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Fluke worms, liver fluke *(Opisthorchis viverrini)*: a review and updated information

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For parasitic worms, the second most common in human beings is fluke (The most common is nematode). Fluke's skin; tegument, is composed of nucleated cells and has the permeability function. Most flukes or treamatodes are hermaphrodites with the exception of schistosome. There are two hosts in flukes' life cycle: one is usually a mollusk in which asexual reproduction occurs, the other is a vertebrate in which generations of flukes are produced through sexual reproduction. Flukes' infective stage is called metacercaria. Usually, during this stage the worm is encysted in or on an intermediate host; some, however, encyst on aquatic objects. In a definitive host, excystation begins with host's digestive enzyme in the host intestine and it starts the migration of juvenile flukes, given that the final habitat is some other hosts' organs.

Liver fluke, Opisthorchiasis viverrini, causes major clinical and public heath problems in Thailand. About 79 % of the people living in endemic areas are infected. In human body, the fluke (7.4 mm x 1.5 mm) usually inhabits the distal bile duct, gall-bladder and sometimes the pancreatic duct. In side the host's GI tract, the fluke releases embryonated eggs (36.7 μ x 15.0 μ) which are finally excreted with feces. The most serious complication of the disease is biliary obstruction with or without cholangiocarcinoma. The host's immune response may occur very early during the infection. Histological studies show that after 3 -15 days, there is acute and sub-acute inflammatory reaction. Antibodies in the serum of patients and experimentally infected animals react with every developmental stage of the parasite. The predominating

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immunoglobulin classes are IgG and, to a lesser extent, IgE, and on some rare occasions trace quantities of IgA and IgM are detected. In bile, it is secretory IgA and rarely other classes that are detected. However, overwhelming evidence leads to a conclusion that the produced antibody has very little protection. Immunodepression is also noted during O. viverrini infection in experimentally infected animals. This phenomenon might be associated with poor activity of immune surveillance system against neoplastic transformation. Vaccines may neither be essential nor practical for the control of the infection, because the infection confers little immunity to re-infection. Moreover, in the process of induction of immunodepression, vaccine may be needed to overcome such depression.

Keywords: *Opisthorchis viverrini, Fluke, Liver fluke, Updated, Review.*

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Objective : To review the general biology of the fluke worms and various presentations including life cycle, clinical manifestations, immunological studies, host immune responses and vaccine of human liver fluke : *Opisthorchis viverrini*

วิไล ศักดิ์ศิริสัมพันธ์. พยาธิใบไม้และพยาธิใบไม้ในตับ *(Opisthorchis Viverrini)*: บทความทบทวนและข้อมูลที่ทันสมัย. จุฬาลงกรณ์เวชสาร 2546 มี.ค; 47(3): 175 – 89

พยาธิใบไม้มีจำนวนมากนับเป็นอันดับสองรองจากปรสิตตัวกลม ผิวหนังของพวกนี้ เรียกว่า tegument ซึ่งเป็นเซลล์พวกที่มีนิวเคลียสและมีหน้าที่ควบคุมการผ่านเข้าออกของสารต่าง ๆ พยาธิใบไม้ส่วนมากเป็นกระเทย ยกเว้นพยาธิใบไม้ในเลือด ในวงจรชีวิตจะมีโฮสท์ 2 พวก พวกแรกเป็นหอย ซึ่งระยะต่าง ๆ จะเพิ่มจำนวนแบบไม่อาศัยเพศ และอีกพวกจะเป็นสัตว์มีกระดูกสันหลัง ซึ่งระยะต่าง ๆ เพิ่มจำนวนแบบอาศัยเพศ ระยะ metacercaria คือระยะติดต่อ มักเป็นถุงซีสภายในหรืออยู่บนโฮสท์ตัวกลาง บางชนิดจะเกาะที่ผิวของวัสดุต่าง ๆ ในน้ำ จากน้ำย่อยในระบบทางเดินอาหารระยะติดต่อนี้จะถูกย่อยออกจากถุงซีส และบางชนิดจะเดินทางสู่อวัยวะอื่น ๆ ต่อไป

