CHAPTER 1

INTRODUCTION AND AIM OF THE WORK

Introduction

Immunology developed from the study of resistance factors towards infections . Recently , it was proved that this defence mechanism could give rise to serious disorders instead of protecting , and wide overlaps between immunology and other disciplines have become apparent. The influence of hormones or metabolic conditions on the immune system and of immune factors on the endocrine system in health and disease has received much attention . A great share of this attention has centrated on diabetes (Mario , et al. , 1983) .

Disturbances of intermediary metabolism undoubtly play a major role in the etiology of complications of diabetic pregnancy . However , abnormalities of immune function may also be responsible (Galbraith and Faulk , 1979) .

Although few immunologic studies have been performed in either pregnant insulin - dependent diabetics or in gestational diabetics (Galbraith and Faulk , 1981) a variety of abnormalities of immune function have been described in nonpregnant diabetic patients (Mario , et al. , 1983) , the immunologic effects of pregnancy per se may also be pertinent . Furthermore , a number of complications , incl-

uding several lesions of the fetoplacental unit that are almost characteristic of pregnancy with diabetes , display manifestations consistent with the involvement of immunologic mechanisms (Galbraith and Faulk , 1979) .

Antibodies are immunoglobulins (Ig) that react specifically with the antigen which stimulate their production. In the human sera there are five major classes of antibodies : IgG , IgM , IgA , IgE & IgD .

Aim of the Work

The aim of the present work is to determine the level of serum IgG , IgM , and IgA as a parameter of humoral immunity in the diabetic pregnant females , compared to normal uncomplicated pregnancy during first , second , and third trimesters .

CHAPTER 2

REVIEW OF LITERATURE

Short Review on the Immune Response

When an antigen comes in contact with cells of the immune system , it initiates a specific immune response . This response takes two forms :

1 - **Humoral Immunity** : depends on the appearance in the blood and other body fluids of immunoglobulins . These combine specifically with antigen of the kind that stimulated their production and this union can lead to neutralization of bacterial toxin , phagocytosis , lysis , after activation of complement (Myrvik and Weiser , 1984) .

The anibody response is a physiological reaction to any foreign material induced into a body , irrespective of whether it is harmful or not . Furthermore , antibodies may be formed against internal antigen that have been liberated from disintegrated microrganisms (Weir , 1983) .

2 - Cell - mediated Immunity : depends on the development of lymphoid cells which are specifically sensitized to the inducing antigen and which react directly with the antigen to bring about cytotoxic eff-

ects as graft rejection , delayed hypersensitivity

(Jawetz , et al. , 1980)

The Immunoglobulins

Definition

Immunoglobulins are antibodies which can react specifically with the Ag which stimulated their production . Immunoglobulins comprise about 20 % of total serum proteins (Jawetz and Melnick , 1980) .

Historical Discoveries

The gamma globulins were first recognized and designated as a distinct group of serum proteins by Tiselius in 1937 . He termed these proteins gamma globulins because they migrated more slowly in an electric field than globulins of the two other groups called alpha and beta . Tiselius and Kabat in 1942 next demonstrated that the Abs of serum are restricted to the gamma globulins . Many years elapsed before the Nobel Prize - winning work of Porter , Edelman and their associated , 1950 , whom revealed much of the fine structure of Ab moleculer and stimulated a wave of research . Since Abs are gamma globulins , they are referred as immunoglobulins . Immunoglobulins are glycoproteins ; they occur as

monomers and polymers and are divided into several classes and subclasses
based on antigenic differences in various constituent polypeptides (Myrvik and Weiser , 1984) .

The Steps of Immunoglobulin Formation During B - Cell

Differentiation

The capacity to respond to immunologic stimuli rests principally in cells of the lymphoid system . during embryonic life , a stem cell develops in fetal liver and other organs . This stem cell probably resides in bone marrow in postnatal life . Within fetal liver and later in bone marrow , the stem cell may differentiate into cells of the red cell series or of the granulocyte series . Alternatively , the stem cell may turn into a lymphoid stem cell that may differentiate to form at least two distinct lymphocyte populations . one population (called T lymphocytes) is dependent on the presence of a functioning thymus ; the other (B lymphocytes , analogous to lymphocytes derived in birds from the bursa of Fabricus) is independent of the thymus .

B lymphocytes constitute only about 30 % of the recirculating pool of small lymphocytes , being mostly restricted to lymohoid tissue . Their life span is short (2 days - 2 weeks) . The gut - associated lymphoid tissue (e.g. , tonsils , peyer's patches , appendix) may be an important source of B lymphocytes . B cells have abundant membrane - bound immunoglobulin molecules . Each cell carries only one type of immunoglobulin , but some carry both IgD and IgM . B cells can differentiate , proliferate and mature into plasma cells - large lymphocytes that synthetize specific antibodies . Some large lymphocytes can probably revert to small B lymphocytes that have a long life and serve as " memory cells " . B cells have various surface receptors , including immunglobulins and Fc receptors . Attachment of Ag to Ig or of Ag - Ab complex to an Fc receptor provides a stimulus for B cell proliferation . B cell populations are largely responsible for specific immunoglobulin and Ab production in the host . With certain antigens that are large polymers (e.g. pneumococcus polysaccharide , anthrax) , B cells alone are stimulated into antibody production , requiring no T cell cooperation . With other antigens that have a smaller number of determinants and require a carrier , T

cell cooperation with B cells is needed for antibody production (" helper " T cells) (Jawetz and Melnick , 1980) .

Structure of the Immunoglobulins

It has been estimated that the immune system has the potential to produce between 10^6 and 10^8 different antibody specificities (Leder , 1983) . Each immunoglobulin molecule is composed of heavy and light polypeptide chains , with two chains of each type linked by disulfide bonds in each monomeric molecule . The familiar antibody structure includes , antibody - combining sites (the variable regions) and a constant protion that subserve a host of biologic function , Fig.(1) , The variable regions are formed from the approximately 100 amino - terminal amino acides of the heauy chains and the 100 amino - terminal amino acids of the light chains . These amino acids have been found to vary widely in sequence from immunoglobulin molecule to the other . Such variable regions provide antigen specificity . In contrast , the remainder of the immunoglobulin chains consist of amino acids that are invariant from molecule to molecule within an immunoglobulin class

(or subclass) . These constant regions contain in their heavy - chain moieties, the class determinants of various class - associated immunologic functions (complement fixation , opsonization , etc.) (Leder , 1983) .

On the basis of marked antigenic differences in the heavy chain it has been possible to define the existance of five classes of human immunoglobulins using the technique of immuno electrophoresis . These are referred to as IgG , IgM , IgA , IgE , and IgD .

Immunoglobulin G (IgG)

IgG comprises about 75 % of Igs in normal human sera . Each molecule of IgG consists of 2 L chains and 2 H chains linked by 25 (- S _ S) bonds , Fig. (2) . (Jawetz and Melnick , 1983) .

IgG is divided into four subclasses IgG_1 , IgG_2 , IgG_3 , and IgG_4 which comprise 66 % , 23 % , 7 % , and 4 % , respectively . This division into the four subclasses , is based on isotypic Ag determinants . The subclasses are structurally similar (90 % homogy) , and there is variability in the number of interchain - disulfide bonds that bind

