IMMUNOLOGICAL CHARACTERIZATION OF CHRONIC LYMPHOCYTIC LEUKAEMIA

T H E S I S

Submitted by

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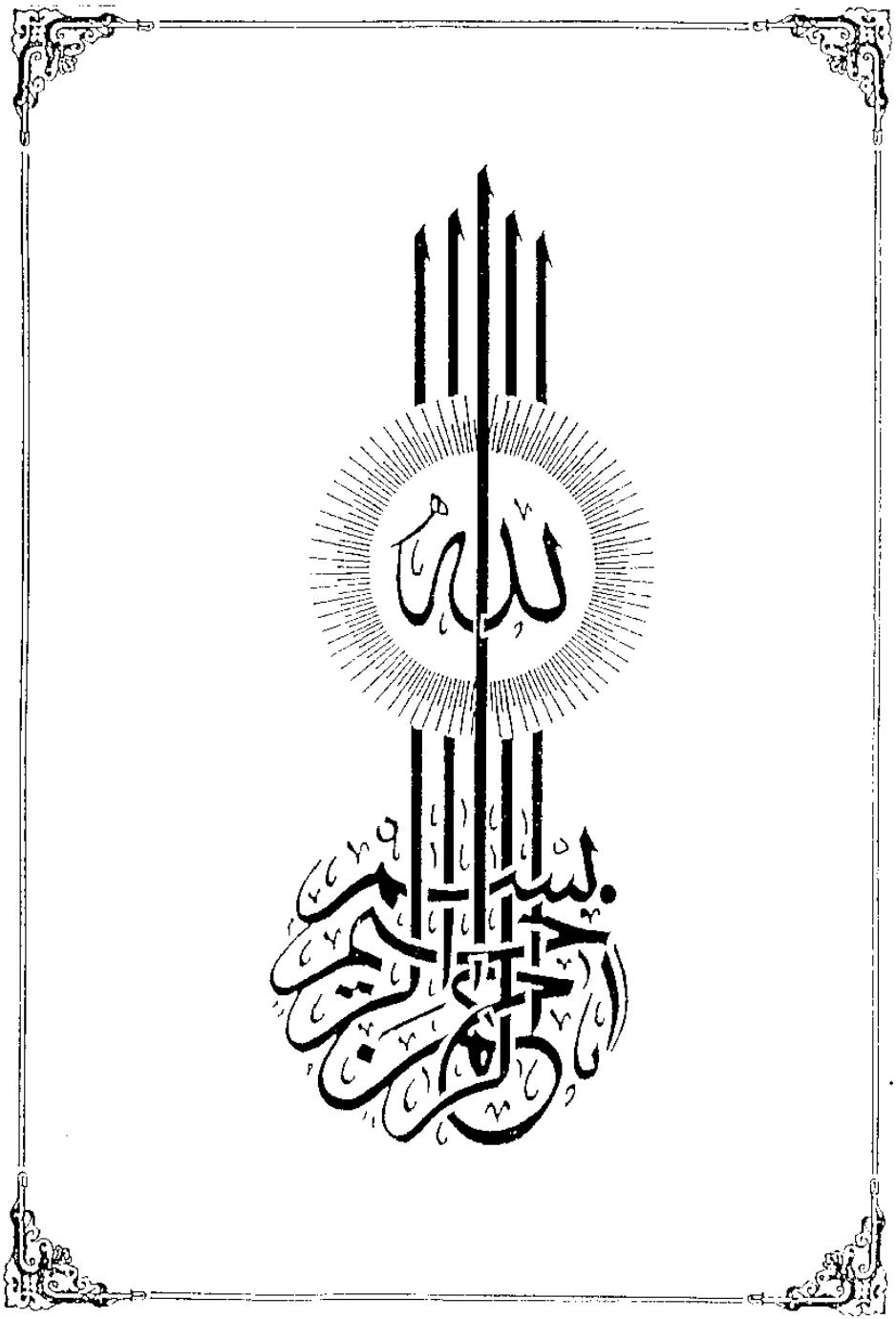

