II. LITERATURE REVIEWS

A. Leukocyte surface molecules

Leukocytes express a large number of different molecules on their surface. Some leukocyte surface molecules appear at the resting stage, some of which are expressed at particular stages of cell differentiation or activation. Monoclonal antibodies raised against human leukocyte surface molecules have become a major tool in determining and characterizing the structures and functions of these molecules. A systematic nomenclature has been developed for these cell surface molecules, designated by the CD system (Knapp et al., 1990). The term CD (cluster of differentiation) was derived from the analysis of monoclonal antibodies generated against human leukocyte antigens in different laboratories worldwide. An international workshop series determines their cellular reaction patterns and the molecular weights of the recognized molecules. Monoclonal antibodies with the same specificity are grouped together and given a CD number. This number can also be used to indicate the molecules recognized by the clustered monoclonal antibodies (Knapp et al., 1990). Since 1982, five international workshops on human leukocyte differentiation antigens have been established. One hundred and thirty of cell surface molecules were already named in CD system. The details of all 130 CD antigens and of the monoclonal antibodies which defined them can be found in the proceedings of the fifth international workshops (Schlossman, S. et al., 1994).

In some cases, functions of leukocyte surface molecules are known. Several of them function as ligand receptors which are of critical importance in the development of the immune response. Antigen-specific receptors on lymphocytes are an example. The recognition of foreign antigens by antigen-specific receptors on lymphocytes is the basis for the initiation of the immune response. Antigen-specific receptors of B-lymphocytes are surface immunoglobulins, which are of mostly IgM and IgD isotypes. Specific interaction of these surface immunoglobulins with foreign antigens results in signal transduction, and finally, the induction of antibody production (Melchers and Andersson, 1986, Cambier and Ransom, 1987). Antigen-specific receptors of T-lymphocytes, termed T cell receptor, are expressed exclusively on the surfaces of these cells (Clevers et al., 1988). T-lymphocyte recognition of antigens through these

T cell receptors is the basis for a range of immunological phenomena including helper and suppresser activities, and lymphokine production. Another example of ligand receptors on a leukocyte surface is interleukin-2 receptor (IL-2R). Interleukin-2 (IL-2), a lymphokine, is the major autocrine growth factor for T lymphocytes (Morgan et al., 1976). The quantity of IL-2, synthesized by activated $CD4^+$ T cells, is an important determinant of the magnitude of T cell dependent immune response. IL-2 also stimulates the synthesis of other T cell-derived cytokines, such as IFN-γ and lymphotoxin (LT). Failure to synthesize adequate quantities of IL-2 has been described as a cause of antigen-specific T cell anergy (Smith, 1988). The action of IL-2 on T cells is mediated by binding to IL-2 receptor proteins (Robb et al., 1987). There are two distinct cell surface proteins on T cells binding to IL-2. The first to be identified is called IL-2Rα, a 55 kDa polypeptide (p55) that appears upon T cell activation and was originally called a Tac (for T activation) antigen (Robb et al., 1984). The binding of IL-2 to cells expressing only IL-2Rα does not lead to any detectable biologic response (Lowenthal and Greene, 1987). The second IL-2 binding protein, called IL-2Rβ, is about 70 to 75 kDa. The affinity of IL-2 binding to IL-2Rβ is higher than to those of IL-2Rα, with a K_d of approximately 10^{-9} M. IL-2Rβ is expressed coordinately with a 64 kDa polypeptide, called IL-2Rγ, forming a complex designated as IL-2Rβγ. IL-2 promotes a growth of cells expressing only IL-2Rβγ (Dukovich et al., 1987). Cells that express IL-2Rα as well as IL-2Rβγ can bind IL-2 much more tightly, with a K_d of approximately 10^{-11} M (Tsudo et al., 1986, Robb et al., 1987). Both IL-2 binding and growth stimulation can be blocked by antibodies to either IL-2Rα or IL-2Rβ, and most efficiently, by a combination of antibodies to both receptor subunits. This means that IL-2Rα forms a complex with IL-2Rβγ, thus, increasing the affinity of the IL-2Rβγ receptor for IL-2, and thereby, allowing a growth signal to be delivered at significantly lower IL-2 concentrations. It is believed that IL-2 first binds rapidly to IL-2Rα, and this facilitates the association with IL-2Rβγ.

