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Opisthorchis viverrini: Influence of maternal infection in hamsters on offspring infected with homologous parasite and their IgG antibody response

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Abstract

We investigated the influence in hamsters of a maternal *Opisthorchis viverrini* infection on their offspring infected with homologous parasites and the kinetics of the *O. viverrini*-specific IgG antibody responses. No significant difference ($P > 0.05$) was found in the specific IgG antibody response and the number of *O. viverrini* eggs per gram feces (EPG) between infected offspring from infected mothers and infected offspring from uninfected mothers. A significant difference ($P < 0.05$) of EPG per worm was found between infected offspring from infected mothers and infected offspring from uninfected mothers only when the offspring were infected with *O. viverrini* after weaning at 5 weeks of age. The worm loads in infected offspring from infected mothers were significantly less than that in infected offspring from uninfected mothers. This study demonstrated that maternal infection effects worm fecundity and the worm load in an infected offspring.

Index Descriptors and Abbreviations: Maternal opisthorchiasis; Offspring infection; Hamster; Worm reduction; Worm recovery; Worm fecundity; Worm load; Antibody response; EPG, eggs per gram feces; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; p.i., postinfection

1. Introduction

Opisthorchiasis, an infection caused by the liver fluke *Opisthorchis viverrini*, is endemic along the Mekong River Basin e.g., in Laos, Cambodia, and Thailand. It is estimated that the number of current human infections is in the order of 9×10^6 cases (World Health Organization, 1995). The disease has become more common in developed countries with the influx of Asian immigrants (Schwartz, 1986; Woolf et al., 1984). In North, Northeast and central Thailand, a recent survey showed that 21.5% of the population were infected (Jongsuksuntigul and Imsomboon, 1998). Infection occurs when raw or inadequately cooked infected freshwater fish are ingested. This fluke is a parasite of the bile ducts and gall bladder. The parasites can produce morbidity including abdominal pain, dyspepsia, and fatigue. In heavily infected cases, pyogenic cholangitis, biliary calculi, obstructive jaundice, and even cholangiocarcinoma can develop (Harinasuta et al., 1984; Pungpak et al., 1994; Schwartz, 1980).

Although the parasite does not invade the tissue or come into intimate contact with the lymphoid tissue, a significant degree of both humoral and cellular immune response can be detected (Wongratanacheewin et al., 2003). It is conceivable that metabolic products or perhaps shed structural components may diffuse through the epithelial lining of the bile duct into the surrounding tissue where they can elicit an immune response (Sripa and Kaewkes, 2000a).

The humoral immune response to *O. viverrini* has been demonstrated in animals and patients with opisthorchiasis (Flavell, 1981a,b; Haswell-Elkins et al., 1991; Janechaiwat et al., 1980; Pinlaor et al., 2004; Sirisinha, 1984; Sirisinha et al., 1983a; Wongratanacheewin et al., 1988). Serum from

