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# REVIEW ARTICLE

## LIVER FLUKES AND CANCER IN SOUTH-EAST ASIA*

STITAYA SIRISINHA

*Department of Microbiology, Faculty of Science, Mahidol University and Laboratory of Immunology, Chulabhorn Research Institute, Bangkok, Thailand.*

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Different lines of evidence currently available suggest that microorganisms and parasites may play a role in the development of a diverse group of human cancers. The involvement and the mechanisms of oncogenic viruses in the development of cancers in man and animals are well documented. Many of these cancers including nasopharyngeal, cervical and hepatocellular carcinomas and cholangiocarcinomas are highly prevalent in Southeast Asian countries. The parasites known to play an etiological role in tumor development in man include liver flukes (*Opisthorchis viverrini, O. felineus* and *Clonorchis sinensis*) and blood flukes (*Schistosoma haematobium*).[1-4] *S. mansoni, S. japonicum*, whipworms and hookworms have also been suggested to be associated with colon and rectal carcinomas and even lymphomas but causal evidence for this is lacking.

The incidence of liver cancer in Southeast Asian countries is among the highest in the world, with a total of more than 30,000 new cases per year.[5-7] In the endemic northeast Thailand, a crude annual incidence of CCA for both sexes is 54/100,000 with an age-standarized incidence of 87/100,000, comparing with 2/100,000 in Western countries.[6] It has been estimated that in Thailand and Loas alone, 8 million people are at risk of developing CCA.[6] On the average, the age of the patients is 50-60 years with a male to female ratio of 4:1. In some parts of Thailand, the incidence among the male population is estimated to be over 135/100,000, with an age-standardized incidence of as high as 334/100,000 (comparing with only 104/100,000 for the female). Of the two major kinds of primary liver cancer, that of the biliary system, i.e., cholangiocarcinoma (CCA) occurs at higher frequency in areas where liver fluke infections are endemic.[3,5-7] For example, in Korea a hepatocellular carcinoma (HCC) to cholangiocarcinoma ratio (HCC:CCA) of 4:1 was found in the Pusan area where *C. sinensis* infection occurs, while a ratio of 10:1 was found in Seoul where *C. sinensis* does not occur.[4] Similarly, a ratio of 3-4:1 was reported in the endemic northeastern part of Thailand where *O. viverrini* infection may be as high as 90% in some villages. This compares with an average ratio of 5:1 to 10:1 throughout the country.[5] It should be noted that in countries where liver fluke infections do not occur, CCA is rarely found and the HCC: CCA ratios as high as 56:1 have been reported.

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## EVIDENCE SUPPORTING AN ASSOCIATION BETWEEN CHOLANGIOCARCINOMA AND *O. VIVERRINI* INFECTION

Prospective and retrospective studies by a number of investigators have clearly demonstrated a strong relationship between CCA and *O. viverrini* infection in humans.[2,7-10] This is supported by the fact that the flukes are found at autopsy in a large proportion of CCA cases. Different lines of evidence suggest that CCA predominantly occurs in those with chronic and heavy infections. More cases of CCA and other hepatobiliary diseases have been noted in villages where there is a high incidence of *O. viverrini* infection.[2] CCA can be readily induced in animals experimentally infected with human liver flukes. It does not occur in uninfected but similarly exposed animals.[11,12] Fermented foods consumed by people in the endemic areas of *O. viverrini* infection contain high concentrations of nitrate and nitrite, and to a lesser extent nitrosamine. These compounds are known to constitute a risk factor for a number of other human cancers.[13] It should be noted that other carcinogens, e.g., aflatoxin, may also be involved. Data presented by Thamavit and his associates clearly suggest that pathological lesions induced in the liver by *O. viverrini* infestation (e.g., bile duct proliferation, cholangiofibrosis and cirrhosis) help to promote carcinogenesis initiated by a subcarcinogenic dose of dimethylnitrosamine.[11] Subsequent tests suggested that the proliferative changes induced in the hamster liver were probably irreversible, since praziquantel administration did not significantly reduce the ability of the induced lesions to develop further along the line to malignancy.[12]

Because there is evidence suggesting that nitrosamines can be derived from both exogenous and endogenous sources,[13] the endogenous nitrosation in subjects with *O. viverrini* infestation was investigated. It was found that infected individuals excreted high levels of nitrate and nitrite in their saliva.[14] These individuals also excreted high levels of nitrate and nitrosoproline (NPRO) in their urine. This suggested a greater exposure to N-nitroso compounds than uninfected individuals and might explain the higher risk for CCA development. Subsequently, Srivatanakul and her associates reported that people living in the high-risk area of Thailand and seropositive for *O. viverrini* antibody excreted significantly more NPRO after proline ingestion than did those who were seronegative.[15] The enhanced nitrosation reported could be inhibited by the ingestion of ascorbic acid. Limited data currently available suggested also that the ascobic acid could alter the course of CCA development in the hamsters (W. Thamavit, personal communications). Altogether, these data suggest that interaction between chemical carcinogens and *O. viverrini* is etiologically involved in the development of CCA. Similar findings and conclusions were also reported for cancer of the urinary bladder associated with *S. haematobium* infection, particularly when the condition was complicated by secondary bacterial infection.[4]