Some surface molecules on human leukocytes function as cell-cell adhesion molecules. The adhesion of various types of leukocytes to other cells is essential for many of their basic functions. Cell surface molecules, which function as adhesion molecules such as integrins, the hetero-dimeric membrane proteins, are composed of two non-covalently linked polypeptide chains, α and β. Three integrins subfamilies were originally defined on the basis: which of three β subunits were used to form

heterodimers pairing with a distinct and non-overlapping set of α chains. More recently, five additional β chains have been defined. β1-containing integrins are also called VLA molecules, referring to 'very late activation'. The β1 integrins are also called CD49a-f/CD29, CD49a-f referring to different α chains and CD29 to common β1 subunits. Most β1 integrins are widely expressed on leukocytes and non-blood cells, and mediated the attachment of cells to extracellular matrices. VLA-4 (CD49d/CD29) is expressed only on leukocytes and can mediate the attachment of these cells to endothelium by interacting with VCAM-1. VLA-4 may be one of the principal surface proteins that mediate the homing of lymphocytes to endothelium at the peripheral sites of inflammation (Hemler, 1990). β2 integrins, also known as the LFA-1 (leukocyte function-associated antigen-1) family, plays an important role in the adhesion of lymphocytes with other cells, such as accessory cells and vascular endothelium (Simmons et al., 1988). This family is also called CD11/CD18, CD11 referring to different α chains and CD18 to the common β2 subunit, for example, both CD11b/CD18 and CD11c/CD18 mediate leukocyte attachment to endothelial cells and subsequently extravasation. CD11b/CD18 also functions as a complement receptor on phagocytic cells, binding particles opsonized with a by-product of complement activation called the inactivated C3b (1C3b) fragment (Holers et al., 1992). Another example of adhesion molecules on leukocyte surfaces is CD28 and CTLA-4. CD28 is an Ig superfamily member that serves an important role in T cell activation response (Freeman et al., 1992, Linsley et al., 1992,). CTLA-4, a homologous molecule, is expressed on activated T cells. Both CD28 and CTLA-4 genes are closely linked on human chromosome 2 (Abbas et al., 1994). One identified counter receptor for both CD28 and CTLA-4 is the B7 molecule, a 60 kDa Ig superfamily member, expressed constitutively on dendritic cells (Larsen et al., 1992) and expressed inducibly on B cells (Freedman et al., 1987) and monocytes/macrophages (Razi-Wolf et al., 1992). When B7 binds to the CD28 molecules on T cells that are concurrently stimulated by TCR ligands, signals are generated that enhance IL-2 gene transcription and perhaps stabilize IL-2 mRNA (Linsley et al., 1993). Besides CD28, B7 binds to CTLA-4 on activated T cells, but the role of this interaction is unknown (Abbas et al., 1994). A recent study demonstrated that a B7/CD28 costimulation can induce IL-5 production by resting human T cells. This activity could be inhibited by both rIL-10 and endogenous IL-10 (Schandene et al., 1994).

As mentioned above, some functions of various kinds of leukocyte surface molecules have been characterized. However, dozens of defined and characterized molecules are still awaiting a functional description to be prescribed to them, as are many undefined leukocyte surface molecules.

