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PHENOTYPIC ANALYSIS OF T-CELL LEUKEMIA IN EGYPT BY MONOCLONAL ANTIBODIES

Thesis

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(Clinical Pathology)

BY

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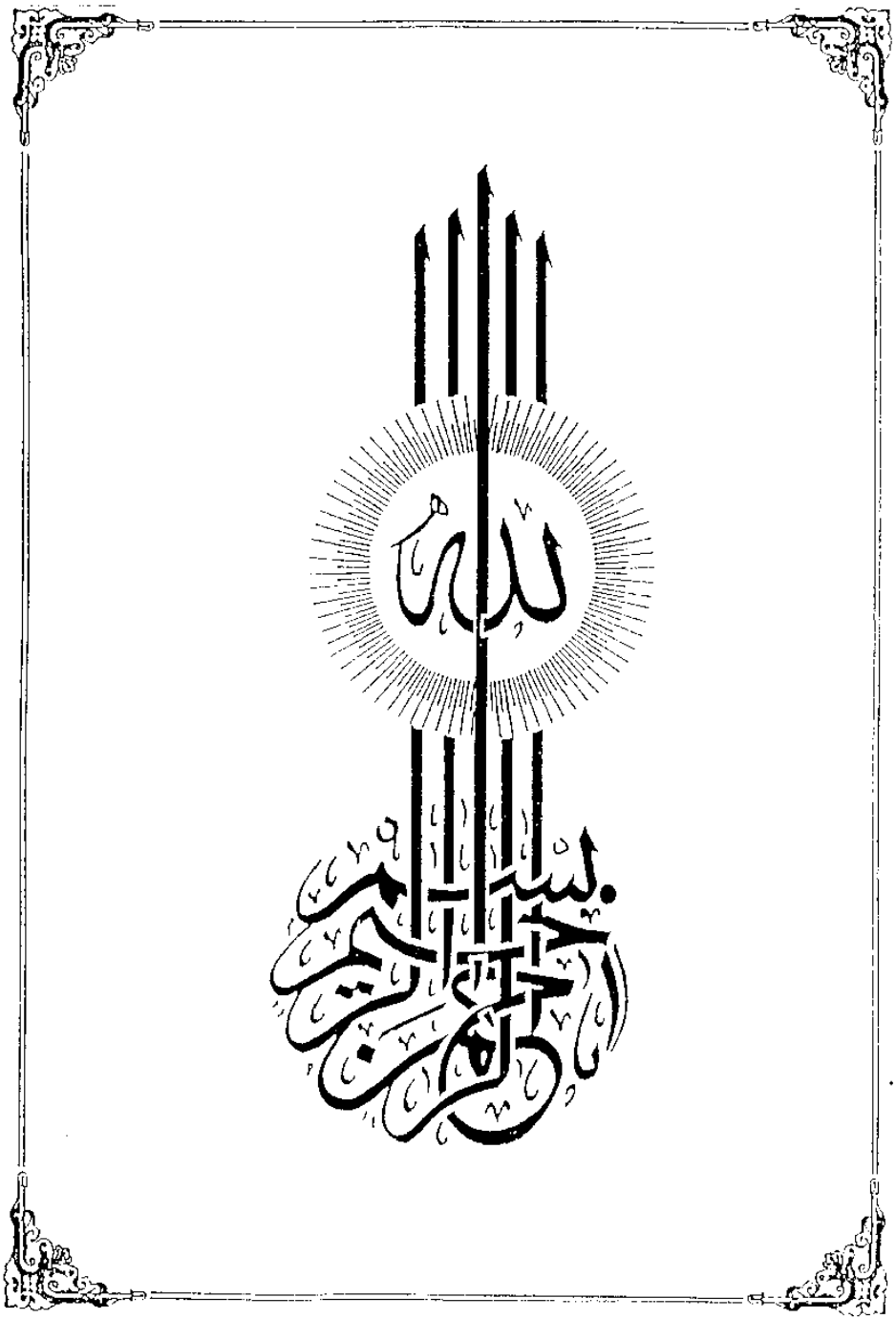

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Introduction

and

Aim Of The Work

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Previous studies performed at the NCI, Cairo University, has demonstrated a relative incidence of immunological subtypes of lymphoid leukemias different from western countries. The main difference was a relatively low incidence of common-ALL (39.5 %) and a high incidence of T-ALL of about 48.6 % (Kamel et al., 1986).

