PLATELET ALLOANTIBODIES

(Review)

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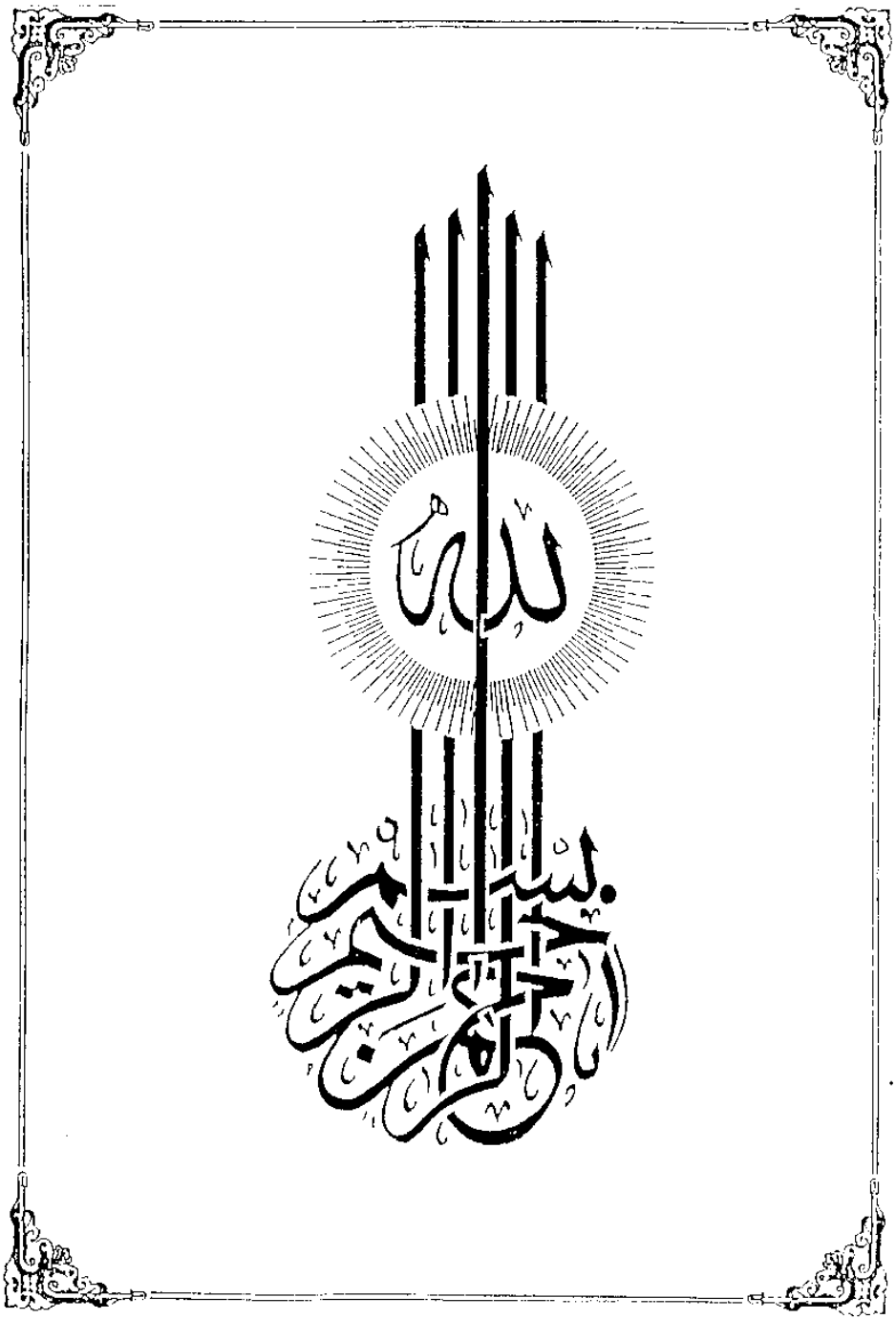
The Candidate