B. Monoclonal antibody

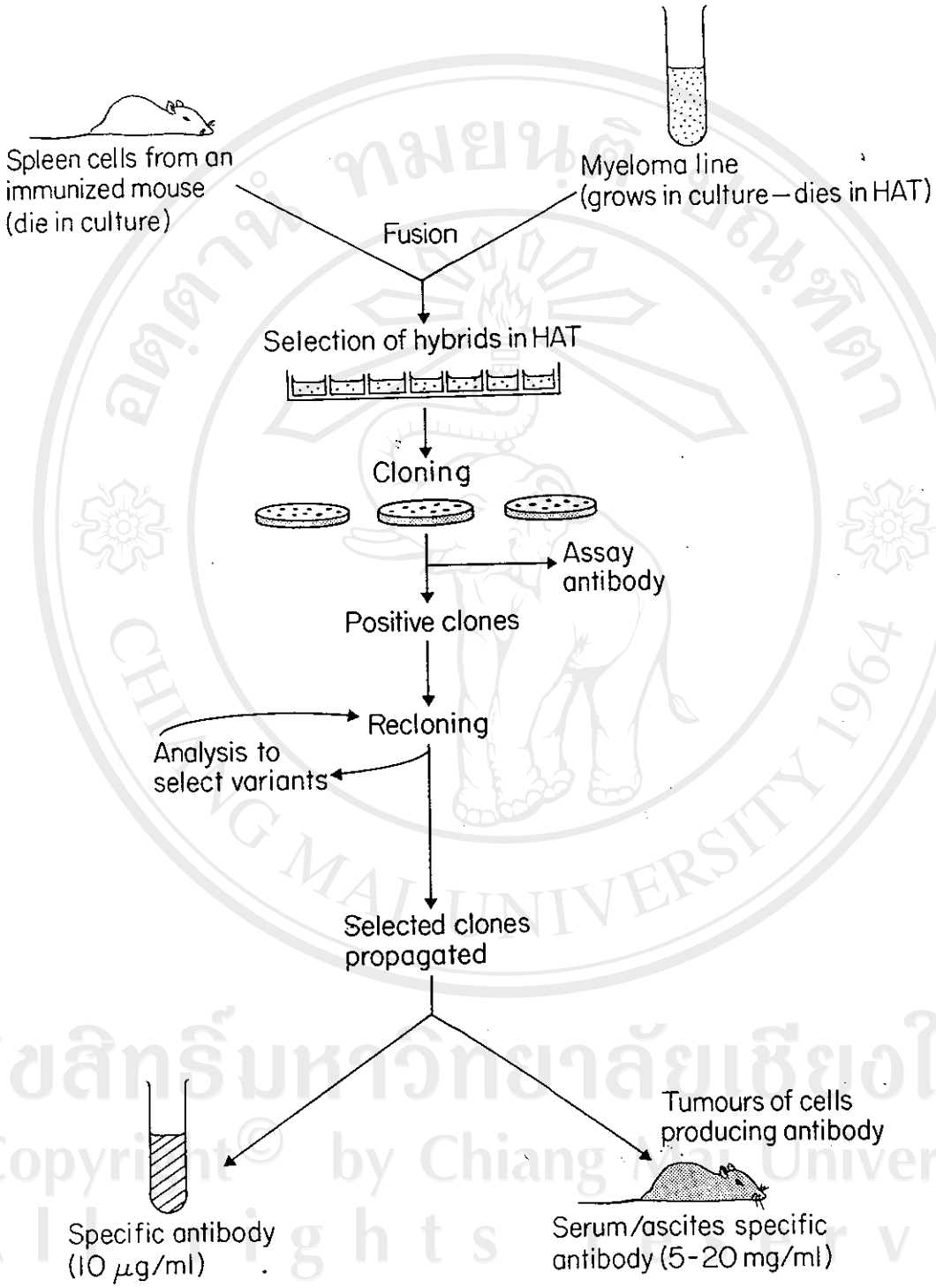
1. Principle of the monoclonal antibody technology

Spleen cells from immunized animals have a finite life span *in vitro*. The antibody activity expressed in that population of cells can be rescued by fusion to a continuously growing *in vitro* adapted myeloma line which confers the malignant, immortalizing phenotype. Resulting hybrids can be selected by growth in a medium which kills the parental myeloma cells. Hybrids which secrete desired antibodies are then cloned by limiting dilution or growing in soft agar. Selected clones are propagated for large scale production of antibodies by culturing in tissue culture medium *in vitro* or injecting them into mice as a tumor *in vivo*, so that antibodies are secreted into the medium and ascites fluid of mice, respectively. Since, the individual clones are generated from a single cell, therefore, antibodies secreted from these clones are called monoclonal antibodies. The broad overview is provided in the following scheme (p. 7).

2. Monoclonal antibodies are powerful tools for the characterization of leukocyte surface antigens

Since 1975, Kohler and Milstein have initially immortalized antibody-secreting lymphocytes by fusing them with cells from a continuously growing cell line. They cloned individual hybrid cells to produce lines of cells (hybridoma), each of which secretes one particular antibody molecule (Kohler and Milstein, 1975). This hybridoma technology, producing a predefined specific monoclonal antibody, is a very useful technique in immunology and other biological sciences.