โรคพยาธิใบไม้ในตับของคน (Opisthorchis viverrini) ยังเป็นปัญหาสาธารณสุขในประเทศไทย ประมาณ 79 % ของประชากรในแหล่งระบาดที่ติดโรคนี้ พยาธิตัวแก่ขนาด 7.4 มม. X 1.5 มม. มักอาศัยในท่อทางเดินน้ำดี ถุงน้ำดีของตับหรือตับอ่อนได้ ไข่พยาธิมีขนาด 26.7 μ x 15.0 μ จะผ่านมาตามลำไส้และขับออกพร้อมอุจจาระ ความรุนแรงของโรคคือการเกิดท่อทางเดินน้ำดีอุดตันโดยอาจมีมะเร็งของท่อทางเดินน้ำดีร่วมด้วยหรือไม่ก็ตาม การตอบสนองทางภูมิคุ้มกันของร่างกายเกิดขึ้นเร็วมาก สภาพเนื้อเยื่อพบปฏิกริยาการอักเสบหลังติดเชื้อเพียง 5 -15 วัน แอนติบอดี้ในซีรั่มของคนไข้และสัตว์ทดลองทำปฏิกริยาจำเพาะกับเชื้อพยาธิทุกระยะได้ IgG เป็นชนิดที่พบผลิตมาก และที่น้อยกว่าคือ IgE บางครั้งตรวจพบ IgA และ IgM ได้ในปริมาณต่ำ ๆ ส่วนในน้ำดีพบปริมาณ IgA มาก ซึ่งชนิดอื่น ๆ มีโอกาสพบในน้ำดีน้อยมาก อย่างไรก็ดี หลักฐานต่าง ๆ แสดงไว้ว่าภูมิคุ้มกันของร่างกายไม่มีอำนาจป้องกันโรค นอกจากนี้ยังพบว่าเกิดภาวะกดระบบภูมิคุ้มกันขึ้นระหว่างการติดเชื้อ ภาวะนี้อาจมีผลเกี่ยวข้องก่อให้เกิดความอ่อนแอของระบบภูมิคุ้มกันที่ต้องต่อต้าน-กำจัดการเกิดเซลล์มะเร็ง ส่วนวัคซีนในทางปฏิบัติอาจไม่จำเป็นหรือไม่เหมาะสม เพราะภูมิคุ้มกันที่เกิดขึ้นจากการติดเชื้อไม่สามารถต่อต้านการติดเชื้อซ้ำ และวัคซีนจะต้องแก้ปัญหาหรือเอาชนะภาวะการกดภูมิคุ้มกันของร่างกายได้ด้วย

There are four classes in the phylum of flatworms (Platyhelminthes) which are namely: Trematoda, Cestoidea, Turellaria and Nemertea. The trematodes or flukes are the second most common parasitic worms in humans (the first is nematode). Some trematodes cause economic loss to society through infections of domestic animals, while others are prominent parasites of humans. This paper summarizes the biology of the group of flatworms, i.e. illustrating their morphology and life cycle, clinical manifestation and pathology, with immunological studies on *Opisthorchis viverrini* which is the most common species in Thailand.

Biology of Trematode (Fluke)

1. General morphology

1.1 Body Form

Most flukes are dorso-ventrally flat and oval in shape; some are however, as thick as they are wide. Usually they have a powerful oral sucker that surrounds the mouth; most of them also have a midventral acetabulum or ventral sucker. If a worm has only one oral sucker, it is called monostome. If it has an oral sucker and an acetabulum at the posterior end of the body, it is called amphistome. If the acetabulum is elsewhere on the ventral surface, the worm is a distome.

1.2 Tegument

The body of a trematode is covered with nucleated cells with living complex tissue. The cells containing the nuclei (cytons) lie beneath a superficial layer of muscles connected to the distal cytoplasm by way of channels **(internuncial processes)**. Because the distal cytoplasm is continuous, without any intervening cell membrane, the tegument is **syncytial. Spines** often appear on certain areas of trematode's body. The tegumental surface of many flukes bears ridges of various configurations: pits and sensory papillae. The spines consist of crystalline actin: their bases lie above the basement membrane of distal cytoplasm and their apices project above the surface.

The tegument of liver fluke is variously interrupted by cytoplasmic projections of gland cells, openings of excretory pores and nerve endings.

1.3 Muscular system

Their muscles are superficial circular, longitudinal and diagonal layers enveloping the rest of the body like a sheath below the distal cytoplasm of the tegument. Often the muscles are most prominent in the anterior part of the body. In the lateral areas there are strands connecting the dorsal to the ventral side of the superficial muscles.

1.4 Nervous system

The nervous system of platyhelminthes is orthogon with longitudinal nerve cords connected at intervals by transverse ring commissures. Several nerves issue interiorly from the cerebral ganglion, and three main pairs of dorsal, lateral and ventral supply the posterior parts of the body. The anterior end, especially the oral sucker, is well supplied with sensory endings.

