S_M ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS (IMMUNOFLUORESCENT STUDY)

THESIS

Submitted By

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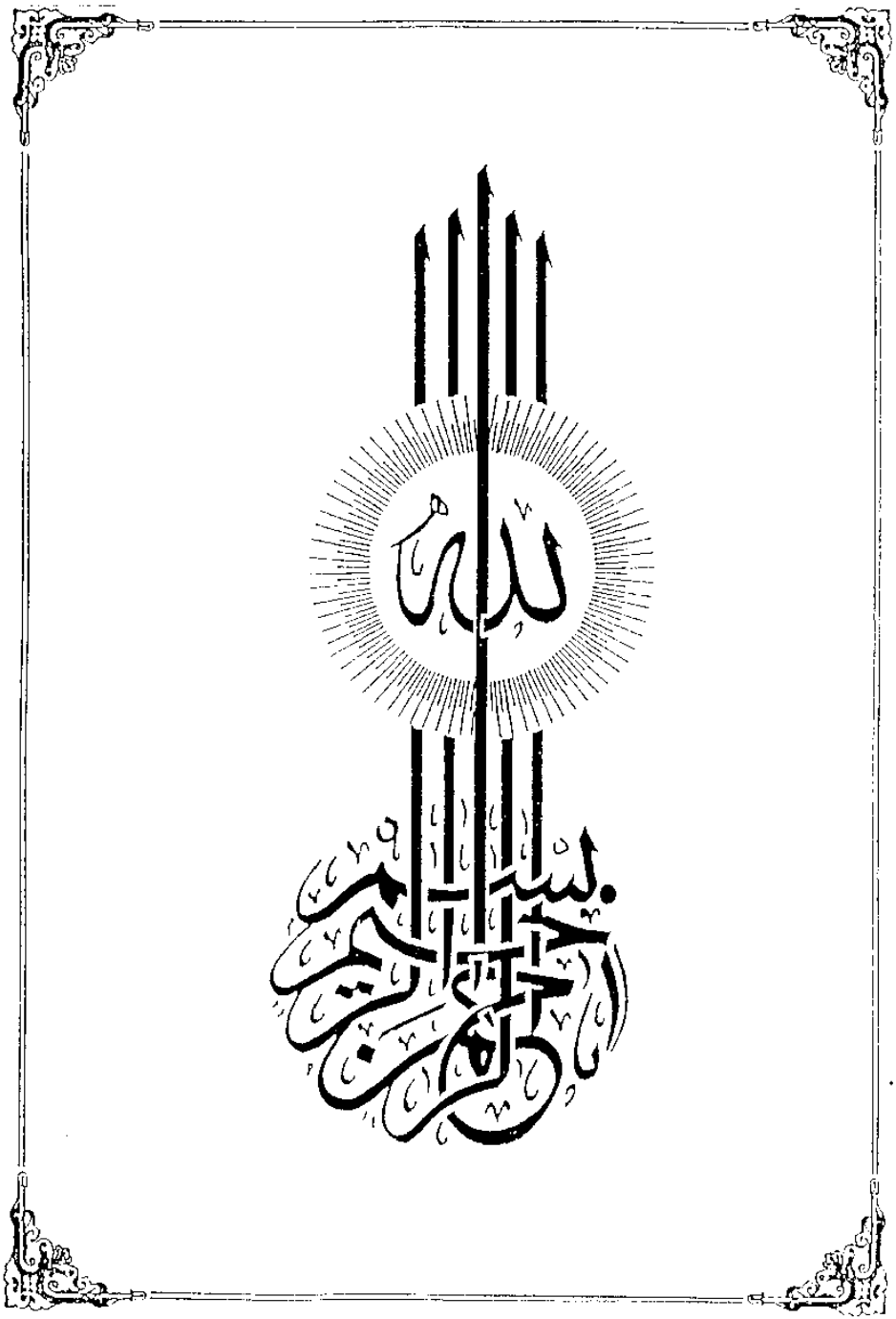
MANAL HASSANEIN



