AUTOANTIBODIES IN IDIOPATHIC THROMBOCYTOPENIA

THESIS

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بسم الله الرحمن الرحيم

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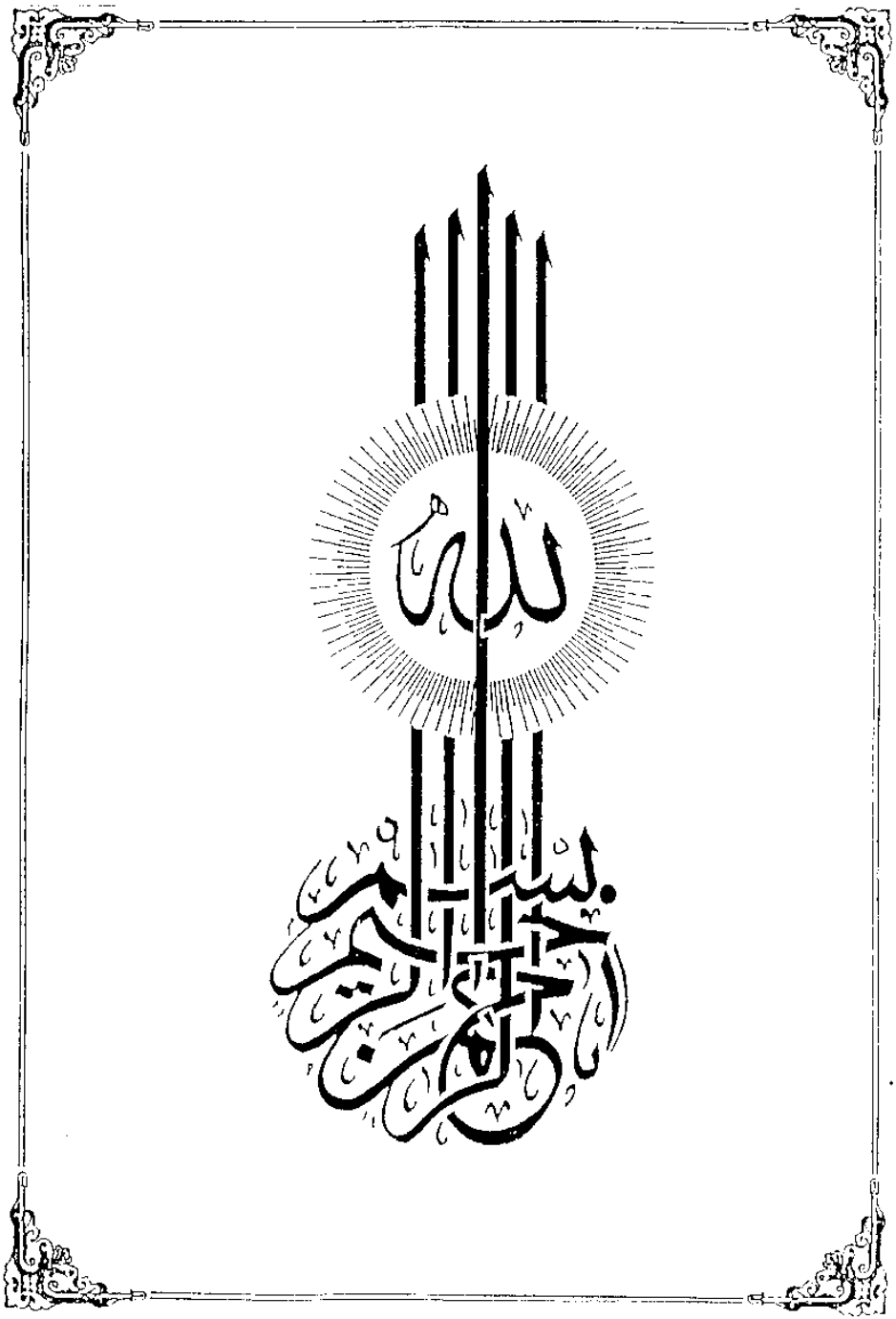
صدق الله العظيم