1.5 Excretion and Osmo-regulation

Excretion is the process for:

1) removal of waste metabolic products,

2) regulation of internal osmotic pressure,

3) regulation of internal ionic composition,

4) removal of unnecessary or harmful substances.

A unit of excretory system closes at the

proximal end and opens to the exterior at the distal end through a pore. These unit is based on a flame bulb (protonephridium). It is flask-shaped and contains a tuft of fused flagella to provide a motive force for the fluid in the system. Ductules of the flame cells join collecting ducts on each side and eventually feed into an excretory bladder in the adult that opens to the outside with a single pore.

1.6 Reproductive systems

Most trematodes are hermaphrodites with the exception of schistosomes. Some are capable of self-fertilization. Some require cross-fertilization to produce viable progeny. Some inseminate themselves when there is only one worm present in the host, but they always seem to cross-inseminate when there are two or more. There are a few known instances when adult trematodes reproduce parthenogenetically.

Male reproductive system: The number of testes of a fluke worm vary from one species to the other, numbering from one testis to several dozens. The shape of testis also varies from round to highly branched. Each testis has a vasefferen that connects with one another forming a vas deferens. The duct then goes toward genital pore. The genital atrium is most often on the midventral surface, anterior to the acetabulum, depending on the species. Generally, it can be found nearly anywhere, including the posterior end, beside the mouth, or even dorsal to the mouth. Usually, before reaching the genital pore the vas deferens enters a muscular cirrus pouch where it may expand into an **internal seminal vesicle** for sperm storage. The cirrus is male copulatory organ. The ejaculatory duct is usually surrounded by numerous unicellular **prostate gland cells**.

Female reproductive system: The female reproductive tract of a fluke worm has only one ovary, usually round or oval in shape. The short oviduct is provided with a proximal sphincter, the **ovicapt** that controls the passage of ova. Seminal receptacle forms an out-pocketing of the wall of the oviduct. At the base of the seminal receptacle is a slender tube (Laurer's canal) which blindly ends in the parenchyma or opens through the tegument. Laurer's canal is probably a vestigial vagina that is no longer functional.

The vitelline cells are produced in follicular vitelline glands, and connected by ductules to the main right and left vitelline ducts. After joining the common vitelline duct, the oviduct expands to form ootype. Numerous unicellular Mehlis' gland surrounds ootype.

When an oocyte leaves the ovary and comes down the oviduct, several vitelline cells and perm emerge from seminal receptacle. For a long time it was believed that Mehlis' gland contributed shell material; the organ was called shell gland in older texts. However, we now know that the bulk of shell material is contributed by vitelline cells, and the function of the Mehlis' gland remains obscure. (Figure 1, 2)

2. Development and Life cycle

There are two hosts in the life cycle of a flat worm: one is a vertebrate in which sexual reproduction occurs; and the other is usually a mollusk in which one or more generations are produced by asexual reproduction.

2.1 Egg (shelled embryo)

The egg shell or egg capsule of most flukes has an operculum at one end. There are variations in the shape, size, thickness, and color of the egg shell.

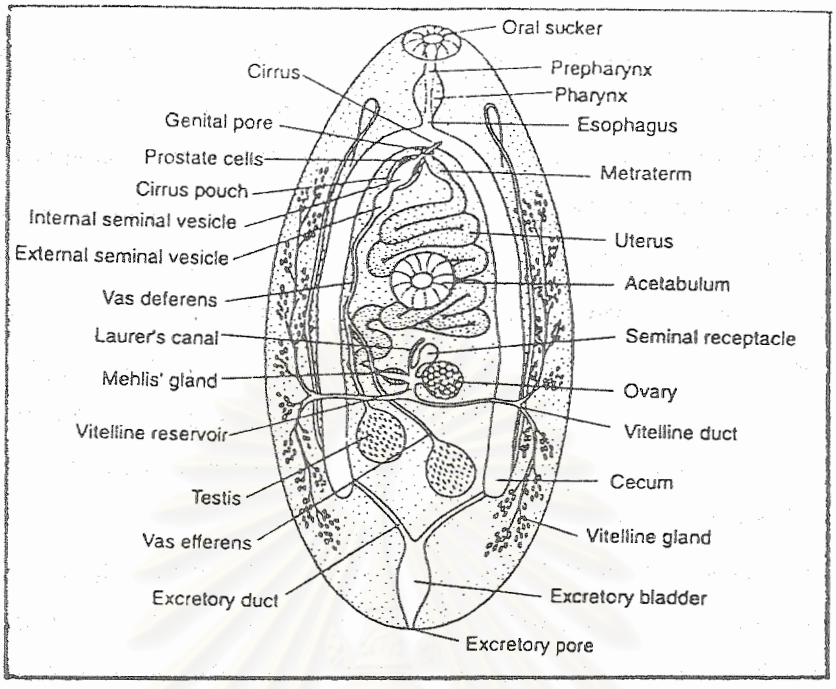

Figure 1. Diagram of male and female reproductive system.