LIST OF ABBREVIATIONS

ANA : *Antinuclear antibody.*

ARA : *American Rheumatism Association.*

CBP : *Complete blood picture.*

CF : *Complement fixation.*

CIE : *Counter immunoelectrophoresis.*

CRP : *C-reactive protein.*

CTD : *Connective tissue disease.*

CTL : *Cytotoxic T lymphocyte.*

DAT : *Differential agglutination titer.*

DM : *Dermatomyositis.*

DNA : *Deoxyribonucleic acid.*

DNase : *Deoxyribonuclease.*

DR : *D-related.*

DRw : *D-related workshop.*

DNP : *Deoxy ribonucleoprotein.*

ELISA : *Enzyme linked immunosorbent assay.*

ENA : *Extractable nuclear antigen.*

ESR : *Erythrocyte sedimentation rate.*

Fc : *Crystalizable fraction.*

HA : *Hemagglutination.*

HLA : *Human leukocyte antigen.*

ID : *Immunodiffusion.*

IF : *Immunofluorescence.*

Ig	: *Immunoglobulin.*
JRA	: *Juvenile rheumatoid arthritis.*
LE	: *Lupus erythematosus.*
MCTD	: *Mixed connective tissue disease.*
MHC	: *Major histocompatibility complex.*
PM	: *Polymyositis.*
PSS	: *Progressive systemic sclerosis.*
RA	: *Rheumatoid arthritis.*
RANA	: *Rheumatoid arthritis nuclear antigen.*
RIA	: *Radioimmunoassay.*
RNA	: *Ribonucleic acid.*
RNase	: *Ribonuclease enzyme.*
RNP	: *Ribonucleoprotein.*
RW	: *Rose Waaler.*
SLE	: *Systemic lupus erythematosus.*
SS	: *Sjögrens syndrome.*
T_H	: *Helper T cell.*
T_S	: *Suppressor T cell.*
TSH	: *Thyroid stimulating hormone.*

Introduction

INTRODUCTION

Antinuclear antibodies (ANA) are among the immunologic features observed in patients with rheumatic diseases. ANA are specific for many different nuclear constituents , including deoxyribonucleic acid (DNA) , deoxyribonucleoprotein (DNP) , histone , ribonucleic acid(RNA),nucleolar materials, and antigens in the soluble nuclear extract which are generally known as extractable nuclear antigens (ENA). The soluble nuclear extract contains different antigens. The Sm antigen reported by Tan and Kunkel (1965) , and the nuclear ribonucleoprotein antigen (RNP) described by Mattioli and Reichlin (1971). The common methods to detect the reactions between these soluble nuclear antigens and their corresponding antibodies are double immunodiffusion (ID) in agar plates , complement fixation (CF) , passive hemagglutination (HA) , and immunofluorescence (IF) (Akizuki et al , 1977).

Antibodies to Sm antigen (a nuclear acidic protein) were found almost exclusively in serum of patients with systemic lupus erythematosus (SLE) , suggesting that this antibody might be a " marker " antibody for this disease. Antibodies to Sm are non-

organ - specific and give a speckled pattern of immunofluorescence on ANA testing (Harmon , 1985).

The ENA (Sm and RNP) have been difficult to distinguish , because they exhibit similar properties , including the speckled pattern by IF. In clinical practice they have been distinguished by the greater RNase sensitivity exhibited by RNP (White et al. , 1982).

The aim of the work is :

To study the prevalence of different antinuclear antibodies in SLE , RA and normal humans by indirect immunofluorescence and serial dilutions.

The effect of dilutions on nuclear staining pattern which may change on progressive dilutions of sera due to the presence of different types of antinuclear antibodies as suggested previously by some workers: Tan (1967) and Northway & Tan (1972).

Since , the occurrence of multiple antibodies showed that , when more than one antibody was present, the probability of SLE was very high (90%).

