COMPLEMENT 3 AND COMPLEMENT 4 IN DIABETIC PATIENTS WITH AND WITHOUT PROTEINURIA

A THESIS

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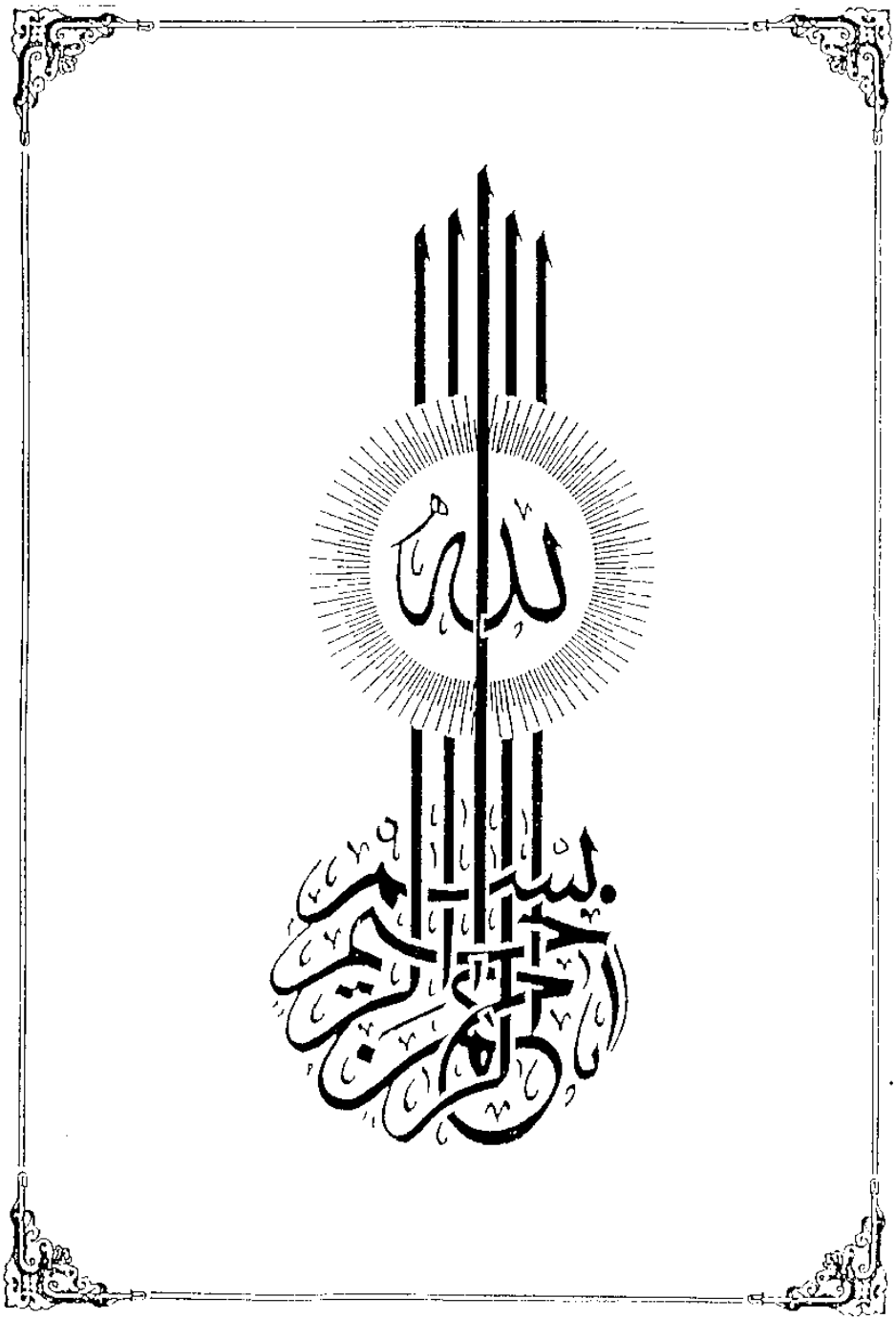
INTRODUCTION



INTRODUCTION

Reduction of serum complement has been found in membraneous glormerulonophritis with proteinuria.

In diabetic patient some degree of glormerulo-ronephritis and proteinurea may occur & this is called Kemilestiel Wilson syndrome.

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AIM OF THE WORK

To study the serum complement 3 and 4 levels in diabetic patients with and without proteinurea. Furthermore, & if changes will be found, to study the mechanism of complement activation i.e. the alternative or the classical pathway .

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or, in more detail:

$$EA + C1qrs \xrightarrow[Ca^{2+}]{} EAC1qrs$$

$$EAC1qrs \xrightarrow[Ca^{2+}]{} EAC1qrs$$

$$EAC1qrs \xrightarrow[Ca^{2+}]{} EAC1qrs + \text{a large C1s Polypetidic fragment.}$$

In addition to IgM and IgG, C1q is capable of reacting with certain polyanions, including DNA, other polynucleotides, (dextran), and chondroitin-sulfates. The pathophysiologic significance of the latter reactions has not been determined. Muller-Eberhard (1968).

