CHAPTER V

DISCUSSION

5.1 Purification of MAbs

There were many methods for purification of the antibody among these the ammonium sulfate precipitation is the common and simple methods. One disadvantage of ammonium sulfate precipitation of antibodies is that the resulting antibodies is not pure because of contaminated with non-specific high-molecular-weight proteins. Ascites production in animal is possible to have contamination of all kinds of organism and proteins (Harlow, 1988).

Affinity chromatography is very effective method for purifying the MAb (Harlow, 1988). It provides also the subclass form but take longer process. The one step elution with citrate buffer, pH 3.0 could elute the IgG2a but the protein A-sepharose binds all class of immunoglobulin. From this study, some of the MAb had other subclass such as IgG1 and IgG2b. Non-specific immunoglobulin will disturb the binding capacity of desire immunoglobulin and after elution the rest immunoglobulin has to be clean at all. According to this reason the one step purification was not selected for this study. The others reason was the MAbs will be used for testing the tumoricidal effect so it is very important to get rid from any proteins which will disturb the immunoreaction. Fukuda (1988), Herlyn (1985) and Hu (1986) used this method for purification MAb that was used for testing the tumoricidal effect.

5.2 Saturated concentration of anti-hepatoma MAbs

Hiraiwa (1990) was used the ELISA method to find out the saturated concentration of MAb. The simplest ELISA method is suitable one confirmed by this attempt. The exact antigen is an important factor in lowering the default. Hiraiwa (1990) means that the cell number must have the same in each well of plate. From this study, the effective concentration of anti-hepatoma MAb for tumoricidal activity was used in higher than the saturated concentration. Such the effective concentration of anti-hepatoma MAb #27 was used 50 fold higher than saturated concentration.

5.3 The tumoricidal effect of anti-hepatoma MAbs

Up to now, the most important characters of MAbs in killing the tumor give by this reasons:

5.3.1 The specificity of MAbs: The most effective MAbs could be counted for those recognized Tumor Rejecting Antigen; TRA (Shimizu, 1991) and growth factor receptor (Sato, 1989) which being up to the negative process for the growth of cell. In selecting this kinds of MAbs it is necessary to have a long way around to find out, except the case that the antigens were already known. The anti-hepatoma MAb #27 and #43 may count for this group, However, it is necessary to be proved.

The tumoricidal effect of MAbs at early period (1979-1985) were randomly selected. Most of them recognized the ODA which commonly are the prominent antigenic epitopes. This anti-ODA was blamed for a factor that caused failure. Later on the MAbs that recognized the Tumor Associate Antigen; TAA were reported for the study of tumoricidal effect (Hellstorm, 1985, Herlyn, 1985), the anti-Tumor Rejecting Antigen (TRA) worked excellently (Shimizu, 1991). Interestingly the anti-hepatoma MAb #27 and #43 which are grouped as anti-ODA as it recognized the antigens on aborted normal fetal liver and new born liver cells, had the

tumoricidal effect. One reason could be explained here is that the HCC-S102 has these antigens and the number are sufficient enough to provide negative process for the proliferation of HCC-S102.

5.3.2 The effective subclass of MAbs : The IgG2a subclass was proved to have killing effect on tumor cell (Hellstorm, 1986, Herlyn, 1982). IgG2a works well with effector cell through the antibody dependent cellular cytotoxicity (ADCC), but IgG2a alone in some case (Fukuda, 1988) such as the anti-hepatoma MAb #27 and #43 also provides good effect.

5.3.3 The anti-hepatoma MAb #27 and #43 could not claimed for the best MAbs as far as both MAbs did not kill over half of treated cells, but both are the best in this series. In further study should determine the tumoricidal effect of those recognize TAA, hepatoma specific and the combination used, cocktails.

The anti-hepatoma MAb #27 and #43 were proved to recognize different epitope by means of competitive inhibition assay. Again the destructive structure function of both which were observing under the electron microscope also confirmed the difference.

5.4 The destructive process of MAb and antigen change

S102 cell line, a human cell line originating from Thai hepatoma, generally demonstrated a cellular morphology similar to that of normal hepatocyte, but with smaller amount of organelles. Thakerngpol (1983) reported that the amount of cytoplasmic organelles was related with the morphological grade of differentiation. Cytoplasmic organelles were decreased in the well differentiated tumor but moderately decreased in the moderately differentiated type. Hepatoma cell of poorly differentiated type had few cytoplasmic organelles. HepG2 cell line, the other human cell line, which is dedifferentiated type expressed mainly as decreased in number of organelles.

The anti-hepatoma MAb #27 firstly attach the RER of HCC-S102 together with the shortening of microvilli soon after 3 hrs of treatment but review on HepG2 cell after 3 days of treatment. The mitochondria was swollen and decreased in number after 1 day of treatment on HCC-S102 and HepG2.

The anti-hepatoma MAb #43 review the swollen and distorted mitochondria on HCC-S102 and milder swollen on HepG2 after 3 day of treatment. Beside the effect to the RER, microvilli and mitochondria there were large prominent vacuoli junction in the cytoplasm of HCC-S102 and HepG2.

According to this data both anti-hepatoma MAbs firstly effected the system of protein transportation and the production whether there is relation or not with the RER system was involved within 24 hrs. The RER is concerned with the transport of proteins which are made by ribosomes on its surface. The morphologic changes in RER related to disturbed protein production and transportation of the cell that leaded to cell death. The morphologic changes in mitochondria relate to disturbed energy production and help explain cell insufficiency and instances of sudden death. After the effect to the RER, microvilli and mitochondria there were follow by vacuole in cytoplasm and fused nuclear membrane including increased of heterochromatin in nucleus.

Anti-hepatoma MAb #27 recognized antigen on HCC-S102 continuously decreased and the gold particle was found in cytoplasm after 3 hrs of treatment. The antigen was totally can not found after treatment for 24 hrs until day 3.
Tian (1989) report that internalization of MAb started after treated cell was incubated at 37 c for 15 min and increasing internalization to 72% after 4 hrs of treatment. In this study the treated HCC-S102 cell with MAb #27 was completely internalization after 24 hrs of treatment.