

PLASMA FIBRONECTIN IN DIABETIC GRAVIDAS
AND IN THEIR NEWBORNS

THESIS

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بسم الله الرحمن الرحيم

قالوا سبحانك لا علم لنا إلا
ما علمتنا انك انت العليم الحكيم

هدى الله العظيم

البقرة - ٢٢ -



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INTRODUCTION
&
AIM OF
THE WORK

REVIEW OF LITERATURE

A. Definition and Nomenclature

The term fibronectin describes a family of structurally and immunologically related high molecular weight glycoproteins that are present in blood, other body fluids, and tissues (Mosesson and Amrani, 1980; Cosio and Bakaletz, 1986; and Hynes, 1986). The name is derived from a Latin origin; fibra means fiber and nectere means to bind or to contact (Kuusela et al., 1976; Mosesson and Amrani, 1980; Saba et al., 1986; and Hynes, 1986).

Prior to the suggestion of the name fibronectin, the protein in its different forms have been designated by a variety of terms including; antigelatin factor (Wolff et al., 1967), opsonic protein (Saba, 1970), large external transformation sensitive protein (Hynes and Bye, 1974), soluble fibroblast antigen (Ruoslahti and Vaheri, 1974), cell surface protein (Yamada and Weston, 1974), galactoprotein (Gahamberg et al., 1974), cold insoluble globulin (Morrison et al., 1948; and Chen et al., 1976), cell adhesion factor (Pearlstein, 1976), lastly cell spreading factor (Grinnell, 1976).

B. Historical Prespective

Two forms of fibronectin have been characterized, a soluble form in blood and other body fluids and another insoluble form is present on surfaces of cells, in extracellular spaces of connective tissue and as a component of basement membrane (Mosesson and Amarani, 1980; and Ruoslahti et al., 1981).

The first fibronectin to be isolated was partially purified from human plasma by Morrison et al., 1948). They described a protein component of fibrinogen containing a fraction that was cold insoluble, and present in the precipitate that forms when plasma stands in the cold hence the name "cold insoluble globulin or CIg", and unlike fibrinogen, was not thrombin coagulable. This protein displayed a more rapid anodal electrophoretic migration rate and higher sedimentation coefficient than fibrinogen. Later, physicochemical analysis reported by Edsall and associates, (1955) led to the suggestion that cold-insoluble globulin (CIg) was a modified dimer of fibrinogen. Shortly thereafter, Smith and Von Koff (1957) discovered a protein in heparin-induced cold precipitate of either normal or pathologic plasma. This protein had properties similar to those of CIg.

Mosesson and his colleagues (1968) investigated a patient with a chronic intravascular coagulation secondary to an occult neoplasm. This syndrome was characterized by persisting pathologic cold induced plasma precipitate termed "cryofibrinogen", the latter was partially coagulable by thrombin, thus confirming the presence of fibrinogen. Two years later, Mosesson and Umfleet (1970) presented a method for isolation and purification of Clg and provided a clear evidence that it was a unique and a major plasma protein of normal concentration $300 \pm 100\mu\text{g/ml}$.

In the early 1970s a wide variety of investigations in different areas of biology had focused on the changes that occurred in cell surface proteins of fibroblasts as a consequence of transformation by oncogenic viruses. Particular attention was paid to a large external transformation sensitive glycoprotein (LETS) of high molecular weight (Hynes and Bye, 1974; Ymada and Weston, 1974; Gahmberg et al., 1974; and Blumberg and Robbins, 1975) that was released from the fibroblast cell surface into the culture medium (Ruoslahti et al., 1973).

In 1975, Ruoslahti and Vaheri reported that Clg was antigenically identical to LETS. This brought to light the uniqueness of fibronectin both as a cell surface / matrix protein and a blood protein, and stimulated numerous investigations on all forms of this protein.

Biological functions of fibronectin

(Mosesson et al., 1980)

- * Cell-cell aggregation
 - Agglutinates fixed erythrocytes
 - Aggregates liver cells.
 - Binds to fixed and live bacteria
- * Cell substratum adhesion
 - Mediates cell attachment to collagen fibrin and plastic substrate promotes cell spreading
- * Reversion of transformed phenotype
 - Promotes cell alignment and decreases overlapping
 - Restores normal surface morphology
 - Restores fibroblastic morphology
 - Promotes microfilament bundle organization
- * Increases cell motility
- * Stimulates reticuloendothelial clearance of particles
 - Liver cell binding
 - Uptake by macrophages in culture
- * Binds to specific macromolecules
 - Actin
 - Cell surface receptors

- | | |
|------------------------|-------------------------|
| - Collagen and elastin | - Dextran sulfate |
| - D.N.A. | - Fibrin and fibrinogen |
| - Gangliosides | - Heparan sulfate |
| - Heparin | - Hyaluronic acid |

Role of fibronectin in cell attachment:

Yamada and Olden (1978) suggested that fibronectin might perform an adhesive function. It agglutinates formalinized sheep erythrocytes (Yamada et al., 1975) and becomes incorporated into the clot in blood coagulation (Mosher, 1979) which may be important in providing a favourable attachment matrix for the cells that grow into the clot (Ruoslahti et al., 1981). The addition of fibronectin to cell culture promotes adhesion of cells to substratum (Pearlstein, 1976). Plasma and cell surface fibronectin are equally active in this respect (Yamad and Kennedy, 1979). Observations in vitro may not accurately reflect findings in vivo. Antifibronectin antiserum produces only 60% inhibition of adhesion to fibronectin covered subendothelial matrices in vivo, as opposed to 100% in vitro (Pearlstein and Hoffstein, 1981). It is some what paradoxical that fibronectin deposit of its cell attachment enhancing properties promotes motility of cells (Ali and Hynes, 1978).