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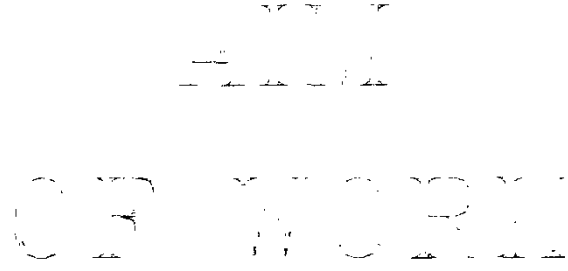
List of Abbreviations

ANA	= antinuclear antibody.
C	= complement.
Fab	= fraction antigen binding portion of the immunoglobulin.
Fc	= crystallizable fraction of the immunoglobulin.
GP	= glycoprotein
HLA	= human leucocyte antigen system
Ig	= immunoglobulin
ITP	= idiopathic thrombocytopenic purpura
MHC	= major histocompatibility complex
nDNA	= native DNA
PAIg	= platelet associated immunoglobulin
PL Abs	= platelet antibodies
SLE	= systemic lupus erythematosus
Tu	= T helper
Ty	= T suppressor
vWF	= von Willebrand factor

TABLE OF CONTENTS



AIM OF THE WORK

Chronic idiopathic thrombocytopenic purpura (ITP) in adults is usually of insidious onset and is characterized by increased platelet destruction due to antiplatelet antibodies (*Szatkowski et al., 1986*). Platelet associated antibodies to platelet membrane glycoproteins can be detected in approximately 75% of patients and serum autoantibodies in about two thirds (*McMillan et al., 1987; Bussel, 1990*).

In children, two forms of ITP occur. A syndrome similar to adult chronic ITP and an acute form of ITP. Several investigators have noted increased platelet associated IgG in acute and chronic ITP, but there are few data on the presence of autoantibodies (*Berchtold et al., 1989*).

Some children and adolescents with ITP develop other autoantibodies and even systemic autoimmune disease, particularly, lupus erythematosus (*McIntosh et al., 1981; Karpatkin, 1985*). In systemic lupus erythematosus (SLE), the presence of autoantibodies is related to defect in immunoregulatory T lymphocytes and family members of affected patients have shown increased expression of autoimmune phenomena and disease (*Miller and Schwartz, 1979*).

Chronic ITP has been associated with immunologic abnormalities including both humoral and cell-mediated immunity. Also, some immunologic abnormalities have been found in relatives of patients with chronic ITP. This has been interpreted as an evidence of a hereditary tendency to develop the disorder *(Stuart et al., 1978)*.

The aim of this study was to evaluate the level of serum immunoglobulins (IgG, IgM and IgA) and serum complement components (C3 and C4) in patients with chronic ITP and their relatives to detect any immunologic abnormality in these patients or their relatives. Also, a trial was done to detect the presence of circulating serum platelet autoantibodies and other serum autoantibodies in patients with chronic ITP and their relatives to establish the autoimmune nature of this disorder, in both children and adults. Also, the relationship between human leucocyte antigens (HLA antigens) and chronic ITP was studied, in order to detect any association between HLA antigens and chronic ITP

REVIEW OF LITERATURE

TOLERANCE AND AUTOIMMUNITY

TOLERANCE

The term tolerance defines a state of specific immunological unresponsiveness to an antigen which arises after an initial encounter with the antigen. Immunological unresponsiveness arising as a result of administration of exogenous antigen is termed "acquired immunological tolerance" whereas the unresponsiveness to endogenous antigens is termed "natural immunological tolerance or self tolerance" (*Male et al., 1987*).

MECHANISMS OF TOLERANCE INDUCTION

1- Repertoire Purging

It is now widely agreed that antibody formation rests on the selective activation and clonal expansion by antigen of B cells with appropriate receptors, and most, though not all observers believe that the T-cell system works through clonal selection as well (*Nosal, 1987*).

Burnet (1957) postulated that immune tolerance to self antigens is subserved by a mechanism that causes fetal immunocytes to be deleted by contact with their specific autoantigens (clonal deletion). *Lederberg (1959)* refined this notion to suggest that all lymphocytes passed transiently through an obligatory tolerizable state before becoming inducible by antigen, and thus self-reactive cells were never allowed to mature.