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LIST OF ABBREVIATIONS

Ag	:	Antigen
α	:	Alpha heavy chain
CD	:	Cluster of differentiation
CLL	:	Chronic lymphocytic leukaemia
CLL/PL	:	CLL with more than 10% prolymphocytes
CyIg	:	Cytoplasmic immunoglobulin
DPFL	:	Diffuse poorly-differentiated follicular lymphoma
DWFL	:	Diffuse well-differentiated follicular lymphoma
EAC	:	3-amino 9-ethyl carbazole
E rosette	:	Sheep erythrocyte rosette
γ	:	Gamma heavy chain
Hb	:	haemoglobin
Ia	:	Class II histocompatability complex antigens
Ig	:	immunoglobulin
k	:	Kappa light chain
λ	:	Lambda light chain
LDT	:	lymphocyte doubling time
LFA-1	:	Lymphocyte function associated glycoprotein-1
LGL	:	Large granular lymphocytes
M rosette	:	(M-RBCs)-Mouse erythrocyte rosette
PAP	:	Peroxidase anti-peroxidase
PBS	:	Phosphate buffered saline
PLL	:	Prolymphocytic leukaemia
PROL	:	Prolymphocyte
SmIgs	:	Surface membrane immunoglobulins
TCR	:	T-cell receptor
TdT	:	Terminal deoxynucleotidyl transferase
Th	:	(Tu or T4)-Helper T-lymphocyte
TLC	:	Total leucocytic count
T_s	:	(To or T8)-Supressor T-lymphocyte
VH region	:	Variable region in heavy chain
VL region	:	Variable region in light chain

INTRODUCTION
AND AIM OF WORK

Chronic lymphocytic leukaemia (CLL) is a lymphoproliferative disorder in which a clonal expansion of small lymphocytes accumulate in the marrow, lymph nodes, blood, spleen, liver and sometimes other organs. The CLL cell is the neoplastic counterpart of an immunologically immature and incompetent lymphocyte (**Williams et al.; 1990**). In over 90% of cases, the clonal expansion is of B-cell lineage while in less than 5%, a proliferation of T-cells is found (**Reijden et al.; 1982**).

B-CLL has always been recognized as a highly variable disease, which includes patients with a slowly progressive evolution and others with a more aggressive illness. The source of this variability may be referred to different clinical stages, different course of the disease within these stages and the confusion of B-CLL with other malignancies (**Melo et al.; 1986**).

Study of the immunologic markers in such patients may prove to be helpful in the definition of certain entities, *e.g.;* B- and T-subsets with distinct markers and functions (**Schroff et al.; 1982**). Furthermore, it is now known that T-cells play an important role in B-cell differentiation. The relevance of T-

lymphocytes and T-subsets in the pathogenesis of B-CLL and some of its complications had been studied by many workers but still under controversy (Lauria et al.; 1983 and Mills et al.; 1987).

Since this disease is usually accidentally discovered, and its incidence in Egypt was found to be relatively higher than we expect (Sherif and Zarate; 1987), the aim of the present work is to study immunologic markers in CLL among Egyptians in order to correlate between these data and clinical and haematological findings.

A trial will be done to find out if cell markers can provide a remarkable aid in prognosis and to be used as an index for follow-up with therapy.

REVIEW OF LITERATURE

I. BLOOD LYMPHOCYTES

The humoral arm of the immune system consists of B-cells which have the capacity to produce immunoglobulin in response to a stimulus. Regulation of this system is controlled by macrophages and more significantly by T-cells. T-cells fine tune the system, with some cells ``helping`` immunoglobulin production and others ``suppressing`` it. Some T-cells participate in the cellular arm of the immune system. Appropriately armed ``cytotoxic`` T-cells can kill certain targets; regulation of this process also requires other T-cells including ``inducer`` cells. Still other cells not easily put in the B- or T-cell category perform other types of the cytotoxic functions (Abo and Balch; 1981).