Other studies at the NCI showed similar differences at the clinical level (Gad-El-Mawla et al., 1984). The results of therapy in Egyptian children with ALL are not matching with the international figures. At the same time, ALL in Egyptian patients children bear a higher prevalence of poor prognostic criteria than those in the western countries.

Hence, it was decided to carry out a detailed study on T cell malignacies to find out if it is also different from that in other areas of the world.

Aim of the work :

1. Study of heterogeneity of T cell malignancies.
2. Study of distribution of T cell malignancies according to their intrathymic origin.
3. Study of reactivity of T cell malignancies with various CDs.
4. Study of sensitivity various Mo Abs in diagnosing T cell malignancies.
5. Defining the smallest panel of Mo Abs that would diagnose all cases of T cell malignancies.

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Review Of Literature

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Most tissues with specialized functions develop and differentiate during fetal and neonatal life. Ontogeny within such systems is complete early in life and cellular replication, renewal, or replacement only involves mature cells with fully differentiated characteristics. These systems remain static throughout life with respect to their function. In contrast, ontogeny within the lymphocytic system continues throughout life with exposure to each new foreign antigen (Parks and Chisari, 1983).

The marrow and the cortex of thymus are the primary lymphopoietic organs which provide a continuous supply of immature differentiating cells through a process of antigen-independent cellular proliferation. The individual cells produced by these tissues are committed to respond to a single antigen but do not become immuno-competent untill appropriately stimulated by that antigen. The cells produced by the marrow and the thymus migrate to secondary lymphopoietic organs (peripheral lymphatic tissue) where lymphopoiesis continues. The cellular proliferation of lymphocytes in peripheral tissue is predominantly a result of antigen-specific stimulation (Parks and Chisari, 1983).

It is now clear that two quite distinct families of lymphocytes exist in the body. These are T cells which are derived from the thymus and B cells which are derived from the Bursa of Fabricious in birds and possibly from bone marrow in mammals. T cells are concerned mainly with cell mediated immunity and they are present in thymus dependent areas of the lymphoid tissue, namely the white pulp of the spleen around central arterioles and in the para-cortical areas of lymph nodes. B cells on the other hand are concerned with antibody production. B cells are present in the germinal centers of spleen and lymph nodes and

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in lymphoid tissue of the gut (Bellanti and Kadlec, 1985).

STEM CELL ORIGIN OF B AND T LYMPHOCYTES:

Both B and T lymphocytes develop from pluripotential stem cell that can give rise to all different types of hemopoietic cells. Despite their common stem cell origin, T and B cells appear to diverge from other hematogenous elements (and from each other) early in their developmental history (Levitt and Cooper, 1984).

Evidence for the pluripotent stem cells was obtained from studies of the myeloproliferative disorders. It was demonstrated that hematopoietic cells in chronic granulocytic leukemia (CGL or CML)bore a unique chromosomal marker, the Philadelphia or Ph' chromosome (Nowell and Hungerford, 1960). This cytogenetic abnormality was demonstrated not only in the granulocyte precursors, but also in erythropoietic and megakaryopoietic cells of the bone marrow (Whang et al., 1963). After a variable period of time, the majority of patients enter a terminal phase that may resemble an acute blastic leukemia (blast crisis) in which maturation no longer occurs. About one-third of blast phase leukemias have a lymphoblastic morphology (Boggs, 1974) and phenotype that correspond to early B cells (Janossy et al., LeBien et al., 1979, Greaves et al., 1979, Griffin et al., 1983, Bettelheim et al., 1985). Although additional cytogenetic abnormalities are common, the new clone of blast crisis cells always retains the Ph', indicating that it was derived from the original CML stem cell (Nowell, 1976). There is also evidence that B lymphocytes are PH' (Fialkow et al., 1978; Martin et al., 1980).

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Until recently, efforts to identify the PH' chromosome in T lymphocytes or blast crisis with T cell markers have largely been unsuccessful. However, Hernandez et al. (1982), Griffin et al. (1983), and Soda et al. (1985) reported cases of CML blast crisis with T cell phenotypes. Fauser et al. (1985) examined bone marrow cells from a patient with PH'CML in chronic phase. They demonstrated that T lymphocytes, generated from the pluripotential stem cell of this patient were Ph'positive. These observations indicate that CML is a clonal disease affecting a cell that gives rise to all hematopoietic cell lines including B and T lymphocytes, i.e. pluripotent stem cell.