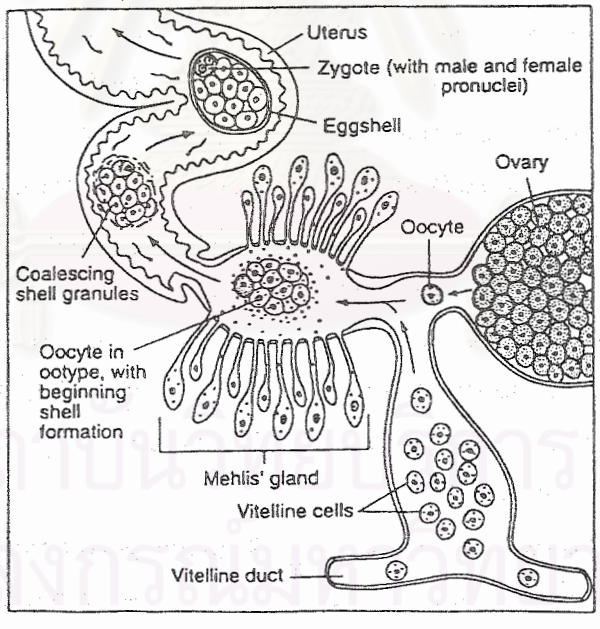

Figure 1.1 Diagram of the oogenotop of a digenetic fluke.

In many species, the egg contains a fully developed miracidium when it leaves the parent. In some,there are only a few cell divisions by the time. Many factors influence the rate of development of the embryo.

In many species, eggs hatch freely in water, whereas in some others they hatch only when they are eaten by a suitable intermediate host. Light and osmotic pressure are important stimuli for hatching. In addition, miracidium releases some factor that alters the permeability of the membrane enclosing the viscous cushion. The viscous cushion is a thin vitelline membrane that stays between the anterior

end of miracidium and the operculum. The increase in pressure within the egg shell causes the operculum to pop open and the miracidium rapidly escapes, propelled by its cilia.

2.2 Miracidium

Miracidia have a variety of sensory organs and endings, including adaptations for photoreception, chemoreception, tangoreception, and statoreception.

The outer surface of a miracidium is covered by flat, ciliated epidermal cells. Underlying the surface are longitudinal and circular muscle fibers. In the posterior half of a miracidium are found propagatory cells or germ balls (embryos), which will be carried into sporocyst stage.

Free-swimming miracidia are very active; they swim at the rate of about 2 mm per second. They have to find a suitable mollusk, since they can survive as free-living organisms for only a few hours.

2.3 Sporocyst

Miracidia undergo metamorphosis into sporocysts near the site of penetration. A sporocyst has no mouth or digestive system; it absorbs nutrients from the host tissue. A sporocyst may be referred to as **germinal sac**. Sometimes sporocysts may become very slender and extended or branched or highly ramified. Embryos in a sporocyst may develop into another sporocyst generation, daughter sporocysts, into a different form of germinal sac (redia), or directly into cercariae.

2.4 Redia

Rediae usually migrate to hepatopancreas or gonad of molluscan host. More active than most sporocysts; they crawl about within their host. They have a rudimentary but functional digestive system, consisting of a mouth, muscular pharynx, and short, unbranched gut. Rediae pump food into their gut by means of pharyngeal muscles. The outer surface of the tegument also functions in the absorption of food, and it is provided with microvilli or lamelloid processes.

Embryos in rediae develop into daughter rediae or into the next stage, cercariae, which emerge through a birth pore near the pharynx. Young rediae can be transplanted from one snail to another through more than 40 generations without cercariae being developed.

2.5 Cercaria

Cercariae represent a juvenile stage of the adult. Most of them have tails that aid them in swimming, but many only have rudimentary tails or no tail at all. Most cercariae have a mouth near the anterior end. The mouth is usually surrounded by an oral sucker and a prepharyns, muscular pharynx. Many cercariae have various gland openings near the anterior margin, often called penetration glands.

