SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)

Project Report of 2022: SVP-2201

"Immunomodulatory Role of Hormones"

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SRIVIPRA PROJECT 2022

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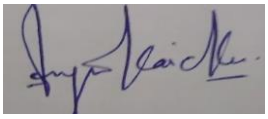
Certificate

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2201titled **"Immunomodulatory Role of Hormones".** The participants have carried out the research project work under my guidance and supervision from21st June 2022 to 25thSeptember 2022. The work carried out is original and carried out in an online mode.



Signature of Mentor

Acknowledgements

We take this opportunity to extend our heartfelt gratitude to our institute, Sri Venkateswara College, for giving us this platform to conduct research on the topic **"Immunomodulatory Role of Hormones"** and sharpen our scientific skills.

We are extremely grateful to our mentor and guide, Dr Anju Kaicker, for showing us how to systematically design a study address the objective of this particular project and write a report. Her valuable guidance and kind supervision throughout the project shaped the present work as it shows.

We would like to thank the Coordinators, Sri Vipra, Principal and Management(Tirumala Tirupati Devasthanams), **Sri Venkateswara College, University of Delhi** for giving us this opportunity under SRI VIPRA.

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Immunomodulatory Role of Placental Hormones in the establishment and maintenance of Pregnancy

INTRODUCTION

Every species living on this planet reproduces. Reproduction is the process by which an organism produces ‘more of its own kind’. During the process of gamete formation, recombination of genetic material takes place, which produces variations in the offspring. Hence, it plays an essential long-term role in the maintenance and evolution of that species. In viviparous organisms, zygote formation and its subsequent development into an embryo and a foetus takes place inside the mother’s body. A successful pregnancy is a rather complex process since only half of the genetic material of the foetus is identical to the mother’s and the other half, which comes from the father is ‘foreign’ to the mother’s body, making the foetus an allograft for the mother. During the course of gestation, tolerogenic immune response is generated by the various immune cells present in the decidua. The hormones released by the placenta, namely hCG, Progesterone (P4), oestrogen, and leptin have an immunomodulatory role. A balance of function of the immune cells and the mediators released by them has to be maintained for the pregnancy to be successful, and a lack thereof leads to various pathological disorders. In this project, we wanted to study the various immune mechanisms that exist at maternal-foetal interface and favour a successful pregnancy. Further, we also wanted to see the role of Placental hormones as an immunomodulator.

FOETAL-MATERNAL INTERFACE

The fertilisation of the sperm and the egg leads to the formation of a zygote which undergoes a series of cleavage reactions converting to 2,4,8 celled stages of morula and finally into a blastocyst. The blastocyst contains the

inner cell mass which is surrounded by the trophoblastic cells which in turn surround the blastocoel i.e. the fluid-filled cavity.
The blastocyst then attaches to the uterine epithelium and the trophoblast undergoes a series of differentiation causing the syncytial fusion of mononucleated cells, which generates the first syncytiotrophoblast. The remaining mononuclear trophoblast cells are called the cytotrophoblast which help in the expansion of the syncytiotrophoblast. Going forward with the gestation period, this syncytiotrophoblast penetrates the endometrium and comes in contact with the maternal capillaries and the venous plexus forming a defined placenta.12-15 days post-conception, the cytotrophoblast leaves the placenta and differentiates into EVT cells which invade the walls of the spiral arteries. The syncytiotrophoblast forms fluid-filled spaces called lacunae and the remaining is the trabeculae which, with time, develops into primary, secondary, and tertiary villi. The extraembryonic mesoderm and the cytotrophoblast form the chorion while the space between the chorion and the inner cell mass is the amniotic cavity which is lined by the amnion. The foetal blood is exchanged throughout the gestation period between the foetal circulatory system and the placental villi via the umbilical veins and arteries which are collectively wrapped under the umbilical cord.