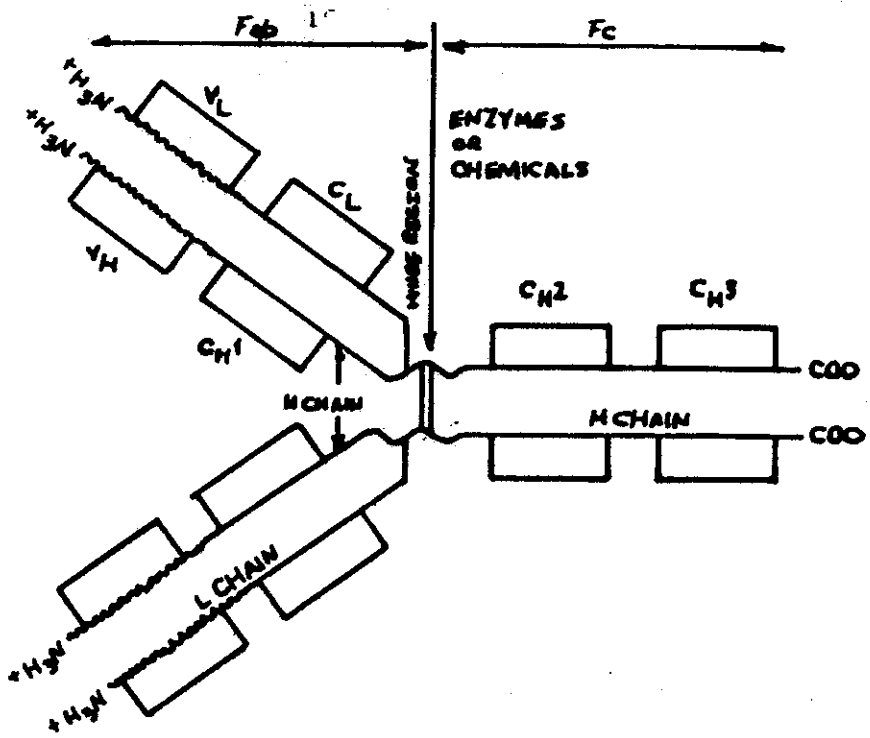

Fig. (1) A simplified for an IgG_1 human molecule (From Fudenberg , H.H. , et al. (eds.) Basic and Clinical Immunology , 3rd Ed. Los Altos , Ca, Lange , 1980)

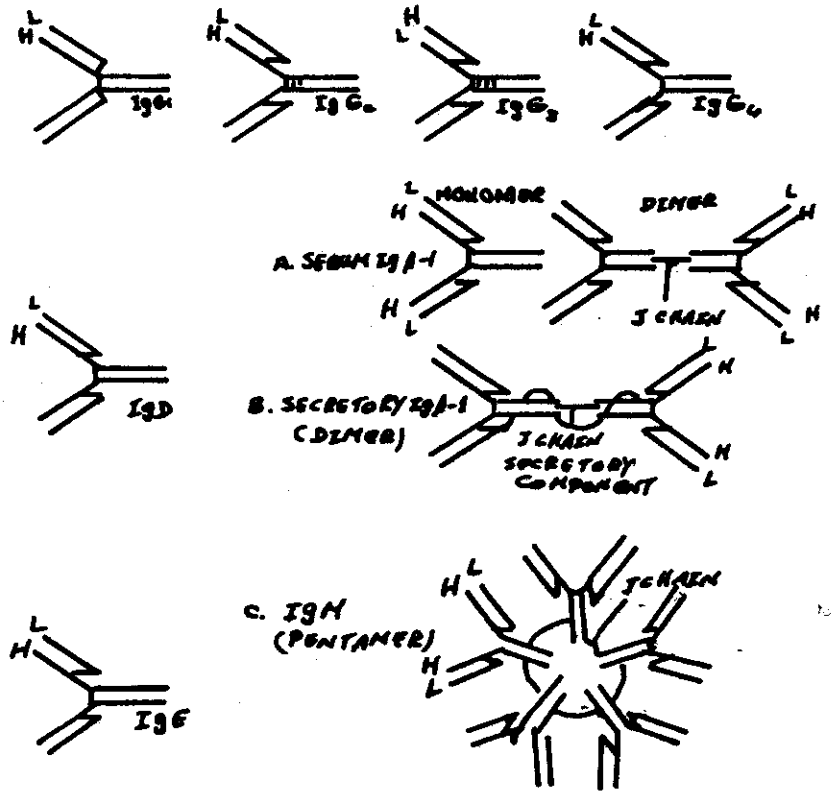

Fig. (2) Diagram Illustrating Human Immunoglobulins (From Fudenberg ,H.H. , et al. (eds.) Basic and Clinical Immunology , 3rd. Los Altos , CA , Lange , 1980)

the H chains in the hinge region (Myrvik and Weiser , 1984) .

IgG synthesis in human is about 35 mg / kg / d , and its half - life is about 23 days much longer than other Ig . It has the ability to control its own catabolic rate (Myrvik and Weiser , 1984) . Normal adult serum levels (1000 - 1500 mg /dl) are reached at 2 years of age and decrease from the fourth decade onwards .

IgG is the only Ig to produce passive cutaneous anaphlaxis . It can probably attach to various tissues through the Fc fragment and participate in anaphylactic type reactions (Jawetz and , 1980) .

Notable variations in the ability to fix complement occur in the order IgG_3 > IgG_1 > IgG_2 . The IgG_4 molecule cannot activate complement by the classical pathway (Myrvik and Weiser , 1984) .

IgG is consisdered the principle serum immunoglobulin . More prolonged antigenic stimulation

is required than for the IgM response , and cooperation with T cell is obligatory . Humoral immune response has two phases : primary phase where the antibodies are IgM , and secondary phase in which IgG becomes the predominant class (Jawetz and Melnick , 1980) .

The subclasses of IgG differ in binding to Fc receptors . IgG_1 and IgG_3 bind firmly to phagocytic cells , and become an important trigger for these cells to " internalize " material bound to their membranes . Also., they are probably important for activating K cells . IgG has the ability to cross the placenta to the fetal circulation which involves an active transport system , and binds to the Fc , and is IgG_1 & IgG_3 specific (Ward and Whicher , 1978) .

IgG_1 is the main subclass involved in passive immunization of the newborn , which has to last for about 3 months , until the baby starts to produce its own IgG (Jawetz and Melnick , 1980) .

IgG may also play an important part in the control of the immune response . When the level of serum IgG is high , there seems to be feed back suppression of all immunoglobulin synthesis . This can lead to a secondary humoral immunodeficiency in IgG myeloma (Ward and Whicher , 1978) .

Immunoglobulin A (IgA)

In human serum, IgA comprises about 15 % of Ig with an average concentration of 100 - 400 mg / dl. The rate of synthesis of serum IgA is about 35 mg / kg / d, and it is rapidly catabolized (Jawetz and Melnick, 1980).

Immunoglobulin A comprises two subclasses, IgA_1 and IgA_2, which occur in serum in the ratio 40 : 1. The two basic molecular forms of IgA, serum IgA and secretory IgA, are present in man. Serum IgA is for the most part a monomeric 4 - chain polypeptide unit, Fig. (2). Serum may also contain small amounts of polymers consisting of monomeric molecules joined together by the J - chain peptide (MW 15,000), with molecular weights ranging from 160,000 to several million. The secretory molecule is a J - chain coupled dimer to which secretory component (SC), a product of epithelial cells, is added. The SC is a polypeptide (MW 60,000) that renders the molecule highly resistant to proteolysis and prolongs its half - life in mucous secretions.

Immunoglobulin A is formed in abundance by the plasma cells in the subepithelial tissues of exocrine glands and in various mucosae.

The plasma cells making IgA are mostly close to the sites where it is secreted . Numerous plasma cells can be found around the collecting ductule in the lobules of the mammary gland , and in the lamina propria of the gut . Most of the IgA in the secretions is in the form $(IgA)_2$. In this form it is much more resistant to enzymatic attack than any other class of immunoglobulin , which is useful for an antibody which has to function in a soup of digestive enzymes . As it passes the epithelial barrier , SC is added to the dimer . Immunoglobulin A is present in all seromucous secretions including saliva , tears , and colostrum .(Ward and Whicher , 1978) .

IgA response matures early , with none in the saliva at birth , and rises to adult levels by age of 3 weeks . The concentration of secretory piece in the saliva is highest at birth , and falls after the appearance of the IgA from mother to young through colostrum or milk seems to be very important in protection (Myrvik and Weiser , 1984) .

Immunoglobulin A is alleged to constitute a first line of defense against certain viral and bacterial pathogens by preventing attachment and colonization on mucous membranes . This mechanism seems to be much less effective in the absence of the normal commensal gut.

flora (Ward and Whicher , 1978) . It may also neutralize toxin in mucous secretions prior to their contact with susceptible host cells (Jawetz and Melnick , 1980) .

Immunoglobulin A does not activate complement by the classical pathway , but when aggregated or denaturated it can activate complement by an alternative pathway (Myrvik , Weiser , 1984) .