ABBREVIATIONS

ACD	: Acid Citrate Dextrose.
ADP	: Adenosine Di-Phosphate.
Allo-NT	: Alloimmune Neonatal Thrombocytopenia.
ANLL	: Acute Nonlymphocytic Leukaemia.
ATP-ase	: Adenosine Triphosphatase.
BSA	: Bovine Serum Albumin.
EDTA	: Ethylene Diamine Tetra-Acetic Acid.
FITC	: Fluorescein Isothiocyanate.
GIFT	: Granulocyte Immunofluorescence Test.
HLA	: Human Leucocyte Antigen.
ITP	: Idiopathic Thrombocytopenic Purpura.
LCT	: Lymphocytotoxicity Test.
LIFT	: Lymphocyte Immunofluorescence Test.
MD	: Multiple Donor.
NAITP	: Neonatal Alloimmune Thrombocytopenic Purpura.
PAGCT	: Platelet Antiglobulin Consumption Test.
PAGE	: Polyacrylamide Gel Electrophoresis.
PA-IgG	: Platelet Associated IgG.
PAT	: Platelet Agglutination Test.
PBS	: Phosphate - Buffered Saline.
PGT	: Platelet β-Galactosidase Test.
PIFMP	: Platelet Immunofluorescence Microphotometry.
PSIFT	: Platelet Suspension Immunofluorescence Test.
PTP	: Post-Transfusion Purpura.
R-E-System	: Reticulo-Endothelial System.
RIA	: Radio-Immuno Assay.
SD	: Single Donor.
SDS	: Sodium Dodecyl Sulphate.

CONTENTS

INTRODUCTION

Platelet Morphology & Ultrastructure

Platelets are non nucleated, discoid or elliptical in shape, on average 3 μ in length & 0.5 to 1 μ in thickness. On dark-ground microscopy they appear colourless and moderately refractile with few immobile granules in the center of the cell. By phase - contrast microcinematography, contractile vacuoles & vacuoles of pinocytosis have been noted (Thompson, 1977). On electron microscopy the platelet was found to be an extremely complex system of membranes, microfilaments, microtubules & organelles. The platelet can be divided into 3 zones namely:-

1- The peripheral zone.

2- The [illegible] zone.

3- The [illegible] zone. (Fig. 1).

[illegible]:-

[illegible]