The usefulness of monoclonal antibodies stems from three characteristics: their specificity of binding, their homogeneity, and their ability to be produced in unlimited quantities. Two features of this somatic cell hybridization make it extremely valuable. Firstly, it is the best method for producing a monoclonal antibody against a known antigenic



Basic protocol for derivation of monoclonal antibodies from hybridoma (taken from Sikora and Smedley, 1984)

determinant. Secondly, it can be used to identify unknown antigens present in a mixture, because each hybridoma is specific for only one antigenic determinant. For instance, if several hybridomas are produced and secrete antibodies that bind to the surface of a particular cell, each hybridoma clone will secrete an antibody specific for only one surface antigenic determinant. These monoclonal antibodies can then be used to identify different cell surface molecules, some of which may be known molecules and others that may not have been previously identified . Some of the most common applications of hybridomas and monoclonal antibodies include the following:

1. Identification of phenotypic markers unique to particular cell types. The basis for the modern classification of lymphocytes and mononuclear phagocytes is the binding of population-specific monoclonal antibodies. This has been used to define "clusters of differentiation" for various cell types.

2. Immunodiagnosis. The diagnosis of many infectious and systemic diseases relies upon the detection of specific antigens and/or antibodies in the circulation or tissues, using monoclonal antibodies in immunoassays.

3. Tumor diagnosis and therapy. Tumor-specific monoclonal antibodies are used for the detection of tumors by imaging techniques and for the immunotherapy of tumors *in vivo*.

4. Functional analysis of cell surface and secreted molecules. In immunologic research, monoclonal antibodies that bind to cell surface molecules, and either stimulate or inhibit particular cellular functions, are invaluable tools for defining the functions of surface molecules, including receptors for antigens. Antibodies that neutralize cytokines are used routinely for detecting the presence and functional roles of these protein hormones *in vitro* and *in vivo*.

For example, Van den berg (1994), investigated a dysfunctional form of CD59 on a $CD59^+$ subclone of a U937 cell line by using an anti CD59 monoclonal antibody. He first evaluated a CD59 expression on various kinds of cell lines stained with an anti CD59 monoclonal antibody, by using the indirect immunofluorescent technique. It was found that besides the $CD59^+$ cell lines tested (K562, HL60, and MOLT4), the U937 showed a two subline difference in the $CD59^+$ expression: $CD59^-$ and $CD59^+$ sublines. U937, a stable promonocytic cell line, has been previously shown as relatively susceptible to killing by complement and responds to non-lethal attack by the removal of membrane-attack-complex (MAC) from the membrane. CD59 is the glycosyl-phosphatidyl-inositol

(GPI) expressed on a cell surface. The binding of CD59 to C8 to form MAC, leads to its potential role in limiting the incorporation of C9, thereby, preventing the formation of a lytic lesion. A monoclonal antibody against CD59, will neutralize CD59 and increase the killing of most $CD59^+$, without enhancing that of $CD59^-$ cell lines. The results of this study showed, however, that an anti CD59 monoclonal antibody did not enhance the killing of a $CD59^+$U937 subline and could not be immunoprecipitated from cell extracts. U937 cell extracts were also negative in Western blotting, when using a variety of anti CD59 monoclonal antibodies. The result indicated that the $CD59^+$U937 cell expressed a form of CD59, which is dysfunctional and structurally abnormal.

Another example is the study of Olson et al. (1994) about the effect of a monoclonal antibody to angiogenin (26-2F mAb) in suppressing tumor growth. Angiogenin, the protein secreted by HT-29 human colon adenocarcinoma cells (Fett et al., 1985) and later from normal mammalian plasma and milk (Shapiro et al., 1987, Maes et al., 1988), exhibited ribonucleolytic activity and was responsible for enzymatic and angiogenic activities. These included a putative receptor binding domain, necessary for functioning. More recently, angiogenin, unlike RNase, was found to support endothelial cell adhesion, a property which may contribute to its role in angiogenesis.