Immunoglobulin M (IgM)

IgM is the most primitave immunoglobulins , and the least specialized . It was the first to appear in the evolution of the vertebrates , it is the first to appear in the fetal development of vertebrates with more than one class of immunoglobulin , and it is the first to appear in any individual immune response . When monomeric IgM first appears on the surface of B - cells , it gives the signal for further differentiation when it binds to an antigen under the right conditions . These conditions seem to involve the proximity of macrophages , T - lymphocytes reacting to different determinants on the same antigen . At this early stage in the B - cell's development it can respond positively to a few types of antigens in the absence of T - cells

(T-independent antigens) but it may respond negatively (become tolerized) to many stimuli . IgM is an effective complement activating agent , and a single molecule bound to a red cell can initiate lysis via the classical pathway .

IgM is not transmitted across the placenta , which is important in preventing haemolytic disease of the newborn due to ' natural ' anti-A or anti-B ABO blood group substances. Though serum IgM is mostly pentameric , the level of monomer may rise in some disease states as S. L. E. (Ward and Whicher , 1978) .

IgM comprises 10 % of Ig in normal human sera . Its molecular weight is 900,000 and the rate of its synthesis is about 8 mg /kg / d , while the half - life in serum is about 5 days . The fetus synthesizes IgM in utero . Since IgM does not cross the placenta , IgM antibodies in the newborn are thus considered a sign of intrauterine infection . Adult serum levels (60 - 180 mg / dl) are reached at 6 - 9 months after birth (Jawetz and Melnick , 1980) .

The 19S IgM molecule is a star - shaped polymer composed of 5 Basic monomers held together by disulfide bonds and a J chain . Each H chain carriers 4 domains on the constant segment . Although the molecule has 10

potential Ag - binding sites , which can be shown by binding studies with small haptens , only 5 sites can be readily demonstrated to bind high - molecular weight Ags . The reasons for this are obscure presumably involve steric hindrance among Ag molecules competing for the 10 identical Ag - binding sites . Because of its multivalence , IgM posseses high avidity for Ags carrying repeating (identical) Ag determinants , such as polysaccharides . It is effecient as an agglutinin and as a C- activating Ab . Other important charateristics are the lack of subclasses and lack of cytophilic properties . The monomeric forms occurs naturally on the plasma membrane of a high percentage of circulating virgin B cells (cells that have Ig receptors , but have not encountered specific Ag) . In this position it serves as intrinsic Ag receptor and thus gives the signal for further differentiation when it binds to Ag under the right conditions . The membrane - bound IgM monomer is usually accompanied by IgD, , which may in some unknown way act in concert with IgM to bind Ag and activate lymphocytes . Since they are synthesized by the cell that carries them , both molecules possess the same Ag - binding specificity , (Myrvik and Weiser , 1984) .

Immunoglobulin D (IgD)

Immunoglobulin D was first found among the myeloma proteins . It comprisess only 0.2 % of the total serum Igs and its production is limited to a small percentage of the plasma cell population . Serum levels of IgD tend to rise in chronic infections . Immunoglobulin D is susceptible to proteolytic degradation and to heat denaturation and has a half - life of only 2 - 8 days . Antibody activity has been shown by indirect methods , but the molecule does not activate complement . Immunoglobulin D serves as an Ag receptor on lymphocytes . During B-cell development , membrane - bound IgD usually follows the appearance of membrane - bound IgM . In human adults , a large percentage of B cells bear both IgG and IgM . On exposure to Ag , membrane IgD disappears . The simultaneous presence of intrinsic Igs of two classes on the B - cell membrane does not necessarily mean that they were synthesized simultaneously by the cell , and does not invalidate the tent that Ig molecules produced by a given cell at any time are all identical . A minor proportion of virgin lymphocytes can bear IgD in the absence of IgM , and vice versa . About 14 % of the peripheral lymphocytes of the newborn bear IgD , whereas the value in adults is 75 % . Another unique feature of IgD concerns the percentage of molecules that contain k chains vs. λ chains . For IgD ,

the k /λ ratio is 20 : 80 as compared to 60 : 40 for all other Igs (Myrvik and Weiser , 1984) .

Immunoglobulin E (IgE)

The presence of a distinct heat - labile (56 °C , 30 min) Ab,, formerly called reagin , in the serum of allergic individuals , which can passively transfer anaphylactic - type hypersensitivity , was demonstrated by Prausnitz and Kustner in 1921 . It was not until , 1966 , when Ishizaka and associates established the identity of reagin with IgE (Myrvik and Weiser , 1984) .

In normal sera , IgE is found only in minute concentration (0,05 mg / dl) . Intact IgE molecules are 8S with a molecular weight of 190,000 . The H chain of IgE are longer than those of IgG by about 100 amino acid residues , perhaps indicating a special function . IgE mediates allergic reactions in skin and other tissues and , with the Fc fragment , binds to most cells and basophils . In persons with allergic reactivity of the antibody mediated type , the serum concentrations of IgE are greatly increased . In such individuals , IgE appears in external secretions and mediates local allergic reactions . The serum level of IgE is increased in parasitic infections (helminthiases) (Jawetz and Melnick , 1980) .

Table (1)

Properties of Human Immunoglobulins

Class	Half-Life (days)	Serum concentration (mg/dl)*	Distribution (%of total in intravascular space)	Synthetic rate (mg/kg/day)	Total immuno-globulin (%)
IgG	23	1000	45	33	80
IgA(serum)	6	200	42	24	16
IgM	5	120	76	6-7	4
IgD	3	3	75	0.4	0.001
IgE	3	0.05	51	0.02	0.00003

dl = Deciliter.

(Myrvik and Weiser:Fundamentals of Immunology, 2nd edition,Lea & Febiger, Philadelphia,1984.)

Table(2)

Reactions of Human Immunoglobulins

Class	Sensitivity to 2-mercaptoethanol	Agglutination	Precipitation	Complement fixation (classical)
IgG1	-	moderate	strong	++
IgG2	-	moderate	strong	+
IgG3	-	moderate	strong	+++
IgG4	-	moderate	strong	-
IgA(serum)	+	weak	weak	-
IgA(secretory)	+	weak	weak	-
IgM	+	strong	variable	++++
IgD	-	?	?	?
IgE	-	?	?	?

(Myrvik and Weiser: Fundamentals of Immunology, 2nd edition, Lea & Febiger, Philadelphia,1984.)

Table (3)

Human Immunoglobulins

Class	Immunologic activities	Opsonic activity	Placental passage	The order of appearance of synthetic ability in infants	Functional valence
IgG	Late response to Ag; antitoxic;antiviral; blood group Abs	+	Yes (all sub-classes)	late	2
IgA(serum)	Block bacterial adherence;viral defense	-	No	intermediate	2(monomer)
IgA(secretory)	Activity in mucous sections;block bacterial adherence;viral defense	-	No	intermediate	2-4 (dimer)
IgM	Early response to Ag; antibacterial;antiviral blood group Abs	+	No	early	5 (10)*
IgD	Present on lymphocyte surface	+	No		2
IgE	Allergic (anaphylactic)reactions;possible respiratory tract defense;mast cell fixation; raised in parasitic infections;cytophilic,for basophils & mast cells	+	No		2

* A valence 10 is the theoretic maximum and could only be achieved with low-molecular weight hapten.

(Myrvik and Weiser: Fundamentals of Immunology,2nd ed., Lea & Febiger, Philadelphia,1984.)

Immunological Study of Normal Pregnancy

Maternal Adjustment in the Immune System in Normal Pregnancy

The concept of normal pregnancy implies the adjustment of the maternal organism to an antigenically foreign fetus and placenta . The observed fact that pregnancies are successful is proof that the mother's immune system is able to adjust to this foreign insult and maintain the pregnancy in an undamaged state . A number of mechanisms have been proposed to explain the non rejection of the fetus and placenta . Some of these include :

1 - Immunologic buffer zone at the maternal - fetal level interface .

2 - Complete separation of maternal and fetal blood circulation .

3 - Masking of the surface alloantigens on the trophoblastic cells :

a - Sialomucin coating on trophblastic cells that decreases or prevents attack by maternal lymphocytes .

b- Acquisition of agents that results in " masking " or " blocking " :

(1) Transferrin that binds to specific receptors on trophoblastic cells .

(2) Antibodies or antibody - antigen complex that bind via Fc receptors to the trophoblastic cell surface .

4 - Synthesis by syncytial trophoblast and maintenance in local high concentrations of hormones and other agents that play an immunosupressive role , e.g. progesterone , estrogens , chorionic gonadotropin , cortisol - binding globulin .