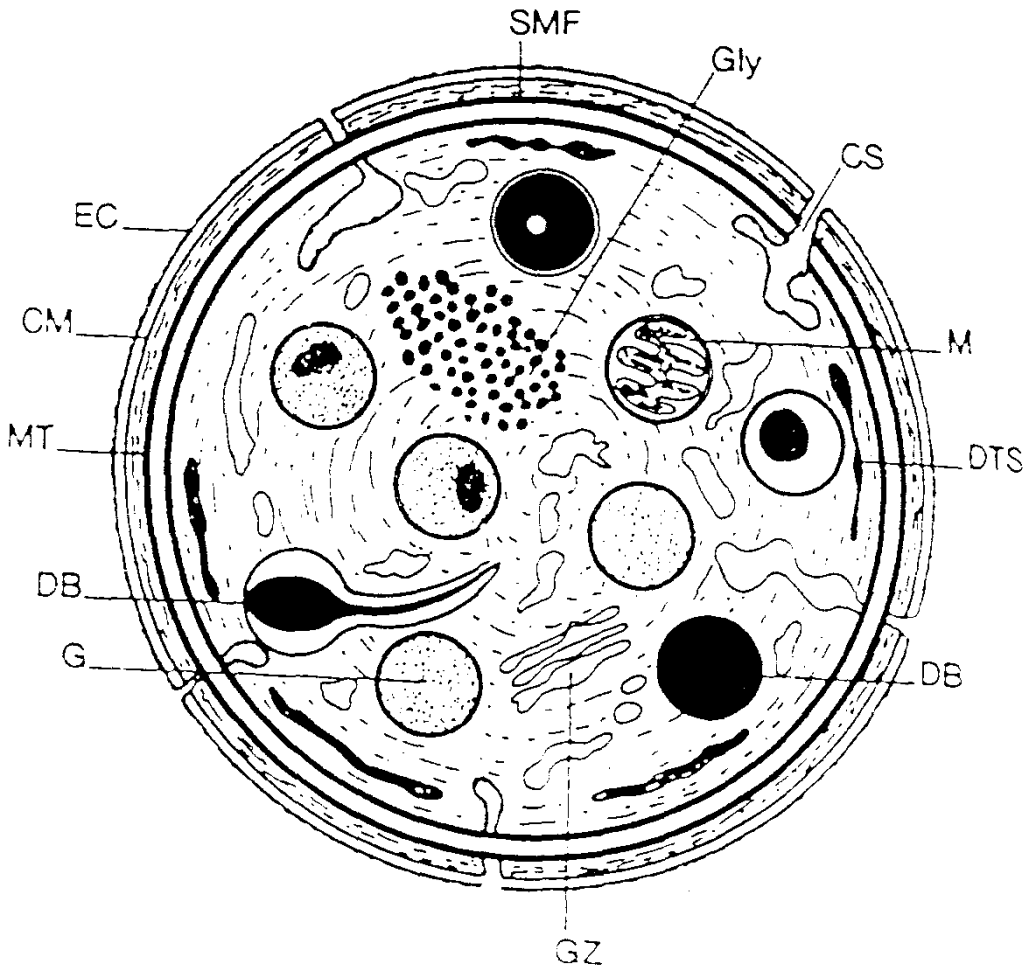


Fig. (1): Diagram to illustrate platelet structure. In the peripheral zone is the exterior coat (EC), the trilaminar cell membrane (CM) and the submembrane filaments (SMF). The organelles and particles consist of the platelet granules (G), the dense bodies (DB), the mitochondria (M), the Golgi zone (GZ), the dense tubular structures (DTS) and glycogen granules (Gly). The system of open channels is indicated by CS.

immediate contact with the surrounding plasma. The coat material is 150 - 200 A in thickness and covers the cell membrane and the linings of the tortuous canalicular system penetrating the platelet substance. It contains acid mucopolysaccharides & glycoproteins. Magnesium - dependent ATP-ase is also present. Adsorbed plasma proteins are important constituents of the exterior coat (White, 1977). This coat material remains on platelets before, during, & after aggregation. The exterior coat is the adhesive site of the platelet. In the submembrane area there are bundles of microtubules which ring the platelet, these microtubules are formed of microfilaments. Both appear to be helical in structure. The microtubules are coiled & appear to exert a spring-like effect on the inside of the cell membrane & maintain the characteristic platelet shape. Treatment of the platelets with microtubule - disrupting agents like colchicine & vincristine leads to loss of shape. The microtubules may orient the wave of contraction during the discharge of platelet contents under appropriate stimuli (Wintrobe *et al.*, 1975; Thompson, 1977).

2. The sol-gel zone:-

Is a gel-like matrix in which the organelles are embedded. There are also masses of microfilaments in the cytoplasm which are involved in platelet contraction. Their appearances are like those of partially purified thrombosthenin, the actinomycin - like protein isolated from the platelets. The open canalicular system is a network of vesicles & interconnecting channels which ramify throughout the entire cytoplasm & communicate with the cell surface (White, 1977).

It may be the route by which the products of platelet secretion reach the exterior as well as a pathway for the uptake of plasma borne substances to the platelet organelles (White, 1977).