Evidence for the presence of pluripotent stem cell is further indicated by analysis of patterns of expression of glucose-6-phosphate dehydrogenase (G6PD). In the somatic cells of females, one of the two X chromosome-linked genes for G6PD is inactivated early in embryogenesis. Consequently, female heterozygotes for the usual gene (GD B) and a variant such as GD A, have two populations of cells, one in which B-type G6PD is synthesized and one in which A-type enzyme is produced. In G6PD heterozygotes, normal cell populations contain a mixture of both cell types and exhibit both B- and A-type enzymes. Neoplasias of unicellular origin, by contrast, show only a single G6PD (Dow et al., 1985). In heterozygous females with chronic myelocytic leukemia, granulocytes, erythrocytes, platelets, and macrophages were shown to express only one of the two G6PD isoenzymes (Fialkow et al., 1977). Furthermore, in a female with idiopathic sideroblastic anemia, isoenzyme analysis demonstrated a single G6PD isoenzyme in hemopoietic cells as well as T and B lymphocytes (Prchal et al., 1978). These data suggest that the disorders in these patients resulted from mutation of a primitive

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lymphohemopoietic stem cell.

Brout et al. (1983) provided another evidence for the origin of B and T cells from pluripotent stem cells. They studied patients with Tn or polyagglutinability syndrome which is a human non-malignant acquired condition resulting from a somatic mutation. They demonstrated the Tn mutation to be expressed by red cells, platelets, granulacytes, and B and T lymphocytes.

Eventually, mature T lymphocytes are reported to be present in human multipotential (GEMM) hemopoietic colonies in culture (Messner et al. 1981; Fauser et al., 1985).

However, it is not known whether there is a common progenitor for T and B lymphocytes or whether they are direct descendants of pluripotent lymphohemopoietic stem cells. The existance of such a common progenitor is suggested by the occurrence of congenital severe combined immunodefeciencies in which both B and T cells are defecient, while the development of the myeloid series is normal (Calvert et al., 1984). Recently, Bodger et al. (1983) suggested the presence the lymphoid progenitor and described its phenotype (vide infra).

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B CELL DIFFERENTIATION AND ITS RELEVANCE TO VARIOUS TYPES OF B CELL MALIGNANCIES

The study of the stages of differentiation pathawy that lymphocytes pass through to become immunocompetent is now possible using highly specific monoclonal antibodies (Mo Abs) that define cell surface antigens and recombinant DNA technology that identify rearrangement of immunoglobulin and T cell receptor genes, together with the more traditional cell markers such as surface membrane (SmIg) and cytoplasmic immunoglobulin (CIg) in B lymphocytes, sheep erythrocyte receptors on T lymphocytes and cytochemical stains.

From the analysis of the marker profiles of leukemias, it has become apparent that these tumours represent arrested stages of cellular differentiation (Minowada, 1985). For this reason, a number of investigators have proposed schemes of normal hematopoietic differentiation based on the concept that the phenotype of the normal lymphoid cell at each level of differentiation is comparable to the phenotype of its malignant counterpart and hence comparable to the immunologic classification of ALL. Rapid advancements in this area has contiually modified and updated the immunologic classification of ALL and at the same time shed more light on leukocyte differentiation and the cellular origins of leukemias and lymphomas.

Sen and Borella (1975) were the first to apply immunologic markers to the subclassification of ALL. They used receptors for sheep erythrocytes to identify a T cell subgroup (15% to 20% of cases). SmIg was also used to identify a B cell subgroup (5% of cases). Both T and B cell subgroups have an unfavourable

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prognosis (Chessels et al., 1977).

The next important progress in identifying ALL was antigen described by Greaves and his associates (1975), termed the cALL antigen or CALLA. CALLA reactivity identified a common-ALL subclass (70% to 80% of cases) with a more favourable prognosis than T-ALL, B-ALL, or non-T-ALL without CALLA `null-ALL, 5% to 10% of cases' (Chessels et al., 1977).

Vogler et al. (1978) identified a subgroup of c-ALL that express Cu heavy chain but not SmIg. They termed it pre-B-ALL. It appears to have a less favourable prognosis (Crist et al., 1984).