5 - Production by the fetus of immunosuppressive agents or cells that enter the maternal circulation , e.g. fetal suppessor T cells , fetoprotein , lymphokines from stimulated fetal lymphocytes .

6 - Production of immunoregulatory agents by the mother that alter the immune attack on the fetoplacental unit :

a - Increased synthesis of adrenal corticosteroids , pregnancy - associted plas-

ma proteins , early pregnancy factor , globulins .

b - Synthesis of " blocking " antibodies .

c - Presence of suppressor cells .

d - Inversion of T - and B - cell number in peripheral blood .

Although these mechanisms , as well as others , have been proposed as explanations for the maintenance of the fetoplacental unit as an allograft , the significance of each mechanism as part of the entire process has not been elucidated . Obivously , some mechanisms are of greater importance and must be considered primary , whereas others are secondary (Gall , 1983) .

Physiologic Alterations During Pregnancy Affecting the Immune System

The maternal blood volume increases dramatically during pregnancy (Chesley , 1972 ; Pritchard , 1965) .

This increase affects formed elements , including lymphocytes . The blood volume expands by 20 - 100 % , starting in early gestation , continuously until 34 weeks when a plateau occurs .

The hemodilution that occurs during pregnancy has an effect on other plasma components . Serum protiens declined by 1 g / dl , most of the loss accounted for by the decline in albumin ; the decline occurring during the first half of pregnancy , with a plateau reached by 24 weeks gestation . Inspite of the expansion of blood volume there is a progressive rise in the periphral lymphocyte (WBC) count during gestation (Pattilo , 1980) .

Quatitative tests of the activity of neutrophils (PMNs) were performed by Bjorksten , et al. , 1978 . They demonstrated decreased chemotaxis in neutrophils from pregnant women , compared with PMNs from nonpregnant women . Phagocytosis of Escherichia Coli by control PMNs was shown to be low in the presence of sera from pregnant women (Bjorksten , et al. , 1987) .

Pregnant women have a depressed endotoxin - stimulated (NBT) test* (Bjorksten , et al. , 1978) . A low NBT test value may indicate a depressed phagocytic capacity in the pressence of normal oxidative metabolism .

* Nitro Blue Tetrazolium Test

Alterations in the levels of complement in pregnancy serum was reported . Plasma levels of C_3 and C_4 are elevated in the second and third trimesters (Teisner , et al. , 1982) . The most likely mechanism for the increased levels of complement components is increased hepatic synthesis (Tedder , et al. , 1975) .

Effect of Placental Hormones on the Maternal Immune System

The concept that human chorionic gonadotropin (hCG) has significant immunosuppressive activity with regard to maternal lymphocytes has been demonstrated by several investigators (Hammarstrom , et al. , 1979 ; Teasdale , et al. , 1973 ; Contractor , et al. , 1973) . As regard to progesterone ,which is a very important hormone in pregnancy , Clemons , et al. , 1978 , have demonstrated with progressive concentration of progesterone , estradiol , and testosterone (1 - 20 µg / ml),inhibition of mixed lymphocyte culture . Mitogen - stimulated cultures were also inhibited by 1 - 20 µg / ml of progesterone .

Immune Complexes (ICs) in Normal Pregnancy

The revelance of circulating ICs to reproduction has been

extensively reviewed (Theofilopoulous , et al. , 1981) . ICs are found when antibodies combine with their corresponding tissue fixed antigens or antigen free in serum or other body fluids . Pope , et al. , 1982 , found that immune complex concentrations in normal pregnancy serum were not elevated , but assays for IgG rheumatoid factor (RF) and IgM RF were increased .

Cellular Immunity in Pregnancy

T - and B - Lymphocytes

The total number of lymphocytes remains throughout pregnancy at 1500 - 3000 / mm^3 . T - lymphocytes forms 70 % ; B - lymphocytes , 25 % ; null cells , 10 % . There is a B / T cell ratio of 1 : 3 (Gall , 1983) . Strelkaukas , et al. , 1975 , described a decrease of T cells percentage to 25 % and increase of B cells to 70 % at 10 - 13 weeks of pregnancy and a return to the normal ratio after 20 weeks . The inversion of T and B cells maybe explained as a physiologic depletion of suppressor T cells , which allows the number of B cells to rise . The increase in B cells may assist in the production of antibodies that function as blocking factors allowing the allograft to be accepted (Gall , 1983) .

In 1983 Gall reported that Bulman and Hancock found a depletion of T cells both by percentage and absolute number of E - rosette - forming cells with a concomitant rise in the IgG bearing cells .

A significant decrease in the percentage and absolute number of helper lymphocytes were found in normal pregnant women throughout pregnancy and during labor . The percentage and absolute number of suppressor cytotoxic T cells and B cells were unchanged from normal controls . The percentage of monocytes was increased throughout pregnancy . The decrease in helper T cells maybe operative with other mechanisms of nonrejection . Several autoimmune diseases have been associated with a high ratio of helper / suppressor cells .

In pregnancy a lower helper / suppressor ratio occurs with a decrease in antibody production and clinical amelioration of disease . A low helper / suppressor ratio is probably normal for the maintenance of pregnancy . The cause of alteration of helper T cells in pregnancy is not known but maybe a consequence of the hormonal changes associated with pregnancy . Immunosuppressive effects have been shown during in vitro studies for hCG , estrogen , progesterone , corticosteroids , fetoprotein , prolactin , and -globulin (Lawrence , et al. , 1980) .

Pregnancy and Alterations in Humoral Immunity

In women antibodies are transferred from the mother to the

fetus prior to birth (Osborn , 1952) . This passive transfer of maternal immunoglobulins is of importance for two reasons . First , a mechanism is provided by means of which protection is afforded to the infant before it has developed an efficient immunologic system . Second , passively transferred antibody interferes with the active synthesis of that antibody by the infant (perkins , 1959) . This principle is being used extensively and successfully on an experimental basis to prevent the development of erythroblastosis (Gall , 1983) .

It is established that infants born at term have normal adult levels of IgG as a result of passage of maternal antibodies across the placenta . However , the placenta is relatively impermeable of IgA and IgM globulins , level of which are very low in the newborn except in cases of intrauterine infection when the infant has been stimulated to produce its own antibody (Alford , 1967) .

Measurement of immunoglobulins , IgG , IgA , IgM , in normal pregnancy has been reported frequently ; but information on IgD and IgE is more limited (Gall , 1983) . The wide range of normal values reflects a variation in antigenic exposure by the mother (Lowrence , et al. , 1980) .

In 1971 , Maroulis , et al. , reported a significant lowering

of serum IgG concentrations during the second , third trimesters of pregnancy and postpartum compared to first trimester as well as matched control group . Benster , et al. , 1970 , compared serum immunoglobulin levels in cases of normal pregnancy with nonpregnant controls , and demonstrated a reduction in all three immunoglobulins at 27 to 33 weeks was clear in the case of IgG .

The mechanism of decline in IgG in apparently healthy uncomplicated pregnancies is not clear .. A dilutional effect on the IgG concentration caused by hydremia of pregnancy does not likely , since concentrations of all other serum proteins except albumin have been shown to remain unchanged or to become elevated during pregnancy (Ganrot , et al. , 1967 ; Mendenhall , et al. , 1970) . Altered metabolic regulatory mechanisms for control of albumin and IgG concentrations during pregnancy must be considered in explanation for these findings . In the nonpregnant state , it has been clearly shown that the plasma IgG concentration is a key factor in determining the rate of catabolism of IgG (Fahey , 1963) . If the IgG concentration drops in body fluids , the catabolic rate decreases accordingly (Fahey , 1963) .

Obviously , the transfer of these protein to the developing fetus could account for the observed changes . It is known that IgG is transfered from maternal to fetal serum and

that the level of IgG gradually increases in fetal serum throughout the last 2 trimesters of pregnancy . Gusdon , 1969 , found a gradual rise in the IgG level during the first two trimesters of pregnancy but by the thirty - third week of gestation the maternal and fetal levels co-incide . During the last 7 weeks of pregnancy the fetal and maternal levels continue to be approximately , with slightly higher fetal than maternal values (Benster , 1970) .