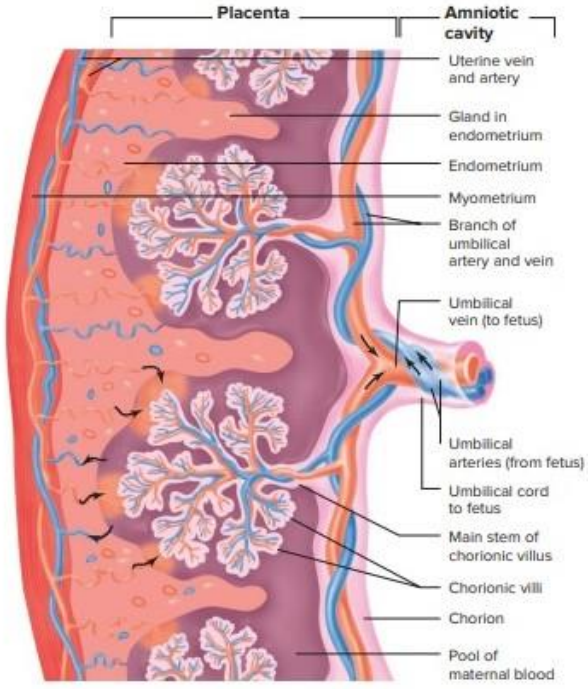

Figure 17.32 Interrelations of fetal and maternal tissues in the formation of the placenta.

Fig 1: *Foetal-Maternal Interface (Source: Vander's Physiology)*

IMMUNE CELLS AT MATERNAL FOETAL INTERFACE

Normal pregnancy consists of special hormonal changes and immunologic challenges. The integration and the balance of immune factors lead to an environment that enables the foetus to escape rejection by the maternal immune system. Immune cells accumulating in the human endometrium at the time of decidualization play critical and diverse roles at the maternal–foetal interface. In the decidua, leucocytes comprise about 30-40% of all cell populations present at any time. The uterine NK (uNK) cells comprise nearly 70% of the leucocytes, while 20% of cells are macrophages and the remaining are various subsets of T cells and dendritic cells (DCs). We first studied the role of these cells and human leucocyte antigens (HLA) in pregnancy.

ROLE OF UTERINE NK CELLS

Found in the maternal decidua during implantation, the uNK cells are abundant during the first trimester of pregnancy. Although they are present in small amounts during the proliferative and secretory phase of the menstrual cycle. Prior to implantation, the uNK cells are about 25-40% only and they increase slowly in number, rising to 70% in the first trimester. Very few uNK cells are present at full term. The uNK cells are different from the peripheral natural killer cells (pNK) which are found in maternal circulation. pNK cells exhibit cytotoxicity and secrete various cytokines to function as the first line of defence. They are CD56 low and CD 16 bright (CD56 CD16++). uNK cells are not cytotoxic and produce cytokines which play a major role in early placental development, trophoblastic invasion, and transformation of spiral arteries. Recurrent miscarriage (RM) or recurrent implantation failure (RIF) are some of the implications of failure in the functioning of uNK cells.

Characteristics of uNK cells

1. In humans, uNK cells are recognised by CD56 expression and lack of the expression of CD3. uNK cells are predominantly represented as CD56bright and CD 16 low (CD56++ CD16).
2. The uNK cells are granular containing granzymes, granulysin, and perforin.

3. uNK cells express the tissue-residence marker CD49a and can be subdivided into three different subsets which can be differentiated by their expression of CD39 and CD103.
4. While uNK are only weakly or not at all cytotoxic against trophoblast cells, this function can alter if decidual or trophoblast cells are infected by any virus or malignant cells during the course of gestation.

There are a number of theories on the origin of uNK which include either differentiation from uterine resident haematopoietic stem cells or recruitment from mature peripheral NK cells (pNK) by chemokine signalling (due to increased level of progesterone). They can also differentiate from immature pNK cells trafficked from the blood into the foetus.

Functions of uNK cells

1. The production of cytokines, growth factors, angiogenic factors, and other chemokines. Cytokines such as Tumor necrosis factor α (TNFα), interleukin-10, interleukin-1β, Transforming growth factor β (TGFβ), interferon-γ(IFNγ), and Macrophage-Colony stimulating factor (M-CSF).

2. Angiogenesis- Angiogenic growth factors such as vascular endothelial growth factor-C (VEGF-C), Placental growth factor (PlGF), and angiopoietin-1 are released by uNK cells.

3. Trophoblast invasion- The uNK cell function is regulated by the expression of receptors such as NKG2A, LILRB1, and receptors of the KIR (killer cell immunoglobulin-like- receptor) family. These receptors bind to HLA class I, HLA-C, E, and G expressed by extravillous trophoblast (EVT cells) and promote penetration of the trophoblast and the villi into the endometrium.

4. Spiral Artery remodelling -In the presence of uNK cells and macrophages, there is a loss of the musculo-elastic structure, and the endothelial cell layer of the decidua breaks off. uNK cells secrete proteases, such as metallomatrix proteases (MMPs) which assist in the invasion of the trophoblast. This causes the foetal trophoblasts to get attracted to the arteries transiently replacing the endothelial lining in the decidua and partly in the myometrium. The resulting consequence is spiral artery remodelling.