2.6 Metacercaria

A metacercaria is usually encysted. Most metacercariae are found in or on an intermediate host, but some encyst on aquatic vegetation, sticks, and rocks or even move freely in water. Before encysting, its tail must be shed off. The encysting metacercariae in intermediate hosts have thinner and simpler cyst walls, with some components contributed by the host. Metacercariae can be classified into three groups, namely:

1. Metacercariae that encyst in the open on vegetation and inanimate objects and that can infect the definitive host immediately after encystment, such as *Fasciola spp.*

2. Metacercariae that do not grow in the intermediate host but require at least several days of

physiological development to infect the definitive host, such as *Echinostomatidae*.

3. Metacercariae that undergo growth and metamorphosis before they enter their resting stage in the second intermediate host and require a period of weeks for this development, such as *Diplostomidae*.

Development of metacercariae in the definitive host begins with excystation. Digestive enzymes largely remove the outer cyst of *F. hepatica*, but when they escape the inner cyst they require the presence of a temperature of about 39°C, a low oxidation-reduction potential, carbon dioxide, and bile. After excystation in the intestine, extensive migration is necessary if the final habitat is in some other organs.

Human Liver fluke : *Opisthorchis viverrini*

Phylum	Platyhelminthes
Class	Trematoda
Superfamily	Opisthorchiodea (Faust, 1929)
Genus	Opisthorchis
	Opisthorchis viverrini

Common name: cat liver fluke

Infection	Opisthorchiasis
Synonym	*Distoma viverrini*

Human liver fluke infection is one of the major clinical and public health problems in many parts of the world. In the East and Southeast Asian countries, the infection is caused by *Opisthorchis viverrini* and *Clonorchis sinensis*. However, in Southern, Central and Eastern Europe and Russia it is *Opisthorchis felineus*. In Thailand, the infection was first reported in 1911 in the province of Chiang Mai by Leiper and the causative agent was later identified by Kerr as *Opisthorchis viverrini*.

Extensive studies on incidence, geographic distribution and biology of opisthorchiasis in Thailand began in 1951 by Sadun who reported that the infection was widespread in the Northeastern provinces and it involved only *O. viverrini*. Subsequently, several other groups of investigators have confirmed the finding. Also, it has been reported that now about 79 % of the people living in the endemic areas are infected; as many as ten million Thais harbor this parasite. The incidence in both sexes is similar and people of all ages are infected. Chronic infection with *O. viverrini* is highly correlated with incidence of bile duct cancer, much more than infection with *C. sinensis*. Although the causative mechanism remain unclear, there is strong evidence that infection with *O. viverrini* is responsible for cancer of the liver.

1. Morphology and life cycle

O. viverrini is a flat, elongated, leaf-like, reddish-yellow colored parasite with an average size of 7.4 mm in length and 1.5 mm in width. Its oral sucker is sub-terminally located whereas the ventral sucker is situated anteriorly about one-fifth of the body length. A small bulbous pharynx leads into a short esophagus which bifurcates to form two ceca extending all the way to the posterior end of the body. The two testes are divided into four lobes by deep fissures, situated in the posterior quadrant. A long seminal vesical pores immediately in front of the ventral sucker. The ovary, situated anteriorly to the excretory bladder, is divided into 3-4 lobes. The uterus is a markedly coiled tubule and terminates in the genital atrium close to the male genital opening.

Man as well as other fish-eating mammals such as cats and dogs can serve as definite hosts

for the parasite. The flukes usually inhabit the distal bile ducts, gall-bladder and sometimes in the pancreatic duct. The operculated oval-shaped eggs, which are yellowish brown in color, have an average size of 26.7 μ by 15.0 μ. Their embryonated eggs pass through the bile ducts of infected men and animals, entering the intestine and they are subsequently excreted with the feces. The eggs, containing ciliated miracidia, do not normally hatch in water. After being ingested by snails. Hence, miracidia develop within 1 month into sporocystes, redia an cercaria. The cercaria leave the snails (their first intermediate host), in Thailand they are *Bithynia goniomphalus*, *B. laevis* and *B. funiculate*. Then they enter the second intermediate host. The latter include species of cyprinoid fishes, e.g., *Cycloheilichys apagon*, *Puntius leiacanthus*, *Hampala dispar* and *Puntius partipentazona*. After attaching themselves to the scales of fish, the cercaria lose their tails and penetrate into the underlying tissue. Then they round up and develop further into metacercaria within 1 month. The metacercaria are oval double-walled cysts with an average size of 201 x 167 μ. They are found primarily both in the body and the fin muscles of infected fish. After being eaten by a suitable mammalian host, the metacercaria excyst in the duodenum. Then the juvenile flukes migrate through the Ampulla of Vater into the common bile duct, cystic duct, gall bladder, intrahepatic duct and finally reach the distal bile duct. Here the juvenile flukes attached themselves to biliary epithelium; it has been estimated that it takes about 3-4 weeks for these juvenile flukes to differentiate and mature into sexually mature adults. The life span of this parasite, as estimated from infection in humans, could be as long as 15-20 years.