Pregnancy results in an enhanced capacity to synthesize antibody (Gusdon , 1969) . Jackson , 1935 , while studying anaphylaxis , found than the highest antibody titers occurred in pregnant rabits . Mitchell , et al. , 1966 , have demonstrated that pregnant women have higher antibody titers against E . coli than nonpregnant .

The fact that high levels of IgG may be transferred to the infant earlier than previously thought becomes more significant when one considers that protective antibodies as well as many immunppathologic proteins in the mother may passively be transferred to the fetus (Gusdon , 1969) , e.g. thyroid autoimmune Ab (Seip , 1960 ; Hall , 1964) . The relatively more rapid rise in the fetal levels of passively trasferred maternal IgG could explain the early onset of haemolytic disease in utero , as indicated by spectro-

photometric analysis of amniotic fluid (Freda , 1965) .

Maroulis , et al. , 1971 , found increased IgM level in the first trimester of pregnancy which could be mother response to fetal antigens early in pregnancy . It is known that fetal cells and globulins can cross into the maternal circulation during pregnancy and stimulate the production of antibodies to fetal red , white cell , platelet , and IgG isontigens . How early this may occur is not known (Maroulis , et al. , 1971) . Certain embryo - specific antigens have also been described , some of which have been shown to elicit an immune response in the mother (Tatarinov , 1965) .

Several workers have found higher levels of IgM in women than in men especially in child - bearing age (Rhodes , et al. , 1969 ; Johansson , et al. , 1968 ; Butterworth , et al. , 1967) . Mendenhall , 1969 , has demonstrated increase in IgM in mother receiving oral contraceptives . The observation that multigravidae have low serum levels of IgM than primigrairdae suggests that successive pregnancies may diminish the effectiveness of IgM synthesis , comparable to the progressive attenuation of the response to antigen in an " hyposensitization " process (Benster , et al. , 1970) .

The values reported on IgA level during pregnancy were conflicting . Although , Gusdon , 1969 , and Marnulis , et al. , 1971 , found little variation than normal , Benster , 1970 , reported increased level of IgA in pregnant women aged over 25 while Studd , 1971 , found a fall in IgA concentration early in pregnancy .

Concerning IgD a definate elevation of the mean concentration in the sera of pregnant women was reported especially at term (Klapper , et al. , 1971 ; Gusdon , et al. , 1972) . Klapper , et al. , 1971 , attributed the increased IgD levels to :

a - An antibody response peculiar to the feto - maternal relationship .

b - The usual metabolism of IgD is altered by the pregnant state .

c - Alterations in the mother's metabolism secondary to increased levels of circulating sex steroids .

Diabetes Mellitus

General Principles

Diabetes mellitus is not a disease and should be viewed as a syndrome - a clinical entity which can involve a long list of symptoms and clinical laboratory findings - which shows a variable response to therapy .Four criteria are in the complete clinical syndrome and these should be considered in clinical diagnosis :

1 - Hyperglycemia : there is an abnormality of carbohydrate metabolism resulting in hyperglycemia and often associated with accelerated fat and protein catabolism .

2 - Large vessel disease : there is accelerated atherosclerosis and medical calcification .

3 - Microvascular disease : there is an abnormality of capillary basement memberans . charaterized by thickness and abnormal function .

4 - Neuropathy : there are peripheral sensory and motor defects , autonomic nervous system dysfunction , segmental demyelination ., and abnormalities of schwann cells .

None of these findings is specific for diabetes , More than one mechanism can produce each of the abnormal findings . Since the primary defect in diabetes is unknown , a patient with any one or all of these abnormalities must be considered a possible diabetic .

Because plasma glucose can be measured simply and accurately , it remains the most often used , but better markers for diabetes mellitus are hoped for in the future (Williams , 1981) .

Diabetes mellitus can be defined as a chronic disease characterized by abnormally high level of glucose in the blood and in the urine . The basic defect is an absolute or relative deficiency of insulin which leads to abnormalities of metabolism , not only of carbohydrate but also of protein and fat (Marble , 1973) .

History of Diabetes Mellitus

Diabetes was described by the ancient Egyptians , 1500 B.C. , as a state of polyuria . The honey urine , in diabetes , was noted by Sushrutha in India 400 B.C. . The first good clinical description of the disease was made by the Roman pyhsician Celsus , and the name diabetes was introduced by Aretaeus , another Roman physician ; both Celsus

and Aretaeus lived in the first century of the Christian Era . In about 1000 A.D. Avicenna , the Arab physician , gave a remarkably good description of diabetes , including complications such as gangarene . Thomas Willis observed that the urine of diabetes was sweet ,while Dobson , 1775) , demonstrated that the sweetness was due to the presence of sugar .

In 1869 Langerhans discovered the islets which later were given his name by Laguesse . In 1889 , a great landmark was reached when Von Mering and Minkowski produced diabetes in dogs by total pancreatectomy . The greatest step forward came in 1921 , when Banting and Best succeeded in extracting from the pancreas a substance with hypoglycemic properties . Since then , many major additions to the knowledge in biochemistry and physiology of the disease were described (Marble , 1973) .

Classification of Diabetes Mellitus , Table (4) .

It has been established in recent years that diabetes mellitus is genetically and clinically heterogenous group of disorders that share glucose intolerance in common (Rotter and Rimoin , 1978) . A classification of the disease was developed by the National Diabetes Data Group of the NIH 1979

in which diabetes mellitus was divided into the following types :

1 - Insulin - Dependent Diabetes Mellitus (IDDM) , Type I , the former terminology of which was juvenile diabetes , juvenile - onset type diabetes , ketosis - prone diabetes , severe diabetes and brittle diabetes .

2 - Non - Insulin Dependent Diabetes Mellitus (NIDDM) type II , the former terminology of which was maturity - onset type diabetes , adult - onset diabetes , ketosis - resistant diabetes , mild diabetes , obesity - hyperglycemia , maturity - onset diabetes , stable diabetes .

3 - Gestational Diabetes

4 - Diabetes Mellitus and Impaired Glucose Tolerance associated with other conditions . the former terminology was secondary diabetes .

5 - Diabetes Mellitus Secondary to Congenital Insulin Receptor Deficiency , the former terminology was congenital insulin resistance with Acanthosis Nigricans .

6 - Diabetes Mellitus Secondary to Insulin Receptor Antibody , the former terminology was aquired insulin resistance with Acanthosis Nigricans .

Table (4)

CLASSIFICATION OF DIABETES MELLITUS (Williams, 1981)

Traditional Clinical Classification (with Alternative Nomenclature)	NIH Diabetes Data Group Classification
1. Juvenile-Onset-Type Diabetes (JOD) (a) Ketosis-prone diabetes (b) Juvenile-onset diabetes (c) Severe diabetes (d) Brittle diabetes	1. Insulin-Dependent Diabetes Mellitus, Type I (IDDM)
2. Maturity-Onset-Type Diabetes (MOD) (a) Adult-onset diabetes (AOD) (b) Ketosis-resistant diabetes (c) Mild Diabetes (d) Obesity-hyperglycemia (e) Maturity-onset diabetes (f) Stable diabetes	2. Non-Insulin-Dependent Diabetes 1. Nonobese NIDDM 2. Obese NIDDM
3. Maturity-Onset-Type Diabetes in the Young (MODY) (a) Familial maturity diabetes (b) Maturity-onset diabetes of youth	
4. Gestational Diabetes	4. Gestational Diabetes
5. Secondary Diabetes	5. Diabetes Mellitus and Impaired Glucose Tolerance Associted with Other Conditions
6. Congenital Insulin Resistance with Acanthosis Nigricans	6. Diabetes Mellitus Secondary to Congenital Insulin Receptor Deficiency
7. Acquired Insulin Resistance with Acanthosis Nigricans	7. Diabetes Mellitus Secondary to Insulin Receptor Antibody
8. Familial Autoimmune Diabetes	8. Diabetes Mellitus Secondary to Familial Autoimmunity

7 - Diabetes Mellitus Secondary to Familial Autoimmunity , the former terminology was Familial Autoimmune Diabetes .