Detection of antibodies to Sm by indirect immunofluorescence in SLE , RA Egyptian patients and in normal humans , which was not studied before. The presence of anti-Sm antibodies can serve as useful serologic marker for SLE and is an important diagnostic aid to the clinician.

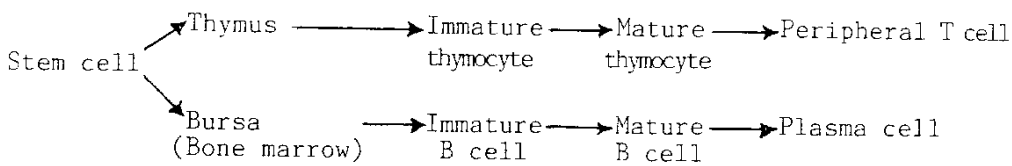
Review of Literature

A. THE IMMUNE SYSTEM AND AUTOIMMUNITY

IMMUNE SYSTEM

The immune system is an extremely complicated one with a variety of roles in maintaining homeostasis and health. Like the endocrine system , it exerts control within the body by virtue of circulating components capable of acting at sites far removed from their point of origin (Katz , 1982).

The lymphoid system is responsible for immune responses and consists of T lymphocytes (thymus dependent ; pass through the thymus during their development) and B lymphocytes (bursa equivalent ; thymus independent). Both T and B lymphocytes are derived from stem cells in the bone marrow and are distributed to the blood and all other parts of the lymphoid system. The differentiation of the thymus derived T lymphocytes and bone marrow derived B lymphocytes as follow :



(Rodnan and Schumacher , 1983).

The B lymphocyte is the central component of

the humoral immune system. It has surface receptors - antibody molecules - that interact with antigen and initiate the transformation of the B lymphocyte (via a succession of intermediate cells) to plasma cells , which produce five major types of immunoglobulin (Ig) (Fig. 1). The basic structure of Ig (Antibody) as in (Fig. 2) (Koffler , 1979).

Certain key factors are important for a basic understanding of the B cell system :

B lymphocytes are genetically programmed to interact with antigen (clonal selection theory). A clone of these cells is stimulated only by antigens that " recognize " and interact with particular antibody receptors on the B cell surface.

The proliferation of antibody-forming B lymphocytes is influenced by T lymphocytes , which can either inhibit or stimulate antibody formation.

B cells may be non specifically stimulated to produce antibodies by substances such as endotoxin (Koffler , 1979).

T lymphocytes also derived from precursor cells in the bone marrow. They pass through the thymus in the process of maturation , and they appear to be activated by thymic hormones. T lymphocytes mediate

