CHAPTER I

INTRODUCTION

During the last 20 years, the leading cause of death in Thailand has changed from infectious diseases to non-communicable diseases (Vatanasapt, 1993). Cancer is the third most common leading cause of death in Thailand. The report in Public Health Statistic (1990), of Ministry of Public Health, Thailand, report that heart disease, appeared as the leading cause of death (28,924). The second was accident (23,634 cases) and the third was cancer (22,154 cases). The incidence of cancer (all sites) has reached 180 to 200 per 100,000 population (age-standardized rate). This reflects an increase in life span, more diagnostic precision because of advanced medical technology and better coverage of the population, and the changes in the life-style of the Thai people (Vatanasapt, 1993). However, death from cancer is also increasing each year in spite of modern trends of treatment.

Primary hepatocellular carcinoma (HCC) is one of the most incurable malignancies in the world. It is much less common in Western Europe, the America and Australia (Boring C.C.,1992), but it is particularly common G.I. tract cancer in the Southeast Asia and in Subsaharan Africa (Di Bisceglie Am, 1988, Fu-Sun, 1986, Okuda, 1986, Robinson, 1984, Sheu J-C., 1985). HCC is found to be the most common of all malignancy of Thai male. It ranks first in male and the third in female as a cause of cancer death of G.I. tract. The male/female ratio is 2.3:1. The average age of the patient is 35 to 65 years (Vatanasapt,1993). Unfortunately,

because the onset of symptoms coincides with advanced stages of the disease, the prognosis is generally poor (Di Bisceglie Am, 1988, Okuda, 1984).

Unless this tumor is detected early (smaller than 3 cm.) by ultrasound or by alpha- fetoprotein screening programs in high risk population (Okuda, 1986, and Tang Z-Y,1985) most of patients only has prolonged life. The complete cure is still far beyond. Mostly, this disease is nearly 100 % fatal even if being treated with modern surgery, chemotherapy and radiotherapy (Ihde,1985). In 1984, the median survival rate of minuted tumor is still less than 1 year (Okuda,1984). According to the studies of Okuda, the 1 to 3 years survival rates of patient with large tumor (more than 5 cm) was vary between 20% to 50% (Okuda, 1984). Most of all patients died from the recurrence tumor and/or hepatic failure. Currently avialable treatment that prolong the survival is still small in number. The effective treatment is restricted to the hepatic resection, when possible, since conventional radiotherapy or chemotherapy results in little benefit (Tang Z-Y,1985). It is an urgent problem to improve the diagnosis in its with such tumors, early stage as guide as possible together with the still on surgical resection of small tumors.

Since 1975, Kohlor and Milstein provided a new hybridoma technology which its monoclonal antibodies had become an amazing tools in immunological field. Surprisingly, it helps to open the secret of tiny molecular reaction of immunology developing immunotherapeutic approaches to malignant diseases (Berge, 1991, Fukuda, 1988) and the vaccination to the tumor. At this moment the monoclonal antibodies are commonly use in helping to give an easily method for the tumor diagnosis. For the usefulness in immunotherapy many monoclonal antibodies have been reported for the tumoricidal effect to a variety of human neoplasms such as the melanoma, B-cell lymphoma (Herlyn, 1985, Houghton,

1985, Oldham, 1984), leukemia (Dillman, 1984), colon carcinoma (Berge, 1991, Michael ,1991), neuroblastoma (Kenshed, 1985) and especially hepatocellular carcinoma (Fukuda, 1988, Order, 1985).

The tumor growth suppression and tumoricidal effect are related to a specific isotype of a monoclonal antibody. Monoclonal antibodies of IgG2a isotype particularly inhibited growth of HCC tumors in vitro with or without effector cells, while other isotypes showed no tumoricidal reactivity (Fukuda, 1988, Herlyn, 1985). MAbs themselves can kill antigen-positive tumor cells by binding to the antigen that inhibit the activity of growth, such tumor rejecting antigen (Shimizu, 1991). Some antitumor mouse monoclonal antibodies which was IgG2a isotype can also mediated antibody-dependent cellular cytotoxicity (ADCC) in the presence of human effector cells (Hellstorm, 1981, Hellstorm, 1985, Herlyn, 1985, Houghton, 1985, Reisfeld, 1985) and /or activate human complement (Hellstorm, 1985, Houghton, 1985, Reisfeld, 1985). During 1980-1985 the tumoricidal effect of monoclonal antibodies alone were concluded in model of nude mice carrying human tumor xenografts and cancer patients as provide less antitumor activity (Hellstorm, 1985, Herlyn, 1985, Houghton, 1985, Reisfeld, 1985).

The method of using the monoclonal antibodies since then have been modified to carry toxin (Blakey,1988) or chemotherapeutic agent (Embleton, 1986) to target them to the tumor masses specifically. The modification of chimeric antibodies seems to promicingly improve the efficiency of monoclonal antibodies. One of reason is the occurrence of human antimouse immunoglobulin (HAMA) that raised the limitation in giving sufficient dose to reach the complete cure. The second is most of those monoclonal antibodies recognized only non-tumor rejecting antigens, mostly recognized only the oncodevelopmental antigen (ODA).

In this thesis, the aim was to find out whether anti-hepatoma MAbs alone that give tumoricidal effect and how they destroy the HCC cell lines observing

under electronmicroscope. All of the anti-hepatoma MAbs used were provided by Dr. Kingkarn Laohathai's collections (Laohathai, 1985). All of them are IgG2a subclass, and did not recognized the normal adult liver (tissue and extracts), normal tissue and blood cell antigens. There are separated into three groups according to the reaction to fetal and new born liver cells or not but not recognized the alphafetoprotein, beside the reaction to other human cancer cell lines. Those recognizes the fetal and new born liver together with the HCC and other tumors were grouped as antibodies that recognized ODA. The second group is those recognizing only a panel of cancer cell lines and HCC. This could be the anti-hepatoma MAbs that recognized the tumor associated antigen. The third is those reacted only with HCC cell lines which could be anti-hepatoma MAbs that recognized hepatoma specific antigen. The studies, firstly anti-hepatoma MAbs were selected the best MAbs that had tumoricidal effect. Secondly, the appropriate concentration of MAbs and incubation time in killing the HCC cell lines were found out in condition of free of effector cells. The effective MAbs were then selected and used to investigate destructive process on HCC under electron microscope, together with the observation on the relation of intracellular change of the organelles and the evidence of surface antigens. The benefits of this study is expected to provide better understanding in using MAbs more efficiently in clinical trail for immunotherapy.