2. Clinical manifestations and pathology

The clinical signs of the liver fluke infection depend on the number of the parasites in the biliary system and the duration of the infection. If the disease manifests by jaundice, pain in the right upper quadrant and frequent causes gastrointestinal disturbances. Some patients may also have enlarged liver, particularly those who have massive infestation or malignant transformation. In most cases, hepatic failure is common and immediate cause of death. Examinations of infected men and experimentally infected animals (e.g. hamsters) show that the bile ducts are usually hypertrophic and dilated. In chronic cases with massive infection, mechanical obstruction, bile stasis and cyst formation may result. In such cases, eggs released from adult flukes may not be found in the feces of the patients. Proliferation of biliary epithelium is common and the walls of the bile ducts become thickened with fibrous connective tissue. Inflammation with cellular infiltration composing chiefly of lymphocystes, monouclear cells, macrophages, polymorphonuclear leucocytes and few eosinophils has been reported. Superlative cholangitis is not infrequent and at times the infection may extend into the parenchyma of the liver tissue, causing hepatitis with formation of micro-and macroabscess. Hosts with a low protein diet may develop a significantly greater degree of bile duct proliferation than age and sex matched animals fed a normal protein diet. Circumstantial current avaible evidences suggest that opisthorchiasis may contribute to the development of liver carcinoma, especially cholgniocarcinoma (intrahepatic duct carcinoma). The unusually high incidence of cholangiocaricinoma in patients with opisthorchiasis, is based on the analyses of

necropsy and biopsy cases. This is more than a coincidence. More direct evidence on the association of infection by Opisthorchis and cholangiocarcinoma was presented. It was shown that the epithelial cells of bile duct was stimulated to proliferate possibly by mechanical trauma or by metabolites released from the parasites. It is known that proliferating cells are more susceptible to malignant transformation by chemical carcinogens than their non-dividing counterparts. Therefore, this altered proliferating activity of epithelial cells of infected animals would be more sensitive to the action of a carcinogen than those of uninfected animals. In addition, it appears that the presence of liver flukes could shorten the latent period for neoplastic transformation by a carcinogen as well as by reducing the doses of the carcinogen required for it induction.

Diagnosis is based on the recovery of the characteristic egg in the feces. Praziquantel is reported to have almost 100 per cent efficacy when given a single dose of 40 mg/kg to a large group of mildly to moderately infected Thais in studies over a 2 - year period.

3. Immunological studies

In Thailand, during the last 15-20 years, a group of scientists has been interested in the immunology and biotechnology of *O. viverrini* and opisthorchiasis. At the beginning, they tried to get basic information on parasite antigens and host responses to infection using a hamster model. Antigens prepared were used to study the relationship between host and parasite, the protective immunity, production of monoclonal antibodies, construction of DNA proves and the use of all those probes for the diagnosis of opisthorchiasis.

3.1 Antigenicity of *O. viverrini*

Parasite antigens are generally divided into two main groups, namely: somatic and excretory-secretory (ES) antigens. For most parasites, both types of antigens are present in all developmental stages including eggs, cercaria, metacercaria, juvenile flukes and adult flukes. In general, excretory-secretory products can be prepared by *in vitro* cultivation of living parasites in protein-free medium whereas the preparation of somatic antigen can be obtained by homogenizing the whole parasite. Each stage of parasite development is antigenically complex. Moreover, the antigen(s) that could effectively induce antibody formation may not be the same as the functioning antigen(s) that induce protective immunity.