Insulin - Dependent Diabetes Mellitus (type I), Table (5)

This type of diabetes was found to be usually characterized clinically by abrupt onset of symptoms , insulinopenia and dependence on injected insulin to sustain life , and proneness to ketosis . Classically , this type of disease occured in juveniles , and it was formely termed juvenile diabetes . However , it can be recognized and become symptomatic for the first time at any age , hence , diagnosis based on age at onset was found to be inappropriate . Genetic determinants were thought to be important in most patients as expressed by the associated increased or decreased frequency of certain histocompatability antigens (HLA) on chromosome 6 . Abnormal immune response and autoimmunity were found to be also important in playing an aetiological role , and islet cell antibodies were found to be frequently present at diagnosis . IDDM is also associated with an increased risk of having autoantibodies to thyroid tissue (Williams , 1981) .

Non - Insulin - Dependent Diabetes Mellitus (NIDDM), Table (5)

In this type , the patients were found to be not dependent on insulin for prevention of ketonuria and were not prone to ketosis . However , they might require insulin for correction of symptomatic , or persistant , fasting hyperglycemia if this can not be acheived with the use of diet or oral agents . The patients of this type of diabetes might develop ketosis under special circumstances . There might be normal levels of insulin , mild insulinopenia or above normal levels of insulin associated with insulin resistance . Non - insulin dependent diabetes was found to be heterogenous in nature . Although in most patients who develop non-- insulin dependent diabetes the onset was found after the age of 40 , the condition was found also to occur in young persons who do not require insulin and are ketotic . Consequently age at onset was again not considered as a criterion by which to classify the type of diabetes . The non- insulin dependent diabetes was found to have a genetic basis , which appeared to be stronger than in insulin dependent diabetes . This was evidenced by a more frequent familial pattern of occurance . Environmental factors super - imposed on genetic susceptibility were found to be involved in onset of the non - insulin dependent diabetes . Intake of excessive calories lea-

Table (5)

CLINICAL CHARACTERISTICS OF THE TWO MAJOR TYPES

OF DIABETES MELLITUS (Williams, 1981)

NIH DIABETES DATA GROUP TERMINOLOGY *	INSULIN-DEPENDENT DIABETES MELLITUS (IDDM)	Non-Insulin DEPENDENT DIABETES MELLITUS (NIDDM)
Alternate Clinical Terminology	Juvenile-Onset Diabetes Brittle Diabetes	Maturity-Onset Diabetes Adult-Onset Diabetes Stable Diabetes
Age at onset	Usually less than 45 years	Usually over 30 years
Genetics	Less than 10% of 1st.degree relatives affected	More than 20% of 1st.degree relatives affected
HLA	Associated with HLA-B-8, BW-15, DW-3, and DW-4	No HLA association
Immunity	Increased incidence of autoimmune phenomena	No increase in auto-immune phenomena
Body Weight	Usually lean	Usually obese
Metabolism	Ketosis prone	Ketosis-resistant
Treatment	Insulin	Weight loss; may need oral agent or insulin

* Diabetes 28:1039-1057, 1979

ding to weight gain and obesity was found to be an important factor in its pathogenesis . Characteristic aggregation of HLA types and islet cell antibodies have not been found (Williams , 1981) .

Stages of Maturity Onset Diabetes

1 - Potential abnormality of glucose-tolerance :
This stage was formely knwon as potential diabetes . It includes individuals presumed to be at increased risk for diabetes on genetic ground , and individuals with circulating islet cell antibodies .

2 - Previous abnormality of glucose tolerance :
This has also been called subclinical diabetes or latent diabetes . The fasting blood sugar and the glucose tolerance test are normal . Diabetes is suspected because of decreased glucose tolerance after cortisone adminstration or after certain other types of drug therapy , during pregnancy , or which stressful illness .

3 - Impaired glucose tolerance
This has also been variously described as chemical diabetes , latent diabetes or asymptomatic diabetes . The fasting

blood sugar is normal but the glucose tolerance test is abnormal .

4 - Overt diabetes mellitus

This has been termed clinical diabetes . Fasting blood sugar is always elevated .

Diabetes in Pregnancy

I - Carbohydrate Metabolic Changes Accompanying Pregnancy

Pregnancy results in a series of endocrine and metabolic changes one of which is in carbohydrate metabolism . The first alternation is in blood glucose levels . There is a constant movement of glucose across the placenta of the fetus making the fetal glucose concentration similar but slightly lower than that of the mother (Spellacy , et al. , 1964) . The woman's blood glucose concentration during the fasting period is slightly lower than when she is not pregnant (Osofsky , 1984) . Following a meal there is a more prolonged hyperglycemia .

Fasting plasma insulin levels are slightly elevated during pregnancy . At term , the normally pregnant woman releases about 3 times the amount of insulin to control her glucose levels than she would if she was nonpregnant (Spellacy , et al. , 1963) .

This pregnancy - induced demand upon ß - cells of the pancreas to hyperfunction in pregnancy has been described as the " diabetogenic stress of pregnancy " . If the woman

is unable to produce the excess insulin , e.g. as with the subclinical diabetic , she has then a period of hypoinsulinism with hyperglycemia during the last portions of the pregnancy and is termed a " gestational diabetic " .Following delivery there is a fairly rapid reverse of this back toward the nonpregnant state (Spellacy , 1971) .

On the other hand , it has been shown that ovarian steriods , cortisol , prolactin and human placental lactogen can all alter carbohydrate metabolism and these hormones are all elevated in normal pregnancy (Spellacy , 1971) . These alterations seem to act at the peripheral target cell via effect on the insulin receptors (Osofsky , 1984) . Recent studies by Pagano , et al. , 1980 , and Tsibris . et al. , 1980 , using blood and fat cells taken from pregnant women , have shown a reduction in the insulin receptors .

So , the diabetogenic factors of pregnancy are :

1 - The placenta produces increasing amounts of hormones that antagonize insulin action .

2 - Maternal serum cortisol increases threefold .

3 - The placenta itself contains enzymes that may increase the degradation of maternal insulin (Jovancvic and Peterson , 1983) .

Thus , the carbohydrate changes occuring during pregnancy have the cumulative effect of making mild subclinical diabetes worsen and become clinically obvious during late pregnancy . They also make the control of blood glucose levels for the insulin - dependent diabetic patient more difficult .

II- Classification of Diabetes in Pregnancy

Pregnancy is complicated by diabetes in 2 to 4% of all cases (Merkatz,et al., 1980).

White Classification of Diabetic Pregnancies (White,1974).

A	Glucose tolerance test abnormal. No symptoms. Euglycemia maintained with treatment by appropriate diet but without insulin.
B	Adult onset (age 20 or older) and short duration (less than 10 years).
C	Relatively young onset (age 10 - 19) or relatively long duration (10 - 19 years).
D	Very young onset (age less than 10) or very long duration (20 or more) or evidence of background retinopathy.
F	Renal disease.
R	Proliferative retinopathy.
RF	Both renal disease and proliferative retinopathy.
G	Multiple reproductive failures (habitual abortions and / or still births).
H	Arteriosclerotic heart disease.
T	Pregnancy after renal transplantation.

Pyke Classification (Pyke, et al., 1973).

1.	Gestational diabetes : diabetes that starts during pregnancy and disappears after pregnancy.
2.	Pregestational diabetes : diabetes that began before conception and cotinues after pregnancy.
3.	Pregestational diabetes complicated by vascular disease: retinopathy, nephropathy, pelvic vessels, or peripheral vascular disease.

Modification of The Pyke Classification (1975)

I.	Gestational diabetes	A. Good diabetic control B. Less than optimal diabetic control.
II.	Pregestational diabetes	A. Good diabetic control B. Less than optimal diabetic control.
III.	Pregestational diabetes complicated by vascular disease (retinopathy,nephropathy, peripheral vascular disease).	A. Good diabetic control. B. Less than optimal control.

Immunological Disturbances in Diabetes Mellitus

I--Humoral Immunity

1 - Serum Immunoglobulin in Diabetes

The abnormal behaviour of serum immunoglobulins in the course of diabetes mellitus is one of the problems related to the immunopathologic framework of the disease . Cheta . et al. , 1982 , investigated serum immunoglobulins G , A , and M by radial immunodiffusion in a group of insulin dependent and non - insulin dependent diabetes . They found a remarkable increase of IgA levels as compared with IgG and IgM whatever the criteria of clinical analysis was . Moreover , all the three Ig classes presented higher levels in females than in males and IgG and IgA showed a slight tendency to increase with the duration of disease . In terms of the patients' age at onset of disease , they pointed out that IgA alone increased gradually with age reaching a " real peek " in senile diabetes . On the other hand , Smith , et al. , 1978 , found prevalence of IgA deficiency in childern with juvenile diabetes but not in insulin - dependent adult diabetes .