## POSSIBLE MECHANISMS FOR THE DEVELOPMENT OF CHOLANGIOCARCINOMA IN INFECTED HOSTS

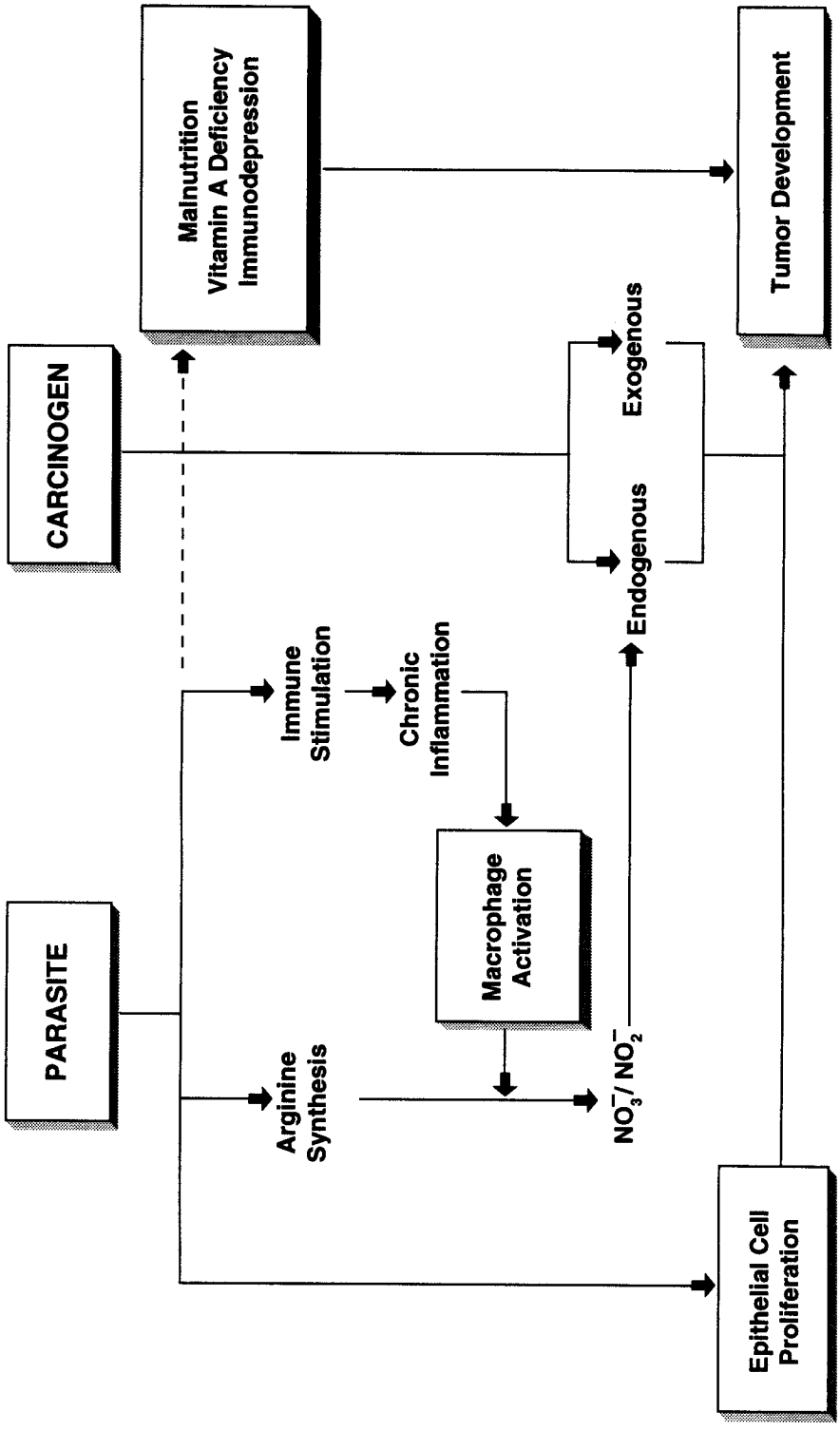
Data from both epidemiological and experimental studies clearly indicate the involvement of *O. viverrini* in the development of CCA in Thailand. It is also likely that the initiator is N-nitroso compounds from either exogenous or endogenous sources (Diagram I). While the role of N-nitroso compounds in the development of cancers is well documented,[13,16] the mechanism of preconditioning provided by the parasite has yet to be settled.