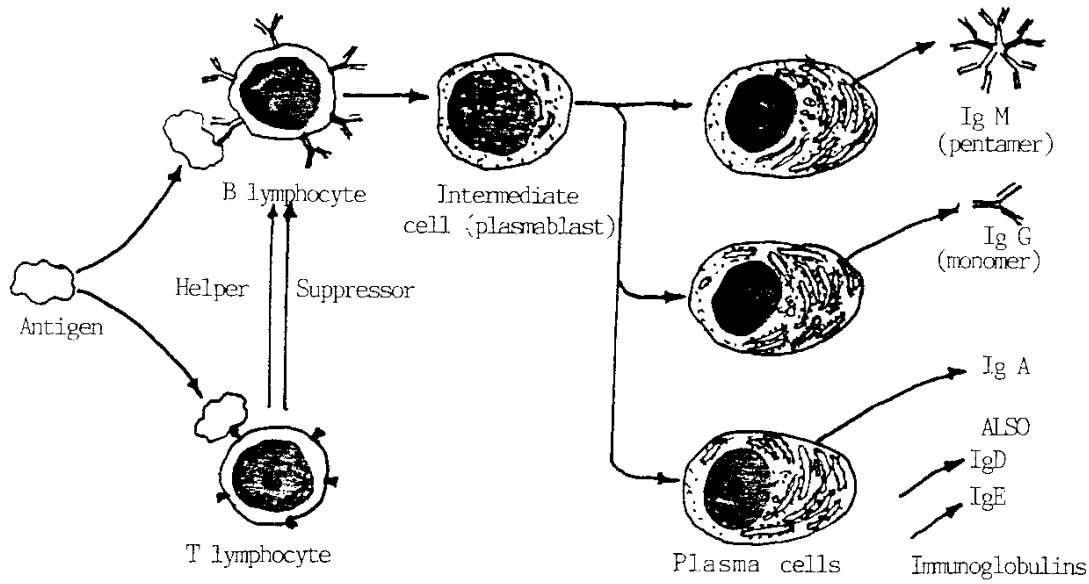


Fig. (1): Immunoglobulin (Antibody production)
(Koffler , 1979)

The basic immunoglobulin molecule consists of 2 heavy (H) chains and 2 light (L) chains held together by disulfide (SS) bonds. The heavy chains have a hinge region which demarcates the antigen-binding segment (Fab) from the crystalizable fraction (Fc). The heavy and light chains both are comprised of constant (C) regions where the amino acid sequence is fairly constant and variable (V) regions where the sequence varies. The heavy chains have 3 constant domains (C_H1 , C_H2 , C_H3) and 1 variable domain (V_H). The light chains have 1 constant domain (C_L) and 1 variable domain (V_L).

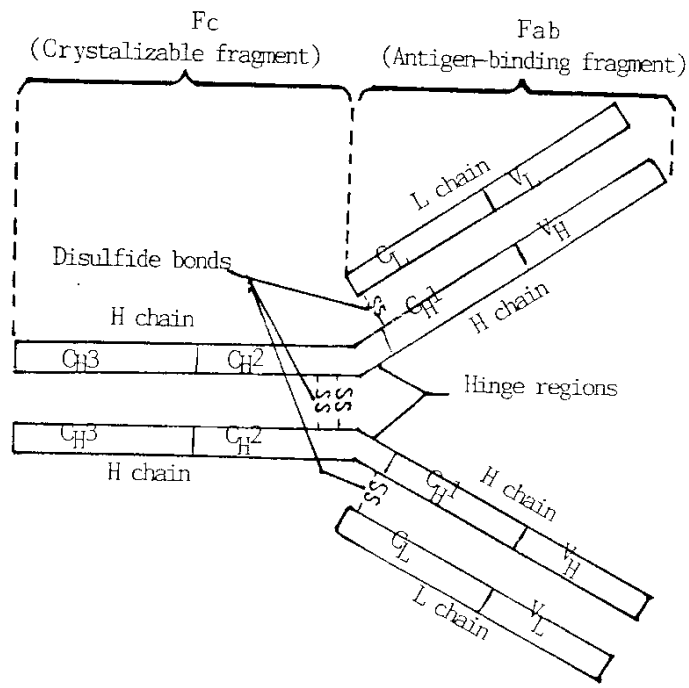


Fig. (2): Basic structure of immunoglobulin (Koffler , 1979).

cellular immunity. The T cell surface has specific antibody like receptors , which are not as readily demonstrated as those on B cells.

Upon reaction with an antigenic determinant specific for its surface receptors , the T cell releases a variety of lymphokines , such as cytotoxins , which combine with cell membranes and are generally toxic to cells ; chemotactic agents , which attract inflammatory cells ; inhibitory factors , which retard the migration of certain inflammatory cells ; and antiviral substances , as demonstrated in vitro. T cells also influence the production of antibodies by retarding or facilitating the proliferation of B lymphocytes (Cantor and Boyse , 1977 ; Reinherz and Schlossman , 1980).

Regulation interaction in immune response (Fig.3): Helper T cell (T_H) capable of exerting positive regulatory effects on B cells and stimulating their differentiation into fully mature antibody-secreting plasma cells. T_H cells also exert positive regulatory influences on precursors of T cells destined become cytotoxic T-lymphocytes (CTLs). The second category of regulatory T lymphocytes , known as suppressor cells (T_S). Ts cells can exert negative regulatory