ROLE OF HUMAN LEUKOCYTE ANTIGEN-G (HLA-G)

HLA-G is a non-classical MHC-I molecule that is expressed on the pre-implantation embryos and EVT cells. It has 2 isoforms: Membrane binding isoform and Soluble HLA-G (sHLA-G). At the transcriptional level, seven HLA-G mRNA transcripts (HLA-G1 to HLA-G7) can be formed from seven exons, that undergo alternative splicing. HLA-G2, G3, and G4 are translated to the membrane binding isoforms, whereas HLA-G5, G6 and G7 are translated to the soluble isoforms i.e., sHLA-G. HLA-G1 can be translated to both isoforms. HLA-G plays a role in, spiral artery remodelling, fetal development and immune tolerance

Role of HLA-G in Spiral Artery Remodelling

The remodelling of the spiral arteries at the time of implantation is essential to accelerate and stabilise the placental blood flow. sHLA-G binds to the KIR2DL4 and gets endocytosed into vesicles of NK cells, where it activates the secretion of pro-inflammatory and pro-angiogenic cytokines (IL-6, IL-1β, IL-8, IL-23, MIP-1-α, and MIP-3-α). KIR2DL4 (or CD158d) belongs to the class of Killer Cell Immunoglobulin-like Receptors. It is expressed by the NK cells and has a different structure, function, and localization than other KIRs. The signalling pathway followed here is the NF-κB pathway.

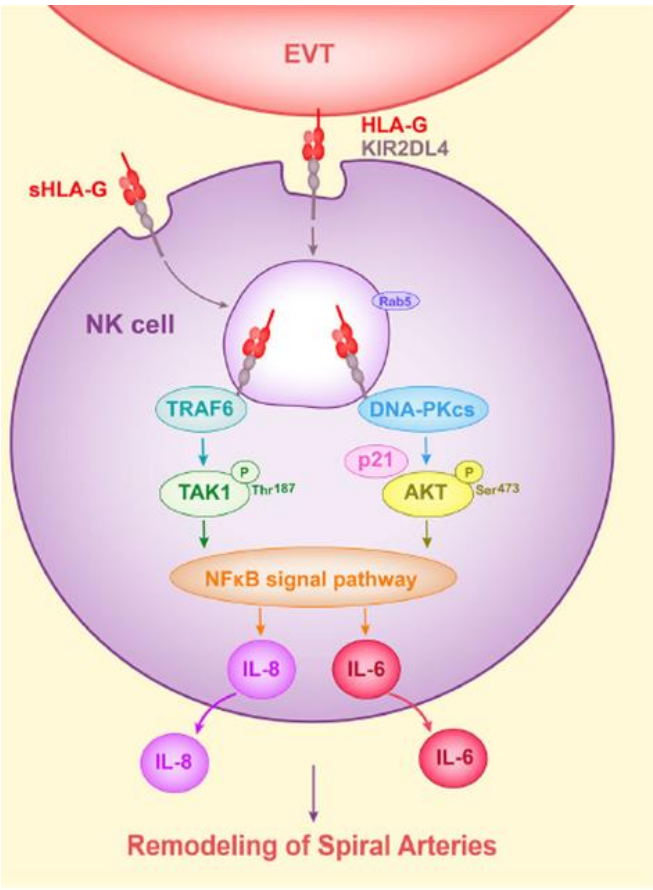

Fig 2: *Binding of HLA-G to KIR2DL4 and activation of NF-κB pathway (Source: Xu et al.)*

According to a study conducted by Long et al., on binding to KIR2DL4, sHLA-G can promote vascular permeability and angiogenesis by stimulating the production of senescence-associated secretory phenotype (SASP) in NK cells. The cytokine IL-8 has been shown to have a role in the invasive property of the trophoblast cell. Its secretion from NK cells is induced by sHLA-G. It is also secreted by decidual NK cells and macrophages, which is stimulated by homodimeric HLA-G on EVT cells. It has also been shown that differentiation of CD4+ peripheral T cells into suppressive CD4+ T cells is induced by HLA-G1+ APCs and decidual Treg cells can increase trophoblast invasiveness. Another example to show the role of HLA-G in spiral artery remodelling is in women with pre-eclampsia. Here, spiral artery remodelling is impaired and it was found that sHLA-G levels in serum of women with pre-eclampsia were reduced.