Nosal and Pike (1975) refined and expanded the above theory. They coined the terms "clonal abortion", "clonal anergy", "clonal silencing" and "clonal purging", all to describe the same event. They proposed that at some stage in their differentiation from stem cells to mature antibody forming precursor cells, B lymphocytes go through a phase during which contact with even low doses of antigen (whether exogenous or endogenous) induces tolerance and not immunity. Further experiments showed that early contact with antigen had rendered the cells refractory to positive signalling, without having actually killed the cells. They have then and subsequently used the phase "clonal anergy" as a more appropriate description *(Nosal and Pike, 1980)*.

Nosal (1983 and 1987) studied the concept of clonal anergy extensively chiefly, for B cells but also for T cells. As far as the B cell is concerned, it is clear that the sensitivity to such negative signalling is greatest when the cell's IgM receptors are just emerging on the plasma membrane, that is during the pre-B to B cell transition. At this stage, surprisingly low concentrations of the antigen can be effective *(Nosal, 1987)*.Immaturity is not an absolute prerequisite for down-regulation. In fact, normal adult hapten-specific B cells can be rendered anergic in vivo or in vitro, but by much higher concentrations of antigen. Even some mature antibody-forming cells can be

caused to stop forming antibody, admittedly by high concentrations of highly multivalent antigens. This phenomenon is termed effector cell blockade (*Schrader and Nosal, 1974*). There is, however, a very large quantitative gulf separating this from the induction of clonal anergy in the immature cells (*Nosal, 1987*).

As regards the T cell, many authors believe that there is a major censorship function exercised in the thymus itself, which prevents auto-destructive T cells from maturating and migrating, but permits cells with the capacity to recognize a foreign antigen X in association with an MHC restriction element to pass the gate. It is probable that some positive selective step is required here, as the selfness of the restriction elements is learnt, not genetically determined (*Nosal, 1983, 1987*).

However, during the last two decades, several groups of investigators succeeded in challenging clonal deletion as a general explanation for tolerance to self by : (1) inducing autoimmune disease by injecting organ extracts; (2) demonstrating the presence of numerous autoantibodies in normal serum coming from a normal population; (3) demonstrating the presence of normal autoreactive B cells; (4) inducing in an animal numerous autoantibodies after

challenging with mitogens *(Hooper et al., 1972; Robert et al., 1973; Mackay, 1983)*.

2- T Suppressor Cells

Most immunologists favor a central role for T cell mediated suppression in the induction and maintenance of self tolerance *(Dorf and Benacerraf, 1984; Gibson et al., 1985)*.

Interesting experiments have shown that memory T suppressor cells exist. They have also shown that tolerogenic doses of antigen could induce T suppressor cells and their maintained activity could be guaranteed by the development of memory T suppressor cells following subsequent exposure to low doses of the tolerogen *(Male et al., 1987)*.

It is thus possible that tolerance could be achieved by a combination of clonal deletion and T suppressor activity *(Good and Nosal, 1983)*. In a model of adult tolerance to hapten-plus-self MHC, used by many as a standard suppression system,clonal analysis showed substantial functional clonal deletion of cytotoxic T lymphocyte precursors to coexist with a suppressor state *(Nosal, 1983)*.

Suppressor T cells have also been found which are idiotype specific. They specifically suppress the production of a particular idiotype, presumably by direct interaction with B cells producing this idiotype. So, in the context of tolerance it can be seen that if an immune response is dominated by expression of a particular idiotype, idiotype-specific T suppressor cells could play an important role in maintaining a state of tolerance to an antigen *(Male et al., 1987)*.

3- Defects in the Afferent Limb of the Immune System

Some view tolerance as mainly due to defects in the afferent limb of the immune response. They argue that many self antigens never enter the afferent limb of immune induction effectively through reasons of accessibility and processing *(Nosal, 1987)* .For example, membrane associated antigens of great importance in autoimmunity, such as the nicotinic acetylcholine receptor at the nerve-muscle junction, exists as integral membrane proteins on the surface of cells negative for class II MHC antigens. In their natural state, such antigens cannot engage the interest of T cell receptors. Indeed, it has been argued that when class II expression is aberrantly induced, the stage is set for autoimmune activation directed against such antigens *(Bottazoo et al., 1983)*. Also, many