In general, tissue damage or functional aberrations associated with any kind of infection may be attributed either directly to the infectious agent itself or indirectly to interactions between the agent and the immune system. For example, some microbial and parasitic agents are known to be immunosuppressive, and could theoretically allow tumors to escape immune surveillance, flourish and metastasize. It is possible for the cholangiocarcinoma that occurs in Thailand that fluke movement along the biliary tree physically damages the epithelial lining and eventually leads to bile duct proliferation and hyperplasia. Replicating stages of cells during proliferative processes are known to be more sensitive to the action of carcinogens. Moreover, the physical and chemical damages might promote the carcinogen-transformed cells to proliferate more rapidly and develop into cancer. This, together with any toxic excretory-secretory products from the parasite might provide sufficient explanation for the preconditioning role of the parasite (Diagram I). However, to date no such toxic products have yet been identified. On the other hand, *O. viverrini* is known to excrete arginine during *in vitro* culture[2] and it is possible that parasite-derived arginine could enhance the production of nitrogen oxide derivatives (Diagram II). The parasite may also metabolize bile acids or exogenous carcinogens making them more carcinogenic. Moreover, bile stasis and stone formation associated with the infection may provide favorable conditions which further enhance carcinogenesis.

The time when the infected subjects are exposed to carcinogen(s) is the time when the immunological response should be fully active. Therefore, the effect of immunological reactions cannot be disregarded. It is known that *O. viverrini* remains localized extracellularly in the hepatobiliary system throughout its life in the human host and can stimulate both local and systemic immune responses. Humans and animals experimentally infected with the liver fluke exhibit both humoral and cell-mediated immune responses against the parasite.[10,17-19] The bile of these infected hosts also possesses IgA antibody to parasite antigens.[18, 19] Both biliary and serum antibodies, together with cell-mediated immune reactivities, can induce intense and chronic inflammatory responses, leading to a number of diverse biological consequences including lymphokine secretion (e.g., TNF, γ-IFN, IL-2, etc). Some of these lymphokines can readily mobilize and activate macrophages and other immune cells (Diagram I). Such an activation process may involve the production of enzyme arginase which can in turn metabolize arginine to citrulline, nitrate and nitrite.[20] Moreover, a number of environmental factors known to have influence on the host immune response can indirectly affect the intensity of the immune interaction in infected animals, e.g., malnutrition.[21,22] It has been demonstrated that a low protein diet makes infected hamsters respond more brisky, possibly making them more susceptible to tumor