Detailed analysis of adult somatic extract shows the presence of a high antigenic mosaic with their M_r ranging from 16 to more than 116 kDa. The predominant components are those with M_r of 16-17 kDa. These components are associated with the parasite tegument. Only the protein of 16-kDa strongly reacts with opisthorchiasis serum. The ES products shows the predominant component at 89-kDa. The ES component not only strongly reacts with all sera from patients with opisthorchiasis but also with a number of sera from patents with clonorchiasis. Monoclonal antibodies (mAb) to the somatic extract of 16-kDa and to the ES product of 89-kDa show intense immunofluorescent staining of the surface tegument. The other mAb that specific to the other somatic component of a M_r of 90 kDa (can strongly react with the parenchymal tissue that underneath the surface tegument especially with those of oral and ventral suckers, pharynx and cecum.

3.2 Host immune responses to *O. viverrini*

O. viverrini residing in the lumen of bile duct may release antigens in the form of soluble components or the excretory-secretory products and egg antigens which gain access into the underlying tissues. There is evidence showing that these antigens could stimulate both humoral and cell-mediated immune responses. Currently available evidence suggests that the immune response could occur very early during the course of infection. Serum from infected hamsters gives positive test in immunoelectrophoresis 30 days after infection.

Patients infected with *Opisthorchis* have antibody responses that can be detected by a number of immunological techniques including ELISA, immunoelectrophoresis, passive agglutination, and immunofluorescence. Antibodies can react with all developmental stages of the fluke. The predominant isotype of serum antibodies is IgG. However, IgE, IgA and IgM antibodies have also been detected but only at trace quantities. On the other hand, a predominant antibodies in the bile of infected individual are secretory IgA.

The histopathological studies of experimental infected animals showed that after 3 -15 days of infection, there was acute and sub-acute inflammatory reaction in the portal area where the flukes were located. The inflammatory cells were predominantly eosinophils, neutrophils, mononuclear cells and foamy macrophages. From one month onward, a non-specific macrophage granuloma could also be noted around the eggs. By 6 weeks, there were epithelioid granulomas with multinucleated giant cells surrounded by small lymphocytes and young plasma cells. Such a reaction was probably associated with the host immune reactivity against adult flukes and their eggs. Between 6 weeks and 4 months, the reactions are infiltrated with lymphoblasts and plasma cells. These pathological findings are consistent with the histological patterns of cell-mediated immune response of the host to invading parasites.

Although the evidence presented above indicate that superficial luminal infection by *O. viverrini* can stimulate both systemic humoral and cell-mediated immune responses. However, the protection of these antibodies and cells are still questionable. *In vitro* studies, these liver flukes are killed in the presence of fresh serum from infected humans or experimentally infected hamsters.

In addition, there is also a report that Opisthorchis could activate complement via an alternative pathway, resulting in tegument damage and parasite killing. Different lines of evidence also suggest that following a primary infection there is little, if any, protection against re-infection by the same parasite. Prior infection failed to confer immunity to re-infection, judging from the number of worms recovered from the challenged doses. In fact, the number found was at times higher than that expected from the combination from the dose used in prior infection and that used in re-infection. Passive transfer of either serum or lymphoid cells or both from infected donor animals fails to confer protection in recipients against a subsequent challenge. Similar results are obtained through the studies on *C. sinensis*. The worm recovered in animals immunized with this human liver fluke antigen was not reduced but seemed to be slightly higher than that of the un-immunized group.

Not only that there was no clear evidence on development of acquired immunity in the liver fluke

infection, but there was also limited currently available information that suggests that such infection may interfere with the immune activity of the host. It is possible that the increased susceptibility of infected hamsters to tumor induction by dimethynitrosamine might be associated with poor activity of the host immune surveillance system against neoplastic transformation or with the modulation of immuno-suppression. However, the higher incidence of cholangiocacinoma in people living in the endemic areas may not be related only to the above observations. It is probably the cumulative and result of several aetiological agents which may include nutritional and environmental factors.

Regarding immunodiagnosis, detection of serum antibodies gave unsatisfactory results. Subsequently, a highly sensitive and specific indirect ELISA was developed to quantify antibody of the 89 kDa. However, it could not distinguish the past from the present infection due to the persisting antibody. A reliable antigen (89 kDa) detection in the feces has been developed by using monoclonal antibody. In parallel with the works on antibody probe, DNA probed was also developed for the detection of parasitic DNA.