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O. viverrini infected hamsters contained specific IgG antibody capable of reacting with somatic, excretory–secretory, and egg antigens from homologous parasites (Sripa and Kaewkes, 2000b). Antibody responses to *O. viverrini* antigens were detected as early as 2 weeks after infection (Sirisinha et al., 1983a; Sripa and Kaewkes, 2000b). In contrast to studies on the humoral immune response, those on the cell-mediated response are limited. No significant reduction in the worm burden of hamsters receiving immune spleen cells and serum was observed compared to the control groups despite a substantial reduction in fecal egg counts (Flavell et al., 1980). Sirisinha et al. (1983b) found that prior infection of hamsters with *O. viverrini*, whether or not eliminated with praziquantel before the time of the re-challenge, failed to confer any protective immunity to re-infection. The lack of protective immunity is possibly due to *O. viverrini* suppressing both cellular and humoral immune responses to heterologous antigen and mitogen as demonstrated by reduced phytohemagglutinin-induced lymph proliferation and response to sheep red blood cell stimulation (Wongratanacheewin et al., 1987). The antibody titers in the infected animals were also depressed during the late stage of heavy infection (Sirisinha et al., 1983a). On the other hand, the potential acquired immunity in hamsters with prior infection was demonstrated by immunizing them with an aqueous somatic extract of adult worms, resulting in a 30% reduction of the worm load (Sirisinha and Wongratanacheewin, 1986). Akai et al. (1994) found significantly higher serum antibody levels of IgG, IgA, and IgM to *O. viverrini* adult worm homogenates and levels of total antibody to *O. viverrini* metacercariae homogenate in the *O. viverrini* egg-negative stool residents in an endemic area of opisthorchiasis compared to the *O. viverrini* egg-positive stool group indicating some protective evidence to opisthorchiasis. In addition, Sirisinha et al. (1986) also reported the ability of the serum from infected animals or from patients with opisthorchiasis to kill the worms. *O. viverrini* was able to activate complement via alternative pathway and the activation caused tegumental damage and resulted in parasite killing. There is less knowledge of the immunological interactions between mothers and their offspring after infection; for example, whether changes in the kinetics of the humoral immune response to egg, excretory–secretory (ES) and somatic antigens of *O. viverrini* or the exposure to *O. viverrini* antigens in utero alters the susceptibility to infection in the offspring. Therefore, the aim of this study was to examine the influence of a maternal *O. viverrini* infection on offspring infected with the same parasite and the kinetics of the parasite-specific IgG antibody responses after infection.

2. Materials and methods

2.1. Parasites

Opisthorchis viverrini metacercariae were obtained from the flesh of naturally infected cyprinoid fish from an endemic area in Khon Kaen Province, Thailand, by pepsin–HCl digestion and filtration. The larvae were washed several times with normal saline and collected using a dissecting microscope.

2.2. Preparation of parasite antigens

Adult *O. viverrini* worms were obtained from the livers and bile ducts of hamsters which had been infected 3 months previously. The fresh worms were washed several times in normal saline containing penicillin 100 U/ml, and streptomycin 100 μg/ml. After washing thoroughly, the viable worms were used to provide ES products and egg antigens. For somatic antigen, the adult worms were homogenized with a tissue grinder in a small volume of 0.1 M PBS, pH 7.4, containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 μM *N*-(*N*-[L-3 transcarboxyoxiran-2-carbonyl]-L-leucyl)–agmatine (E-64). The suspension was then sonicated with an ultrasonic disintegrator and centrifuged at 10,000*g* for 30 min at 4 °C. The supernatant was used as the source of antigen.

The ES antigens were obtained as described previously by Maleewong et al. (1999). These were prepared by in vitro culture of viable flukes in modified Tyrode's buffer containing penicillin G (100 U/ml), streptomycin (100 μg/ml), 0.1 mM PMSF, and 1 μM E-64 at 37 °C under 5% CO_2 for up to 7 days. The culture fluid was changed every 24 h, pooled and centrifuged to remove the eggs. The supernatant and pellets were stored at −80 °C for preparing ES and egg antigens, respectively. For ES antigen, the medium was concentrated by ultrafiltration using an Amicon YM 3 membrane filter (Grace, Danvers, MA, USA), dialyzed against distilled water containing the same proteinase inhibitors as above, aliquoted and stored at −80 °C until being used.

For egg antigens, eggs were washed several times in normal saline to remove any residual ES products. After centrifugation, the egg pellets were homogenized with alumina (Sigma St. Louis, MA) in 0.1 M PBS, pH 7.4, containing the same proteinase inhibitors. The ground suspension was prepared as above and the supernatant was aliquoted and stored at −80 °C before being used. The protein concentrations of all *O. viverrini* antigens were determined by the standard method (Lowry et al., 1951).

2.3. Animals and experimental design

Syrian Golden hamsters (*Mesocricetus auratus*) were used as the experimental hosts of *O. viverrini*. These hamsters were maintained under standard laboratory conditions in an animal section at the Faculty of Medicine, Khon Kaen University. Hamsters were housed in groups of five in plastic box cages and provided with rodent chow and water ad libitum. The maintenance and care of all animal experiments complied with the ethic regulations set by the Animal Ethics Committee of Khon Kaen University, Thailand. All experimental offspring were weaned at 3 weeks of age.