Activation Phase:

FORMATION OF THE C4b2a C3-CONVERTASE

The formation of the C4b2a convertase is the result of action of C1 esterase (C1s) on C4 and C2 components.

$$EAC1 + C4 \begin{cases} EAC14b + C4a \\ C4i(\text{or } L) \end{cases} \qquad (3)$$

$$EAC14b + C2 \begin{cases} EA14b2a + C2b \\ C2i \ (\text{or } C) \end{cases} \qquad (4)$$

This reaction develops in two time phases; Cls cleaves C4 into two fragments: C4b is the heavier of the two (mol. wt. 130,000) and binds to specific receptors present on the erythrocyte surface, while C4a is a polypeptide of unknown function.

When C4b and C2a fragments are present in the fluid phase, they are rapidly inactivated (C2i and C2i)

C4b molecules may also directly bind to the hemolysin molecule and be hemolytically active, that is, play a further role in complement activation, leading finally to hemolysis. Cochrance and Kofflor (1973).

C2 is adsorbed onto C4b, already bound to the erythrocyte wall; adsorption occurs only in the presence of Mg^{2+} ions. Immediately after, C2 is cleaved by Cls molecule; only the heaviest of its two fragments (C2a, mol wt, 84,000) remains fastened to C4b, together forming the C4b2a convertase.

C4b2a is very labile; its half-life at 4°C is several hours, but it is less than 10 min at 37°C. After this step, C2a detaches from C4b and loses all hemolytic activity. This "decay" reaction may be schematized as follows:

$$EAC14b2a \longrightarrow \overline{EAC14b} + C2ai \qquad (5)$$

Once reformed, the EAC14b complex has all of the properties of the initial complex and may react with new C2 molecules to form (in the presence of Mg^{++}) a new labile EAC14b2a complex. Turk (1973).

Oxidation of C2 by iodine increases by 10-folds its hemolytic activity. Convertases formed with $C2^{oxy}$ are much more stable than C4b2a convertases. Bokisch et al (1973).

C42 Convertase Interacts With C3 to Form A C423 C5 Convertase:

C42 convertase has an enzymatic activity of unidentified nature that acts on C3 (B C globulin) to cleave it into two fragments, C3a (molecular weight 8700) with anaphylatoxinic, and chemotactic properties, and C3b (molecular weight 223,000), which binds to specific receptors of the cell membrane. If these receptors are not available, C3b in the liquid phase is rapidly inactivated (C3i) A single C42 convertase site because of its enzymatic action binds several hundred C3b molecules.

$$EAC14b2a + C3 \begin{cases} \overline{EAC14b2a3b} + C3a \\ C3i \end{cases} \qquad (6)$$

A high proportion of bound C3b molecules are hemolytically inactive but possess important immunologic properties,

A high proportion of bound C3b molecules are hemolytically inactive but possess important immunologic properties, such as immunoadherence and opsonization. Hemolytic activity is possessed only by C3b molecules that bind on EA142 sites, forming EAC1423 sites, in which C3b induces allosteric modifications of C42, to which it confers a peptidasic activity. This mechanism explains why despite the large number of C3b molecules bond to red blood cells, C3b possesses only weak hemolytic activity. The C423 set, or more precisely C4b2a3b, forms the C5 convertase of the classical pathway. Ruddy et al. (1972).

Membrane Attack Phase: Formation of the C56789 Complex:

The attack process requires the presence of a membrane surface complex composed of the last five complement components (C5 to C9) and C5 convertases of the classical and alternative pathways. Two main mechanisms have been proposed for the formation of this complex. Freedman (1971).

Formation By Successive Steps:

Under experimental conditions, C5-7 complex may be formed stepwise, and the cellular intermediates EAC14235, EAC1 EAC142356, and EAC1423567 are detected; C5 convertases cleave

C5 into two fragments: C5a (mol wt 15,000); like C3a, has anaphylatoxinic and chemotactic properties; C5b (mol wt 170,000) may either directly enter the liquid phase and be rapidly inactivated (C5bi) or remain in contact with the convertase, subsequently reacting with C6 to form the C5b6 stable complex (see below). If C5b does not react with C6, it is rapidly inactivated (C5bi) despite a brief period of contact with the convertase before detaching into the activat on of C5 by C5 convertases cleaves C5 into C5a and C5b and induces conformational changes in C5b that permit an irreversible adsorption of C6 and C7 onto C5. The trimolecular C567 complex realizes a critical molecular arrangement that permits binding of C8 and C9. The C567 portion of the complex is then capable of combining with membrane receptors and penetrating the inner hydrophobic medium of the membrane, in which it now proceeds to rearrange the microenvironment and iduce functional changes in the membrane. The C5-9 complex can bind to membrane receptor sites only during a very brief period (less than 0.1 sec). If binding does not occur, the complex remains in the liquid phase and becomes hemolytically inactive. It has no activities other than the cytolytic function. Gell et al. (1974).