IgA immune complex positive diabetes patients were found to have elevated serum IgA levels as compared to immune complex negative patients and controls (Triolo , et al . , 1984) .

According to insulin doses , Cheta , et al. , 1982 , found the highest IgG and IgM values in patients with liver involvement , while the highest IgA values were in those with renal complications . The lowest values were recorded in dyslipidemia group for IgG and in the group with recent ketoacidosis for IgA and IgM .

The explanation of the IgA value in diabetic patients is not clear . However , Paunescu , et al. , 1981 and Kehoe , et al. , 1987 , demonstrated a higher daily catabolic rate of IgA (25 %) than IgG and IgM .

Charles , et al. , 1981 , studied the behaviour of the immunoglobulin in the non - insulin - dependent (type II) diabetic patients . They found no significant difference between the mean IgG and IgM levels while a significant high level of IgA was recorded , regardless of age , sex , duration of disease , and type of treatment (insulin / diet or oral hypoglycemic agents and / or diet) . IgA level was significantly different between diabetic patients with infections versus diabetic patients without infection .

However IgA levels of uninfected diabetic patients remained significantly higher than those of normal controls with bacterial infection . They suggested that the elevation of IgA levels in diabetic patients without infection maybe secondary to subclinical infections , particularly urinary and intestinal tract , or secondary to metabolic disturbance (Lopez , et al. , 1978 ; Farid , et al. , 1973) .

Hoddimott , et al. , 1982 , found no overal decrease in serum IgA levels in Type I (insulin - dependent) diabetic patients who were not completely IgA deficient . In addition they found patients with HLA - B8 had IgA levels non - significantly lower than those of patients without this antigen , indicating that quantitative reductions as well as absence of IgA are associated with B8 .

Low IgG levels have been noted in type I than Type II diabetic patients (Hoddinot , et al. , 1982 ; Farid , et al. , 1973) . In addition , others reported low IgG and IgM as well as in IDDM (Yuldashev , et al. , 1980 ; Duric , et al. , 1976) .

2 - Insulin Antibodies

It has been accepted for a long time that the majority of

diabetics treated with insulin develop insulin - binding antibodies , where as patients who have never received insulin do not normally posses such antibodies (Faulk , et al. , 1971) . Consequently , it has been assumed that these insulin antibodies are not the primary cause of islet cell damage in diabetes , although they are undoubtly a cause of insulin resistance . Furthermore , insulin antibodies have apparently developed without adminstration of exogenous insulin in a small group of patients , without evidence of islet cell damage has been demonstrated (Anderson , et al. , 1978) .

3 - Islet - Cell Antibodies

Insulin - dependent diabetic patients also possess antibodies to antigens other than insulin that originate in the pancreatic islets . One group of such islet cell antibodies , described originally by Bottazzo . et al. , 1974 and MacCuish , et al. , 1974 , is predominantly of IgG class and react with all cell types of pancreatic islets . These antibodies are present in the majority of juvenile - onset insulin - dependent patients at the time of diagnosis (Lendrum , et al. , 1975) . However Lendrum , et al. , 1976 and Irvine , et al. , 1976 , found their prevalence declines rapidly as subsequent to clinical

onset of disease . Moreover , islet cell antibodies may even be detected prior to clinical presentation (Irvine , et al. , 1976) , implying a predictive and possible pathogenic role for such antibodies . The antibody reacts with all types of islet endocrine cells and is related to cytoplasmic antigen (William , 1981) .

Recently , another antidody which reacts with surface antigens from rat B - cella has been described . A group of investigators demonstrated serum antibodies reacting with cell surface of a viable insulinoma cell in almost 90 % of insulin - dependent diabetic patients regardless of duration of disease (William , 1981) .

As regard to maternofetal relationship , IgG antibodies to both insulin and islet cells would be expected to cross the placenta (Brambell , et al. , 1970) and could potentially cause damage to the fetus (Galbraith and Faulk , 1979) .

II- Cellular Immunity

Investigations employing blast transformation assays to examine specific cellular immune reactions have revealed evidence of cellular immunity to insulin in diabetic

Patients (Faulk , et al. , 1975 ; MacCuish , et al. , 1975) . Similarly , leukocyte migration assays using antigens other than insulin have yeilded positive results in a proportion of juvenile - onset insulin - dependent cases (Nerup , et al. , 1974 ; MacCuish , et al. , 1974) .

Cell - mediated immunity to islets was observed in the migration - inhibition test using antigens extracted from the pancreas , and this immunity was associated with a delayed - type hypersensitivity reaction in skin testing . The response was not to insulin but could be obtained from the whole pancreatic homogenates or human insulinoma extracts (Nerup , et al. , 1974) .

Quantitation and function of lymphocyte populations using phytohemaglutinin (Hann , et al. , 1976 ; MacCuish , et al. , 1974 ; Ragab , et al. , 1972) , appear to be essentially normal in diabetic patients . However , a number of defects at various stages of the leukocyte phagocytic process have been demonstrated . In addition , there have been a number of studies demonstrating reduced ingestion or intracellular killing of micro - organisms (Tan , et al. , 1975 ; Bagdade , et al. , 1974 ; Marble , et al. , 1938) .

Furthermore , impaired chemotaxis was found by Mowat and Baum , 1971 , while Miller and Baker , 1972 , reported

defective generation of chemotactic activity in serum from diabetic patients . Molenaar , et al. , 1976 , demonstrated that comparable defects in chemotaxis may occur in nondiabetic first degree relatives of diabetics , impling that defects in phagocytic function may involve intrinsic geneticalIy determined abnormalities .

However , other abnormalities of phagocytic cell function have been found to be largely or entirely reversed by careful treatment and maintenance of good diabetic control (Galbraith , et al. , 1979) . This observation may be important for management of pregnant diabetic patients , since defective phagocytic function during gestation might theoratically expose the fetoplacental unit to an increased risk of infection and its consequences .

More recently , specific cytotoxicity was demonstrated with cultured human insulin cells and lymphocytes from insulin - dependent diabetic patients (William , 1981) .

Inhibition of insulin released by lymphocytes from patients with type I diabetes associated with other endocrinopathies has been reported , thus suggesting for the first time a direct effect on beta cell function in vitro by lymphocytes obtained from diabetics (Boitard , et al. , 1980) .

Analysis of lymphocyte subsets in type I diabetics at different intervals after diagnosis using monoclonal antibodies confirming the existence of an increased number of cytotoxic lymphocytes and a low number of suppressor cells at the time of clinical diagnosis (Sensi , et al. , 1981) .

Anthor interesting piece of information indicates that suppressor cell function is strongly reduced in recently diagnosed diabetics , which could explain in the abnormal antibody production and increased cytotoxicity observed (Buschard , et al. , 1980 ; Galluzzo , et al. , 1980) .

Factors Arising During Pregnancy

With a particular reference to insulin - dependent diabetics obstetric events may suggest an impairment of host defense mechanisms and possible abnormalities in immunologic function . For instance , asymptomatic bacteriuria (Macourt , et al. , 1974) , and urinary tract infections during pregnancy (Peterson , et al. , 1974) , appear to be more common in diabetic than in nondiabetic women (Peterson , 1965) . Moreover , abortion and preeclamsia , both diseases in which immunologic factors have recently been implicated , (Faulk , et al. , 1978 ; Bresnihan , et al. , 1977) , are common complication of the diabetic pregnancy (Abell , et al. , 1976 ; Barns , et al. , 1949) .

Gestational Diabetes

Carbohydrate intolerance , termed gestational diabetes , may also develop transiently during pregnancy in normally nondiabetic subjects . Although from a clinical point of view , the frequency of bacteriuria , abortion , and preeclampsia appear to be similar as in juvenile onset , the pathophysiology in gestational diabetics may differ . Gestational diabetes appears to involve peripheral insulin resistance , similar to that occuring in maturity - onset diabetes . It is characterized by normal or elevated serum levels of insulin (Kuhle , et al. , 1976 ; Gabbe , et al. , 1972) ; and by adequate release of insulin following intravenous tolbutamide (Noteovitz , et al. , 1977) .

