Chapter I

Introduction

Cancer is considered as one of the most contingent disease that endangers human life. It has been discovered that the earlier cancer diagnosed, the higher chance of being cured. Therefore the determination of tumor marker which can be defined as a substance present in a body fluid will qualitatively or quantitatively reflect the presence of malignancy.

The model for diagnosis and treatment of cancer are rapidly changing. In the recent past, the knowledge about tumor markers has widened not only cancer research, but also stimulated the clinician to look into a new step for cancer diagnosis and treatment(1). Immunodiagnosis of cancer seems promising, unfortunately, with a few exceptions such as alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG) and carcinoembryonic antigen (CEA), markers are not widely used at the present time due to the clinical applications of them have not fulfilled all the optimistic expectations that many predicted, these makers have achieved a place in cancer diagnosis and management.

Alpha-fetoprotein (AFP) is one of a group of oncofetal substance useful as tumor markers. It is a product of specific fetal tissue and of neoplastic cells of hepatocyte

or germ cell origin in adults(2). This protein belongs to a germ family that is phylogenetic most closely related to serum albumin but they do not crossreact immunologically in their native forms. Its primary, secondary and tertiary structural aspects appear similar to the three-domain concept proposed for the latter protein(3).

Determination of AFP in maternal serum and in amniotic fluid during pregnancy has proved to be an important tool in prenatal diagnosis of spina bifida and congenital nephrosis(4). Production of AFP by germ cell tumors and primary liver cancer is utilized to monitor cancer patients(5). The recent observation that AFP exhibits microheterogeneity, which can be exploited diagnostically, may further expand the indications for the assay of AFP. Further immunochemical characterization of AFP and development of improved assays for its measurement is therefore of great importance. The radioimmunoassay techniques normally used for the measurement of serum AFP are being superseded in precision, accuracy and reliability by two-site immunoradiometric assays particularly since the advent of high-quality monoclonal antibodies.

The hybridoma technique allows production of large quantities of homogeneous antibody from malignant cell hybrids of antibody producing lymphocytes and myeloma cells(6). It has been commonly assumed, but not proved, that such antibodies will by virtue of their monospecificity replace conventional antisera used in various immunochemical tests. However, immunoradiometric

assay (IRMA) and other sensitive immunological assays place special requirements on the antibodies used, not only in terms of specificity but also of affinity, because sufficient sensitivity can only be achieved with antibodies of high affinity(7).

Two-site IRMA as introduced by Addison and Hales(8) in 1971 does not require purified antigen, but need a relatively large quantity of antibodies both for solid phase adsorption and labelling. Monoclonal antibodies will overcome this limitation and their application in immunoassay will allow two-site IRMA to become the prevailing assay procedure in the future. Therefore the production of monoclonal antibodies to human AFP and the development of two-site IRMA using a monoclonal antibody as radiolabel will also be described in this study.

Objective

The objectives of the study are

1. To produce specific monoclonal antibody to AFP

2. To develop the system for monoclonal anti AFP on to solid phase particles

3. To develop the system for labelling monoclonal anti AFP with I^{125}

4. To optimize the IRMA assay for AFP using solid phase separation technique