Conclusion

Many factors may contribute to the fluke's survival within the biliary system. Although, both IgG antibodies and complement activation are reported to kill the fluke, but these only present in only trace quantities in bile. Moreover, suppressive serum factor that interferes with macrophage function has been reported in patients with opisthorchiasis. One other liver fluke (*Fasciola hepatica*) is also known to secrete lymphotoxic substance (s). It is possible, that these factors may be responsible for immunodepression found during *O. viverrini* infection in experimentally infected animals. Following a course of praziquantel treatment, the immune responses to both homologous and heterologous antigens reappear. In addition, these parasites can readily shed their surface tegument following a damage by immune mechanisms, thus provides them with another strategy to evade host's defenses.

Vaccines may not be essential nor practical for liver fluke infection. Although several attempts have been made to induce immunity in experimental animals, using a number of parasite preparations, but the results have been largely unsatisfactory. The infection itself is known to confer little immunity to re-infection. Moreover, if the infection induces immunodepression in humans, as it occurs in experimental animals, the vaccine will be needed to overcome such depression.

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กิจกรรมการศึกษาต่อเนื่องสำหรับแพทย์

ท่านสามารถได้รับการรับรองอย่างเป็นทางการสำหรับกิจกรรมการศึกษาต่อเนื่องสำหรับแพทย์ กลุ่มที่ 3 ประเภทที่ 23 (ศึกษาด้วยตนเอง) โดยศูนย์การศึกษาต่อเนื่องของแพทย์ จุฬาลงกรณ์มหาวิทยาลัย ตามเกณฑ์ของศูนย์การศึกษาต่อเนื่องของแพทย์แห่งแพทยสภา (ศนพ.) จากการอ่านบทความเรื่อง "Fluke worms, liver fluke *(Opisthorchis viverrine)*: a review and updated information." โดยตอบคำถามข้างล่างนี้ พร้อมกับส่งคำตอบที่ท่านคิดว่าถูกต้องโดยใช้แบบฟอร์มคำตอบท้ายคำถาม แล้ว ใส่ซองพร้อมซองเปล่า (ไม่ต้องติดแสตมป์) จ่าหน้าซองถึงตัวท่าน ส่งถึง (สามารถตรวจจำนวนเครดิตได้จาก www.ccme.or.th)

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คำถาม - คำตอบ

1. Parasite antigens are generally divided into two main groups, namely....
   - A Adult and infective stage antigens.
   - B. Somatic and excretory-secretory antigens.
   - C. Tegument and organ antigens.
   - D. Specific and non-specific antigens.
   - E. Cercaria and juvenile fluke antigens.
2. The basic factor that may contribute to the *O. viverrini* fluke's survival within the host...
   - A The fluke could activate complement via an attemate pathway, not classical pathway.
   - B. Passive transfer of either serum or lymphoid cells or both from infected donor animals fails to confer protection in recipients.
   - C. Reside in the bile-duct.
   - D. Induce immunodepression.
   - E. All of the above.

คำตอบ สำหรับบทความเรื่อง "Fluke worms, liver fluke *(Opisthorchis viverrine)*: a review and updated information."

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ชื่อ - นามสกุลผู้ขอ CME credit ..... เลขที่ใบประกอบวิชาชีพเวชกรรม.....
ที่อยู่.....

1. (A) (B) (C) (D) (E)
2. (A) (B) (C) (D) (E)
3. (A) (B) (C) (D) (E)
4. (A) (B) (C) (D) (E)
5. (A) (B) (C) (D) (E)

3. Evidences for vaccine failure of experimental opisthorchiasis are
   A. The ES component show no reaction with all sera from patients.
   B. The fluke could not stimulate cell-mediate immune responses.
   C. The inflammatory cells in histopathological studies were predominantly with neutrophil.
   D. The infection itself is known to confer little immunity to reinfection.
   E. All of the above.
4. Which is correct about opisthorchiasis. ?
   A. Prior infection of *O. viverrini* can confer immunity to reinfection.
   B. The increased susceptibility of infected host to cholangiocarcinoma might be associated with the poor activity of the host immune surveillance system against neoplastic transformation or with the modulation of immunodepression.
   C. The immune response could occur very late during the course of infection.
   D. The histopathological studies of experimental infected animals showed that after 1 month of infection, there was acute and subacute inflammatory reaction in the portal area where the flukes were located.
   E. Both B and D
5. The predominant antibodies in the bile of opisthorchiasis individual is.....
   A. IgG isotypes.
   B. IgE istotype
   C. secretory IgA
   D. IgM isotype
   E. Both A and C

ท่านที่ประสงค์จะได้รับเครดิตการศึกษาต่อเนื่อง (CME credit)
กรุณาส่งคำตอบพร้อมรายละเอียดของท่านตามแบบฟอร์มด้านหน้า

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