Gestational diabetes thus seems more akin in terms of pathophysiology to maturity onset insulin - independent diabetes than to the insulin - dependent form of the disease . Consequently , gestational diabetes could be expexted to be vulnerable to comparable defects in phagocytic function although unnecessarily to demonstrate evidence of cellular or humoral immunity to pancreatic antigens (Galbraith , et al. , 1979) .

Immune Complexes (ICs) in Diabetes

There is evidence that immune complexes are present in the peripheral circulation of diabetic patients (DiMario , et al. , 1981) ; Triolo , et al. , 1984) . Jayaroa , et al. , 1974 , found immune complexes of insulin anti - insulin in insulin - treated diabetes , and it is possible that other antidodies , such as those directed against glucagon and somatostatin (Bottazzo , 1976) and islet cell antibodies (Lendrum , et al. , 1976 ; Bottazzo , et al. , 1974 ; MacCuish , et al. , 1974) may also form immune complexes with the homologous antigen .

Some reports have postulated a relationship between ICs and the late diabetic complications (DiMario , et al. , 1981 ; Irvine , et al. , 1978) . Insulin anti - insulin ICs were thought to contribute to the microangiopathy production (Andersen , et al. , 1976 ; Page , et al. , 1971) . Increasing evidence is accumulating that IgA containing ICs may be involved in the vascular damage of some immunologically related diseases (Kauffmann , et al. , 1980 ; Hal , et al. , 1980) .

The liver plays an important role in the sequestration of antigens derived from the intestinal tract and , since a

large component of circulating IgA is derived from the gut , it is possible that IgA - ICs are of this origin . An increased rate of production , a decreased rate of phagocytic clearance , or an impaired hepatic clearance (by a receptor) of the secretory component might cause the IgA - ICs occurence in diabetes (Triolo , et al. , 1 1984) .

Transplacental Passage of Maternal Antibodies in Diabetic Pregnancy

A number of antibodies charateristic of the diabetic patient are of IgG class and would be expected to be transported across the placenta into the fetus (Galbraith and Faulk , 1979) .

Thus although Palumbo , et al. , 1964 , were unable to find any variation in insulin antibody titer during pregnancy , Exon , et al. , 1974 , demonstrated a progressive decrease in maternal blood levels of antibody to insulin during pregnancy and a rise following delivery , compatible with transplacental passage of insulin binding antibodies . Spellacy and Goetz , 1963 , and Jorgensen , et al. , 1966 , found evidence of such antibodies in cord samples of diabetic infants . It is generally accepted that insulin does

not cross the placenta in significant amounts , and Kalhan , et al. , 1975 , have furthermore demonstrated in a small number of diabetics that the possession of insulin - binding antibodies does not induce insulin transfer .

Another group of antibodies that maybe transported across the placental barrier are islet - cell antibodies . Gamlen , et al. , 1977 , reported detection of such antibodies in an infant born to a mother with idiopathic Addison's disease and gestational diabetes , and it was further found that islet cell antibodies were no longer detectable at 1 year of age .

The pathogenic potential of maternal antibodies remains obscure . It is interesting to speculate that transplacental passage of insulin - binding or islet cell antibodies maybe responsible in part for the fetal pancreatic islet cell damage that occurs . Freytag , et al. , 1971 , found that repeated injection of insulin antibodies into pregnant mice resulted in pancreatic islet hyperplasia with marked degranulation . This sequence of events may occur in human diabetic pregnancy and could be responsible in part for transient or permanent diabetes of the newborn (Gentz , et al. , 1969) or the development of diabetes later in childhood (Yssing , et al. , 1975) .

Moreover , if stimulatory interactions analogous to the type - V stimulatory immune response proposed by Roitt , 1977 , occur between islet cells and such antibodies , these could account in part for the hypoglycemic episodes that frequently occur in the neonatal period (Gentz , 1969) .

CHAPTER 3

MATERIALS AND METHODS

Materials and Methods

Patients Studied

30 diabetic pregnant patients were investigated, 30 diabetic nonpregnant, 10 normal pregnant females,and 10 nonpregnant non-diabetic control. They were grouped as follows:

Group 1 : Diabetic pregnant (N=30) : each trimester (N=10)

Group 2 : Diabetic nonpregnant (N=10)

Group 3 : Normal pregnant (N=30) : each trimester (N=10)

Group 4 : Control (N=10)

(i) Age

The age of the investigated females was between 21 and 42 yrs.

(ii) Blood Sugar Level

The postprandial blood sugar was done as an indicator for the presence of diabetes. 65% of our diabetic pregnant patients had postprandial blood sugar above the normal value (70-110

mg/dl), while 35 % had controlled blood sugar.

(iii) Duration of Diabetes

70% of the pregnant ladies are diabetic from 1 to 4 yrs.; 23% had the disease during the last yr., while 7%are suffering from diabetes for more than 8 yrs.

(iv) Contraceptive Pills

23% of our cases had previous history of taking contraceptive pills, while 77% did not.

(v) Diabetic Treatment

All the cases took insulin as a treatment for diabetes during pregnancy. Only 3 patients (13%) were taking insulin as a treatment for diabetes before pregnancy, while the rest were taking oral hypoglycemic drugs.

(vi) Pariety

Only 3 patients were primgravidae and the rest were multigravidae.

(vii) Other Accompanying Complications

Only 3 patients were suffering from hypertension for long time (8 to 12 years) and the rest of the patients were free from any complications other than diabetes.

Venous blood samples were obtained from 30 diabetic pregnant females during the three trimesters of pregnancy. Control samples were taken from 10 normal pregnant , 10 diabetic nonpregnant females and 10 normal nonpregnant. The cases were chosen from the medical out-patient-clinic of Ain Shams University Hospital together with Benha University Hospital. The level of the serum immunoglobulins (IgG, IgM, IgA) were measured by the Single - Radial Immunodiffusion Method of Mancini (1965) using antibody agar plates (Nor-Partigen immunodiffusion plates). Anaylses were performed on all samples from a given subject at one time in order to avoid differences which could be attributed to day-to-day variability in the testing procedure.

Preparation of The Sample (Serum)

1) 2 c.c blood was taken by venepuncture from each patient without adding anticoagulent in test tubes.

2) Incubation at 37°C in water bath for ½ H.

3) Then the clot is dislodged from the sides by a stiff wire or needle.

4) Centrifugation at 1500 r.p.m .

5) Pipetting the serum by pasteur pipette. (Cruickshank,et al.,1975)

Storage of The Sample

The samples of serum were stored deep frozen at -20°C.

Materials

1) Serum

2) Nor-partigen immunodiffusion plates (Behring Institute) glass capillary tubes for dispenser 5µ l.
Behring dispenser 5µ l.
(Behring wake measuring viewer) scaled magnifying glass.

The Method Used

Principle : Single Radial Immunodiffusion

When antigen (sera) diffuses from a well into an agar containing suitable diluted antiserum initially it is present in a relatively high concentration and forms soluble complexes; as the antigen diffuses further the concentration continuously falls until the point is reached at which the reactants are nearer optimal proportions and a ring of Ag x Ab precipitate is formed arround the well. The diameter of the precipitate ring reflects the concentration of the antigen (Roitt,1980).

Preparation of The Plates

The cover of the aluminium container was pulled off using the tab provided for the purpose; the plastic container was removed and the opened plate was allowed to stand for about 5 min. at room temperature for evaporation of any condensed water which may have penetrated into the wells.

Filling The Wells

1. Both the control serum and the specimens were applied undiluted.

2. The volume required per well was 5 µ 1 (0.005ml).
3. For the dosage of the exact volume Behring Dispenser 5µ 1. was used.

Procedure

1) Control serum for Nor-partigen was introduced into well 1 in order to check the accuracy of the plate.

2) Wells 2 - 12 were filled with the specimens to be examined.

3) After introduction of the specimens, the plate was allowed to stand tightly- closed at room temperature.

Evalution

1) After 48 h the diameters D of the precipitates were measured to an accuracy of 0.1 mm using a scaled magnifying glass against a black background with lateral illumination.

2) Using the Table Calibration Values , the concentration of the immunoglobulin tested was read in correspondence to the precipitate ring diameters measured.

3) The accuracy of these results was checked by means of control serum for Nor - Partigen.