Normal human cells are rich in surface fibronectin and are able to spread in the absence of exogenous fibronectin. Virus transformed cells and cells of neoplastic origin have either

reduced or no surface fibronectin and are dependent on serum or plasma for spreading (Rajarman et al., 1983).

Role of Fibronectin in Differentiation:

Fibronectin appears early in development and seems to be abundant in embryonic tissues. Fibronectin is first detected in the differentiation of the endoderm and is found in the mesoderm but not in the differentiated ectoderm (Vaheri et al., 1985). Several lines of evidence suggest that fibronectin plays a role in directing differentiation and morphogenetic movement (Ruoslahti et al., 1981). Fibronectin is found in undifferentiated mesenchymal tissue and it disappears on differentiation. Thus, it is lost from mesenchymal cells of kidney following differentiation to epithelial cells (Linder et al., 1975) and from myoblasts prior to their fusion into myotubes. Differentiated chondrocytes and adult cartilage do not contain fibronectin (Dessau et al., 1978).

Fibronectin and Haemostasis

The role of fibronectin in haemostasis is indicated by its interaction with fibrin and its presence in platelets. Plasma fibronectin can be cross-linked to itself and to fibrin by plasma transglutaminase (Factor XIII). It is thus covalently incorporated into the fibrin clot, (Mosher, 1975). Fibronectin has been

demonstrated in platelets in association with granules (Zucker et al., 1979). Thrombin and also collagen cause release of platelet fibronectin antigen and increase its surface expression (Zucker et al., 1979; and Ginsberg et al., 1980).

Since platelets function in vivo by aggregation and by adhesion to subendothelial collagen in an injured vessel wall, attention has been directed towards the role of fibronectin in these processes. Plasma fibronectin promotes adhesion and spreading of platelets on collagen in vitro (Hoffman and Hynes, 1979).

Role of fibronectin in mononuclear phagocyte system:

There is now substantial evidence that plasma fibronectin can act as an opsonin promoting reticuloendothelial cells clearance of a variety of particulates as injured cells collagenous debris, fibrin, actin, and aged cells, (Saba and Jaffre, 1980).

Fibronectin is found to be identical to a circulating non-immune globulin which augments phagocytosis. Impairment of reticulo endothelial function in patients following major surgery, trauma, burns or sepsis is associated with diminished plasma fibronectin. Plasma fibronectin measured by immunological assay is decreased also in severely ill persons with disseminated intravascular coagulation. The depression in phagocytic activity is caused in a major part by a depletion of a circulatory opsonin

protein that is indistinguishable from fibronectin. The deficiency of fibronectin in these cases may reflect utilization in reticuloendothelial system disposal of particulates and/or binding protein to areas of tissue injury due to its high affinity for actin and denatured collagen (Blikmenstock et al., 1978). Administration of plasma cryoprecipitate rich in fibronectin to such patients restores plasma fibronectin and results in clinical improvement (Saba et al., 1978). Opsonic protein (fibronectin) in the blood modulates the rate of phagocytic uptake of bacterial and non-bacterial particles by fixed reticuloendothelial cells, mobile macrophages and polymorph-nuclear leukocytes (Saba, 1982).

Alitalo et al., (1981) showed that cultured macrophages could synthesize and secrete fibronectin.

Bevilacqua et al., (1981) provided an evidence that human peripheral blood monocytes had plasma membrane receptors for plasma fibronectin and these receptors were not expressed on other leukocytes. Binding of plasma fibronectin at sites of injury via fibrin or collagen affinity promotes monocytes retention and subsequent enhancement of their phagocytic capacity.

They added that monocytes-plasma fibronectin binding leads to enhanced functional expression of their plasma membrane receptors for the F_C portion of immunoglobulin G site for macrophage fixation and for third component of complement C_3b .

Remold et al., (1981) reported that fibronectin augments macrophage response to migration inhibitory factor (M.I.F.).

Fibronectin has also been known to bind to staphylococci suggesting a possible role in macrophage mediated host defense against bacteria, (Kuusela, 1978; and Mosher, 1980).

Fibronectin: A modulator of the oropharyngeal bacterial flora:

Certain clinical evidence may suggest that serum fibronectin may play a protective role against serious bacterial infection in patients who have suffered extensive burn, undergone major surgery or suffered other forms of serious trauma. Preliminary studies suggest that fibronectin may promote the ingestion of staph. aureus cells by phagocytic cells and attachment of certain strains of pseudomonas pyogenes to human polymorphnuclear leukocytes (Simpson et al., 1982).

An alternative explanation for the clinical significance of fibronectin was suggested by the work of Woods et al., (1981), who have shown that pseudomonas aeruginosa cells adhere in high numbers to oral epithelial cells from seriously ill patient and this increased adherence is correlated with a greatly reduced level of fibronectin on the epithelial cell surface. In this regards, Simpson et al., (1982) have recently presented evidence to suggest that fibronectin on the surface of oral epithelial