Using monoclonal antibodies, some of the cell surface antigens or markers allow the characterization of B- and T- lymphocytes (Brooks et al.; 1980).

Life Span Of Circulating Lymphocytes:

The circulating lymphocytes can be divided into two groups according to their life span. The first includes the long lived lymphocytes (life span from few months to five years) and these constitute about 65-85 % (Little et al.; 1962). They are mainly T-cells in the resting phase ``Go`` (Baserga; 1981). The second short lived category (life span from few hours to five days) constitutes the remaining 15-35 % of circulating lymphocytes and include the remaining T-, B- and non T-, non B- cells (Leucocyte Typing III; 1988).

Classification Of Surface Markers :

Surface markers can be conventially classified into two general groups :receptors and antigens. Although this classification is artificial, it is useful in studying different types of markers (Koepke et al.; 1984). Receptors are molecules on cells which have particular affinity for a specific compound or group of compounds. The best example of receptors on cells are hormone receptors whose function in the mechanism of action of

hormone well known. In contrast, haemopoietic cell receptors don`t have clearly defined functions. The best known lymphocyte receptor is probably the sheep erythrocyte receptor (Erosette receptor) on T-cells (Kaplan and Clark; 1974).

The examples of haemopoietic receptors include F_c portion of immunoglobulin molecule (Christensson and Biberfield; 1978). Complement components (Cossman and Jaffe; 1981) and surface immunoglobulin of B-lymphocytes (sIg) (Gathings, et al.;1977). Although it acts as a receptor, sIg is considered surface antigen because it is always detected with an anti-immunoglobulin antibody (Warner; 1974).

Surface antigens are defined as any molecule on the surface of cells to which antibodies can be made. These antibodies when labelled, serve as probes used to recognize the antigen and thus a particular type of cells. It should be recognized that the antigen`s presence may not have anything to do with the ability of cells to carry out a specific function. In contrast to receptor, antigen is defined by its antibody reactivity and not by its function (Koepke et al.; 1984).

Surface Membrane Markers Of Blood Lymphocytes :

Normal blood lymphocytes possess many membrane antigens or markers which allow the differentiation and characterization of B- and B-Lymphocytes using monoclonal antibodies. The *International Workshops On Leucocyte Differentiation Antigens* have grouped the available monoclonal antibodies into many clusters of differentiation (CD units) (Milstein; 1987).

Surface Membrane Markers Of B- and T-Lymphocyte Lineage :

The lymphocytic progenitor cells arise from the marrow stem cell that share a common lineage with the haemopoetic system. This progenitor cell differentiates sequentially into T- and B-lymphocyte subsets (Cooper; 1987).

B- cell Antogeny :

The very first stages of B-cell development occur in the fetal liver during the 8^{th} to 9^{th} weeks of gestation. Shortly

thereafter, migration to the spleen and bone marrow occurs. In later life, the bone marrow becomes the main repository for precursor B-cells (Cooper; 1987).

The differentiation of B-cell precursors passes by two stages. In the first stage, the stem cell is transformed into pre B- and then to mature B-lymphocyte in a resting phase. This process is completely antigen independent (Roitt; 1988). The various stages of B-cell development are marked by the expression of different proteins on their surface. Pre B-cells with cytoplasmic IgM positive and surface Igs negative ($CyIg^{+}sIg^{-}$) phenotype first appear in the fetal liver in the 8^{th} week. $SIgM^{+}$ B-cells appear 1 week later. By the 12^{th} week and throughout the fetal life, pre B-cells are found in large number in the bone marrow (Cooper,; 1987).

The second stage of differentiation moves the B-cell from the resting stage to a phase in which the responding clone increases in number and each cell arranges its synthetic machinery to produce specific antibody (Calvert et al.; 1984). The process is initiated by two steps (signals); one occurs at the moment of