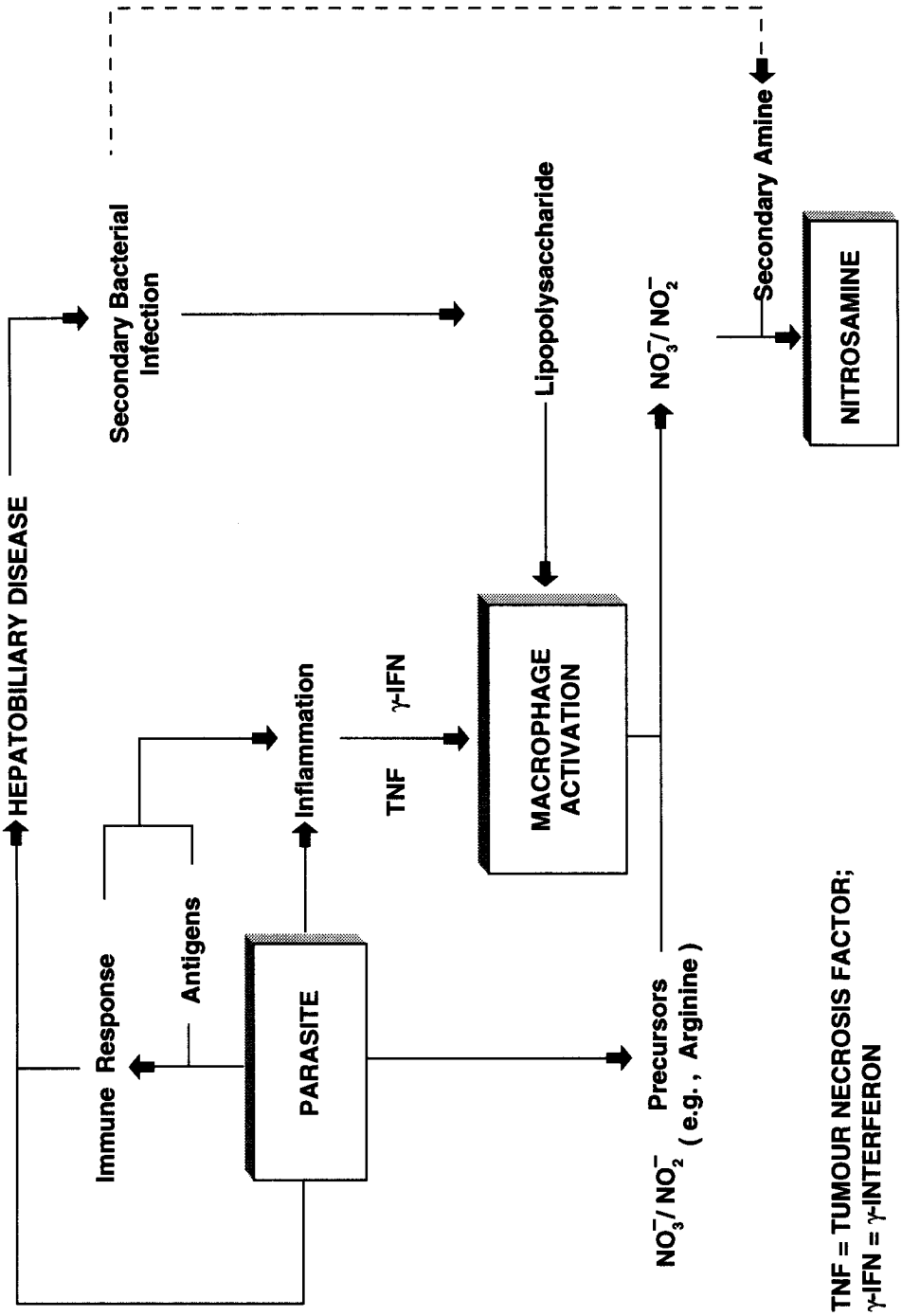
# DIAGRAM I

## DEVELOPMENT OF LIVER FLUKE-ASSOCIATED CHOLANGIOCARCINOMA



# DIAGRAM II

## ENDOGENOUS NITROSATION BY IMMUNOSTIMULATED MACROPHAGES




TNF = TUMOUR NECROSIS FACTOR;
γ-IFN = γ-INTERFERON

development.[22] Aflatoxins found to contaminate foods consumed by local people are known to have the capacity to induce cholangiocarcinoma in primates. Lastly, secondary bacterial infections together with bile stasis and stone formation may further complicate the situation (Diagram I). This condition can enhance endogenous nitrosation not only directly but also indirectly by lipopolysaccharide-induced macrophage activation (Diagram II). LPS and γ-IFN are known to synergistically activate mammalian macrophages.[20] Once the carcinogenic process has been turned on, progression or elimination of the transformed cells depends in part on the ability of the host to mount a response to suppress or to reject it. If the response is weak, the tumor may progress. Whether or not this occurs in CCA development remains to be determined. It should be mentioned that, like other chronic parasitic infections, *O. viverrini* infected animals may be immunodepressed.[21] It would be interesting to see if the tumor would develop more extensively in immunodeprived hamsters. The latter are known to have diminished inflammatory response to *O. viverrini* infection.[22] However, it is not known if this also occurs in man, or, if so, whether the degree of depression is sufficiently severe as to allow the tumor to proliferate unopposed at its own pace.

Very recently, the role of oncogenes and tumor suppressor genes in the development of cholangiocarcinoma has attracted the attention of a number of investigators. As found in other cancer models, the oncogene for example has been turned on in CCA that occurs in the absence of parasite and it exhibits point mutational activation of the c-Ki *ras*.[23] However, the CCA associated with *O. viverrini* in Thailand does not seem to exhibit this phenomenon.[24] The availability of cholangiocarcinoma cell lines should serve as a valuable tool to accelerate the progress in this area of investigation.[25] Research along this line is now being conduct by our collaborators here in Thailand.

## CONCLUSION

Although cancer of the biliary tract is relatively rare in most parts of the world, it is a predominant liver cancer in developing countries. In northeastern Thailand, where both the liver fluke (*O. viverrini*) and cholangiocarcinoma are very common, it appears that the pathological consequences of infection, together with chronic exposure of dietary or endogenously formed N-nitrosocompounds, predispose humans to CCA. There are many hypotheses regarding the mechanisms whereby the liver fluke enhances carcinogenesis. These include induction of a chronic inflammatory reaction, bilary proliferation and hyperplasia, alteration of the metabolism of endogenous and exogenous carcinogens and stimulation of a host immune response which can directly or indirectly make the host more susceptible to cancer induction and progression.

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