mouse encephalomyelitis virus, mouse hepatitis virus (MHV), pneumonia virus of mice, Sendai virus, clia-associated respiratory bacillus, Citrobacter rodentium, Clostridium piliforme, Corynebacterium kutscheri, dermatophyte, Mycoplasma pulmonis, Pasteurella pneumotropica, Salmonella enterica serovar Typhimurium, Giardia muris, Spironucleus muris, and Syphacia sp. infections by monitoring of sentinel mice. The sentinel mice were housed with SCID mice tested for more than 4 weeks, and the pathogens described above, or their antibodies, were monitored by serological tests, cultures, microscopic observations, and/or PCR (7).

Human tumor xenografts. Thirteen human tumor xenografts, derived from the human lung, stomach, and mammary gland and aseptically resected from SCID mice, and 157 frozen tumor samples cryopreserved from 1976 to 1997 in LN2 were examined for contamination by H. hepaticus.

Cryopreservation of the tumors. Several sections (1 by 1 by 1 mm) of human tumors resected from the mice were placed in vials containing Ham’s F-10 medium (Gibco BRL, Grand Island, N.Y.) supplemented with 10% dimethyl sulfoxide (Sigma, St. Louis, Mo.) and 10% calf serum. The vials were kept at 4°C for 2 h, stored at −80°C overnight, and then immersed in LN2.

Passage of tumors contaminated with H. hepaticus. Xenografts grown in SCID mice were aseptically resected in a safety cabinet, cut into small pieces by scissors, and subcutaneously transplanted into other mice. Cryopreserved tumors were thawed quickly in a 37°C water bath and subcutaneously transplanted into other mice. The tumor-bearing mice were accommodated in different vinyl isolators (1,900 by 1,600 by 1,600 mm). The animals were individually maintained. At 3 to 4 weeks posttransplantation, the recipient mice were sacrificed and xenografts and ceca, including feces, were used as PCR samples. Animal experiments were all performed in animal experimentation and rearing rooms in a specific-pathogen-free environment, and when procedures were performed, all materials—e.g., scissors, forceps, and needles—introduced into the animal rooms were autoclaved (9).

Detection of H. hepaticus. PCR was used to detect H. hepaticus in biomaterials. RNAs from feces and ceca, including feces, were extracted with RNAzol (Tel-Test Inc., Friendswood, Tex.) according to the instructions provided by the commercial source. cDNA synthesis and amplification of the cDNA were carried out as described previously (5). The thermal profile involved 25 cycles of denaturation at 93°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1 min. For the second (nested) PCR, 10-fold-diluted first-PCR samples. The thermal profile involved 25 cycles of denaturation at 93°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 1 min. For the second (nested) PCR, 10-fold-diluted first-PCR products were used as the template for the second PCR, along with H. hepaticus-specific second-PCR primers (5’ GAA ACT GTT ACT CTG 3’ and 5’ TCA AGC TCC CCG AAG GG 3’). The expected size of the PCR products was 405 bp. All tests were performed in duplicate.

H. hepaticus was detected in 13 of 13 implanted tumors (nonfrozen samples) and feces of all mice bearing the tumors (100%) (Table 1). Of 157 frozen samples, 10 tumors (6.4%) obtained from 1990 to 1994 were positive for H. hepaticus (Fig. 1). The 13 nonfrozen and 10 frozen samples that were positive for H. hepaticus were transplanted into specific-pathogen (H. hepaticus)-free SCID mice. At 3 to 4 weeks posttransplanta-
tion, *H. hepaticus* was detected in 10 of 13 ceca (76.9%) of the animals bearing the 13 nonfrozen samples and from 8 of 13 tumors themselves (61.5%). On the other hand, the organism was not detected in the ceca of any animals bearing the 10 frozen samples and the tumors themselves (Table 1).

We reported previously that MHV, *M. pulmonis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and lactate dehydrogenase-elevating virus are significant contaminants of xenografts (4, 6, 10, 11), and it has been reported that MHV is not only one of the microorganisms that affects the growth of xenografts in athymic mice (8) but also a lethal pathogen of the mice. In 1996, Fox et al. suggested that *H. hepaticus* may cause an increased risk of hepatic cancer induction in susceptible strains of mice (3). Based on these findings, we showed the necessity of quarantining transplantable tumors for contaminating pathogens (11). Our results showed that *H. hepaticus* is also a significant contaminant of xenografts, and this is the first report of contamination by *H. hepaticus* in human tumor xenografts.

Of 157 frozen samples, *H. hepaticus* was detected only in samples obtained from 1990 to 1994. In 1990, a new SCID mouse stock was reintroduced from outside to our facility without being checked for *H. hepaticus*, suggesting that *H. hepaticus* was brought into our colony by these mice and spread via the xenografts. *H. hepaticus* was detected in 10 of 13 ceca (76.9%) of mice at 3 to 4 weeks after transplantation of the contaminated specimens. On the other hand, the organism was not detected in any xenografts or ceca of the animals bearing *H. hepaticus*-positive samples from LN2, indicating that freezing-thawing has the ability to reduce the numbers of organisms to levels insufficient for subcutaneous infection of SCID mice. El-Zawahry and Greecz (1) reported that freezing at −18°C and −75°C for 1 h resulted in 7 and 42% *Yersinia enterocolitica* inactivation and 55 and 83% cell injury, respectively. Freezing conditions in our experiment were 4°C for 2 h, −80°C overnight, and then in LN2. Although these conditions were suitable for tumor cell viability, they caused the numbers of *H. hepaticus* in brain heart infusion broth to be reduced to 10−5 to 10−8 CFU on brain heart infusion agar plates (data not shown).

The results of this study suggest that *H. hepaticus* spreads via contaminated materials, such as transplantable tumors, and that freezing-thawing treatment of *H. hepaticus*-contaminated materials is effective for inactivation of the organism. In addition we recommend that *H. hepaticus* be added to the list of quarantine items for xenografts in mice.

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<th>Tumor</th>
<th>Detection of <em>H. hepaticus</em> by PCR</th>
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<tr>
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<td>Feces</td>
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<td>Frozen samples</td>
<td>14–170</td>
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\(^a\) Including feces.  
\(^b\) No. positive/no. of samples tested.  
\(^c\) No. positive/no. of contaminants implanted.

FIG. 1. Detection of *H. hepaticus* in cryopreserved tumor samples by PCR. □, negative; ■, positive.

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