A circle of 8 to 10-nm diameter that contains a dark center is observed on electron microscopy and probably corresponds to the molecular rearrangement induced by the C6-9 complex within the erythrocyte membrane. Similar situations can be demonstrated in all cellular membranes during development of a complementary reaction. These circles occur in lymphocytes, platelets, bacteria, viruses, mycoplamas, tumor cells, and even in artificial bilayer lipid membranes (liposomes). It has been suggested that these circles may be holes in the membrane created by the C5-9 complex, establishing a communication between the intra-and extracellular media. This belief is not certain, however, because identical lesions have been observed in lymphoic all cell membranes transformed by the Moloney virus that had been incubated with specific antibodies and complement in the S, C2, and M phases of their cellular cycle, at which time total resistance to the hemolytic action of complement is present. Alper and Posen (1971)

Irrespective of this finding, it is clear that changes induced by the C5-9 complex make the membrane semipermeable, permitting by a donnan effect the passage of extracellular water and salts into the intracellular milieu. The cell now swells and bursts (cytolysis). If the binding of a single C5-9 comples suffices to create the local membrane "lesion"

that provokes cell lysis ("one-hit hypothesis"), it is most likely that under physiologic conditions, especially because of the enzymatic nature of some of the steps of the complement reaction, red blood cell lysis results from multiple lesions that are simultaneously created on the membrane ("multihit reaction"). Gordon (1974).

Alternative Pathway of Activation:

As in the classical pathway, the alternative pathway includes a series of component parts, the activation of which leads to the production of several convertases that react with both C3 and C5. Despite recent extensive studies, the mechanisms of the alternative pathway are still obscure or controversial. It should be obvious that the scheme given below is not definitive and does not account for all experimental data recently brought to our attention. Roitt (1974).

Alternative Pathway Activators:

The alternative pathway may be activated by immunologic substance (Ig agregates and, by inference, immune complexes in whose formation immunoglobulins also participate) and also by nonimmunologic substances (polysaccharides). Alexander and Good (1977).

Of the inulin type, gram-negative bacterial endotoxins, yeast walls, zymosan, cobra venom (factor).

Of the human Ig, IgA was formely believed to be the only Ig capable of activating the alternative pathway.

The activation site of the alternative pathway is present on the F(ab) = 5S portion of molecules. C3b formation induced by the activation of the classical pathway could lead to factor-B recruitment, independently of alternative pathway intervenion. Fudenberg et al . (1976).

Cobra venom factor (CoF) inducing C3 cleavage and activation of the terminal C5-9 components by the alternative pathway would be cobra C3, modified in such a way as human C3b and therefore capable of activating the alternative pathway. Park and Good (1974).

Components of the Alternative Pathway:

Al least five components are implicated in activation

of the alternative pathway, four proper components (factors B and D, IF, or "initiating factor," and properdin) and C3. Factor B intervenes probably in all steps of this activation, whereas C3 intervenes in the native state in the first step and in the c3b state in subsequent steps. Weiser et al (1979).

Factor B (C3PA, GVG, or glycin-rich glycoprotein) is a B globulin (mol wt of about (100,000) that is thermolablile at 50 °C for 10 min. Its concentration in normal human serum is relatively high (100 to 200 g/ml).

Factor D(C3PAase, GBGase) is a globulin (mol wt 25,000), the active site of which is a serine esterase. Its specific function consists of cleaving factor B into two fragments.

Properdin is a B globulin (mol wt 223,000) with an isoelectric point higher that 9.5 that contains four apparently identical subunits, each of 45,000 molecular weight. Properdin activity is linked to its ability to react with C3b and to delay the decay (inactivation) of C3b-dependent convertase. Properdin seems, therefore, to play a stabilizing role for this convertase. Alper and Rosen (1971).

It is a pseudoglobulin of an electrophoretic migration,

with a 6S to 7S ultracentrifugation index, present in trace amounts in human serum. When activated, it may be analogous to the nephritic factor of membranoproliferative glomerulonephritis. Gotze and Muller-Eberhard (1974).

Activation Phases of the Alternative Pathway:

Schematically, activation occurs in three phases.

The first phase consists of formation of the initial convertase, which has the function of inducing the production of C3b molecules necessary for the formation of the second C3 convertase. The initial convertase is composed of factors B, native C3. The formation of this intial convertase and the C3b production it induces may be written schematically as follows. Gotze and Muller-Eberhard (1971).

$$\text{Activating substance} + \text{IF} + \text{B} + \text{C3} + \text{D} \longrightarrow \underset{\text{(initial convertase)}}{\text{S1-IF,B,C3}} \quad (1)$$

$$\text{S1-IF, B, C3} + \text{C3} \longrightarrow \text{S2-C3b}. \quad (2)$$

S1 designates the site of the activating substances on which the initial convertase is formed, and S2 is the site to which C3b molecules bind, which have been generated by the action of the initial convertase on native C3. The