In 1982, Foon et al. summarized the advances in the classification of ALL and related it to the understanding of normal leukocyte differentiation. They subclassified ALL into:

* Unclassified ALL (or null ALL) which represents the lymphoid stem cell and have the phenotype:

(Ia +, CALLA - , B1 -, Cu -, SmIg -, E -, Leu1 -)

* Common ALL which have the phenotype:

(Ia +, CALLA + , B1 +, Cu -, SmIg -, E -, Leu1 -)

Common ALL cells are cells that have not yet acquired the capacity to synthesize cytoplasmic immunoglobulin but have been shown by immunoglobulin gene rearrangements to be committed to B-cell differentiation (Korsmeyer et al., 1981). These data suggest that c-ALL cells may be the earliest B cell (pre-pre-B) stage.

* Pre-B-ALL which most likely corresponds to and have the same phenotype of pre-B cell stage of differentiation:

(Ia +, CALLA + , B1 +, Cu +, SmIg -, E -, Leu1 -)

* B - ALL which represents early B lymphocyte

(Ia +, CALLA +/-, B1 +, Cu -, SmIg +, E -, Leu1 -)

* T - ALL which have the phenotype.

(Ia -, CALLA - , B1 -, CU -, SmIg -, E +, Leu1 +)

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ARRANGEMENT OF IMMUNOGLOBULIN (Ig) GENES AND GENERATION OF ANTIBODY DIVERSITY

The terminally committed B cell, the plasma cell, is capable of secreting large amounts of antibody with extraordinary specificity for antigen. to accomplish this, a B cell must undergo a series of events that allow it to (1) recombine Ig genes encoding the antigen binding specificity, (2) synthesize Ig, (3) embed Ig protein into the cell surface membrane, (4) use surface Ig as an antigen receptor and proliferate in response to antigenic stimulation, and (5) secrete Ig (Lipford and Cossman, 1986).

During the pre-B stage of development, immature B cells acquire the capacity to manufacture a vast number of antibodies with fine specificity for antigen by employing a recombinational mechanism in which the genes encoding the antibody molecule rearrange their positions on the chromosome, thereby greatly expanding the antibody repertoire (Tonegawa, 1983). The genetic information encoding the antigen-binding determinants of Ig protein (the variable region) is composed of multiple gene segments dispersed along the chromosome. In the case of heavy chain gene which is located on chromsome 14 (Croce et al., 1979), rearrangements involve three seperate loci, known as variable (VH), diversity (DH), and joining (JH) regions. These three segments (encoding for the variable region) are then linked to the constant region locus CH (one for each Ig class or subclass). Only variable (VL), joining (JL), and constant (CL) regions are involved in rearrangements for light chains. These light chain genes are found on human chromosome 2 `kappa' and 22 `lambda' (McBride et al., 1982). By selecting one coding region from

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multiple choices for each of these segments and recombinig them into a continuous VH DH JH or VL JL gene, many potential combinations are generated (Tonegawa, 1983).

Further diversity in the variable region amino acid sequences is provided by additional mechanisms, which provide flexibility in the precise nucleotide sequence at the joining sites as well as insertion of nucleotides. This may be the function of the enzyme terminal deoxynucleotidyl transferase (TdT) present in pre-B cells and lost as B-cell maturation progreses (Alt and Baltimore, 1982). In addition, somatic point mutations frequently occur within the V genes following rearrangements (Tonegawa, 1983).

Studies of leukemias of B-cell precursors have demonstrated a sequential hierarchy of rearrangements in which heavy chain genes rearrange prior to light chain genes and kappa light chain rearrange prior to lambda light chain (Korsmeyer et al., 1981). This sequence is reflected by Ig gene expression, since Ig heavy chains are found in the cytoplasm in the absence of light chains in pre- B ALL (Vogler et al., 1978).

These rearrangements can be detected by Southern blot analysis of DNA from B cells using appropriately radiolaelled heavy or light chain probes. In a monoclonal neoplastic proliferation of B cells, the Ig genes which rearranged will all have undergone the same rearrangement and this can be readily detected by gene analysis. However, no Ig gene rearrangements are detected in polyclonal B cell proliferations, since no single rearranged pattern occur at a sufficiently high frequency. The demonstration of Ig gene rearrangement thus provides evidence that a lymphoproliferative disorder is both monoclonal and B cell derived (Arnold et al., 1983). Heavy chain rearrangements have

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