Firstly, they studied the effect of suppressing 26-2F on a tumor growth *in vivo* in athymic mice. The mice were injected subcutaneously with HT-29 tumor cells mixed with 26-2F mAb. The results showed that antibody treatment prevented tumor appearance significantly. Secondly, the anti tumor activity was studied *in vitro*. HT-29 cells were cultured in the presence of 26-2F mAb and [^{3}H]thymidine. The *in vitro* study indicated that 26-2F mAb neutralized the activities of angiogenin in a dose dependent manner and caused the inhibition of tumor growth.

From the results mentioned above, plus the fact that human angiogenin can be detected in the serum of mice bearing HT-29 xenographs, it is most likely that the antitumor effects exerted by 26-2F mAb, resulted from the specific extracellular inactivation of tumor-derived human angiogenin and a consequent disruption of the angiogenic process. Angiogenesis, known to be critical for solid tumor growth (Folkman, 1990), is also a prerequisite for the development of metastasis. It permits the transport of cells shed from the primary tumor and the subsequent growth of micrometastasis at distant sites (Mahadevan and Hart, 1990).

Interference in the process of tumor-induced angiogenesis should, therefore, have therapeutic efficacy in the treatment of metastatic disease. The results suggested that the growth of HT-29 tumors can be delayed and even prevented by the inhibition of angiogenin at an early stage of tumor development.

Most studies in many different fields use the advent of monoclonal antibodies to find answers to the questions. It can be said that monoclonal antibodies are the powerful weapons used to destroy the great wall of problems to enter the molecular land of an immunological world.

C. Cell lines

Cell lines, lineages of cells, which possess the properties of an immortal and continuous pattern of proliferation and differentiation, and self-renewal, were established from various sources of both human and animal origins, e.g. ascites fluid, pleural effusion, tissues, etc. These original cells were cultured in a culture medium appropriate for each type. Cell lines that proliferate continually with appropriate markers for differentiation, provide useful models for studying the regulation of cell proliferation and differentiation. These cell lines can be induced *in vitro* by a wide variety of compounds to form morphologically and functionally mature cells. The study of the interaction of these compounds with cultured cells, may not only provide a better understanding of the nature of the tumor cell phenotype, but may also imply an alternative approach to the understanding of cell functions and the therapy of certain types of tumor origins.

Example for the uses of cell lines, the monocytic line JOSK-1 constitutively produces IL-1 and is a useful source of native IL-1 (Furukawa et al., 1987). Another monocytic cell line THP-1 produces IL-1 in response to LPS and has been used to study the regulation of IL-1 gene expression (Fenton et al., 1987). HL-60 cells produce TNF-α when treated with TPA and have been used as a source of mRNA in the preparation of cDNA for the TNF-α gene (Pennica et al., 1984).

Many cell lines can also be useful in the assay of growth factors. For example, RC-2A cells show increased ^{3}H-thymidine uptake when cultured with G-CSF or GM-CSF (Lyons and Ashman, 1987). The immature myeloblastic cell line KG-1, which responds to GM-CSF and IL-3 by increased proliferation and colony formation in soft agar, was used

in the identification of human IL-3 and in the cloning of the corresponding gene (Yang et al., 1986). In addition, the KG-1 and HL-60 lines have been used in the characterization of the receptors for GM-CSF (Gasson et al., 1986; Park et al., 1986). These cell lines are potentially valuable as a source of relatively homogeneous cells for studies of the mechanism of signal transduction involved in the response to cytokines, and of the intracellular events regulating cell proliferation. Myeloid cell lines, especially HL-60, have also been used in the study of oncogene expression in relation to proliferation and differentiation (Collins, 1987)

Many cell lines have been used in the study of differentiation antigens. They provide the large quantity of cells needed for immunization and screening in the production of mAb (Lyons et al., 1988). Cell lines are also useful sources of DNA or mRNA for cloning genes for cell surface antigens. Genomic DNA of several cell lines were used to produce transfectants expressing human differentiation antigens. These have been used to determine CD groups of mAb (Knapp et al., 1989). Frey and Engelhardt (1987) used U-937 cells to characterize the FcR for IgG. U-937 and other cell lines were used to investigate accessory cell function in T lymphocyte mitogenesis (Wakasugi et al., 1980).

Several cell lines of human origin were used in this study. Source, characterization, morphology and other descriptions of some of which are selected and mentioned in appendix.