3. The organelle zone:-

A variety of formed organelles & particulate elements are embedded in the sol-gel matrix of the platelets, these include the alpha granules, the dense bodies & other storage granules of various size & shape (Thompson, 1977). Dense bodies are relatively few in number. They are the primary secretory organelles of the platelets. During the internal

transformation following exposure to aggregating agents, some dense bodies move towards the platelet surface while granules are shifted to the cell centers. Dense bodies rapidly decrease in number during early viscous metamorphosis and are absent from platelets in late metamorphosis and clot retraction. Serotonin, ADP & catecholamines have been associated with dense bodies, (White, 1977). Alpha granules serve as storage site for platelet fibrinogen, platelet factor 4, β-thromboglobulin and platelet derived growth factor (Kaplan et al., 1979). Mitochondria are not numerous but of vital metabolic importance. They maintain the energy requirements of the platelets in the absence of anaerobic glycolysis (Thompson, 1977; Wintrobe et al., 1975). In addition to their metabolic activity platelet mitochondria may function as platelet repositories similar to mitochondria of the smooth muscle (White, 1977). Golgi apparatus is present in a few platelets and is probably derived from the megakaryocyte (White, 1977). In addition there is a dense tubular system which is usually associated with the peripheral rings of microtubules. It may play a role in the formation of microfilaments. Numerous Glycogen granules are also present.

PLATELET ANTIGENS

Platelet Antigens

Platelet antigens can be classified into 2 groups:-

1) Platelet specific antigens.
2) Antigens shared by other cells.
   a) HLA antigens
   b) Non HLA antigens

1) Platelet Specific Antigens:- (Table 1)

Moulinier (1957) using the antiglobulin consumption technique with papaine - treated platelets, had demonstrated platelet antibody in the serum of a woman whose four children had died from neonatal purpura. The corresponding antigen, termed "Duzo" was shown to be present on the platelets of about 22% of randomly - selected subjects.

Van Loghem et al., (1959) described the system (Zw), they found a serum which agglutinated some samples of platelets but not others; the antigen was named (Zw^a) when a second antigen (Zw^b) was recognized (Van der Weerdt et al., 1963). 98% of subjects were found to be Zw(a+) and 26% Zw (b+). Zw^a and Zw^b appear to be alleles.

Shulman et al., (1961) described a complement - fixing antibody reacting with an antigen PL^{A1} and was subsequently

shown to have the same specificity as anti - Zw^a. Antibody of this specificity (most commonly referred to as anti - PL^{A1}) is associated with the syndrome of post - transfusion purpura and with most cases of alloimmune neonatal purpura.

A second system (Ko) with allelic antigens was described by Van der Weerdt et al., (1962). 16% of subjects were found to be Ko (a+) and 99% Ko (b+). Like anti - Zw^a, anti - Ko^a was detected by platelet agglutination.

Shulman et al., (1964) described the PL^E system who concluded that a relatively common gene PL^{E1} was allelic to a rarer gene PL^{E2}. About 99% of samples are PL^{E1} +ve and 5% are PL^{E2} +ve.

So far, 3 bi-allelic platelet - specific allo-antigen systems are known Zw(PL^A), Ko and PL^E all showing a co - dominant inheritance. Recently another system, Bak, has been detected, from which only one antigen Bak^a, can be recognized at present (Von dem Borne, AE et al., 1980).

Table (1): Platelet - Specific Antigens: Frequency in the Netherlands Population.
(Von dem Borne et al., 1981)

System	Antigen	Phenotype frequency %	Gene Frequency
Zw	Zw^a	97.6	0.855
	Zw^b	26.8	0.155
Ko	Ko^a	14.3	0.0743
	Ko^b	99.4	0.9225
BaK	BaK^a	90.8	0.696

2) Antigens shared by other cells:

a) HLA antigens:

The alloantigens most strongly expressed on leucocytes are those of the HLA (human leucocyte antigen) system which, together with those of the ABO system, have proved to be the most important in determining the compatibility or otherwise of tissue grafts.

The HLA antigens determined by the A, B and C loci are presumed to be present on all nucleated cells and are also expressed on platelets, but only some HLA antigens were