Role of HLA-G in Maternal-Fetal Immune Tolerance

HLA-G, along with a number of cells and pathways, plays a role in regulating the immune system at the maternal-fetal interface. This is crucial because NK cells are the most abundant leukocytes in the decidua during the first trimester of pregnancy. Studies were able to show that it was HLA-G and not HLA-C that prevented the cytotoxicity of NK cells. Antibodies against HLA-C and HLA-G were added in a co-culture medium, which restored the cytotoxic activity of NK cells. However, only anti-HLA-C antibodies were not able to do so, which meant that it was HLA-G that protected the cytotrophoblast cells from NK cell lysis. Additionally, cells overexpressing HLA-G, have been reported to upregulate HLA-E, which interacts with CD94/NKG2A to inhibit NK cell cytotoxicity. Treg cells can promote the expression of HLA-G in trophoblasts and inhibit the cytotoxicity of decidual NK cells, and hence facilitate the production of IL-4 and IL-10 by decidual NK cells. It was shown that HLA-G specifically inhibited cytolytic T cell function and this inhibition could be rescued by anti-HLA-G1 mAb.

Role of HLA-G In Fetal Growth

HLA-G stimulates the secretion of growth-promoting factors (GPFs) in NK cells such as pleiotrophin (PTN), osteoglycin (OGN), and osteopontin (OPN), which facilitate fetal growth. Its action is at the mRNA and protein levels in the decidual NK cells. In a study conducted by Fu et al., it was observed that in patients suffering from RSA (Recurrent Spontaneous Abortion), a small percentage of the decidual NK cells obtained were trNK cells (Tissue Resident NK cells), which showed decreased secretion of GFPs. CD49a+Eomes+ trNK cells are GPF-positive NK cells. Fu et al. also showed that GPF secretion in trNK cells is promoted by HLA-G in EVTs by acting on the receptors ILT2 and KIR2DL4.

ROLE OF UTERINE MACROPHAGES

These are the leukocytes produced from the differentiation of monocytes and are located in almost all tissues of the human body. Although macrophages are present in the placental bed at all times during pregnancy, the number of decidual macrophages vary with gestational age with the highest numbers found in the first and second trimesters. Macrophages are actively involved in trophoblast invasion, tissue and vascular remodelling during early pregnancy, besides their role as major antigen-presenting cells in the decidua.

They are subdivided into 2 groups: M1 (classically activated macrophages) and M2 (alternatively activated macrophages).

M1 macrophages are microbicidal and pro-inflammatory and responsible for inflammatory signalling, while M2 are anti-inflammatory macrophages that participate in the resolution of the inflammatory process, M2 macrophages produce anti-inflammatory cytokines, thereby contributing to tissue healing and induce tolerance.

Functions of Macrophages :

1. Trophoblast Invasion:
   Activated macrophages facilitate trophoblast invasion by degrading the extracellular matrix by secreting IL-1β.

2. Spiral Artery Remodelling:
   Macrophages prepare spiral arteries for further remodelling by trophoblast cells. This is shown by their presence around spiral arteries in the human placental bed that show disruption and disorganization of vascular smooth muscle cells and endothelial cells.Decidual macrophages regulate vascular remodelling by secreting vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and their receptors fms-like tyrosine kinase.
3. Phagocytose Apoptotic Cells:
   Apoptotic cells have immunosuppressive effects. Macrophages phagocytose apoptotic cells to promote trophoblast invasion and spiral artery remodelling and provide a balanced microenvironment at the maternal-fetal interface during the process of pregnancy.

T CELLS AND CYTOKINES

T lymphocytes can be broadly categorised into CD8+ cytotoxic T cells (CTLs) and CD4+ T helper cells (Th cells). Treg cells have a suppressive effect and modulate the activity of both these cell types. Th cells can be further classified into, Th1 cells which produce inflammatory cytokines, and Th2 cells which primarily produce anti-inflammatory cytokines. Th1-type cytokines, such as IFNγ and TNF, have important roles in homeostasis of the pregnant uterus and placenta, and in normal pregnancies, Th2 cytokine levels are high at the maternal–fetal interface, and antibody responses in the mother are also very strong. High numbers of Th1 and Th17 cells are accountable for spontaneous abortion. Further Th17 cells are pro-

inflammatory and affect anti-pathogen immunity in. the mother. Th 17 cells also secrete IL-22 another cytokine important for maintaining immune response at MFI.

A healthy pregnancy is associated with an enhancement of humoral immunity and a down-regulation of Th1 response. There is a downregulation of Th1 cytokines and upregulation of Th2 cytokines, as Th1- type cytokines have a deleterious effect on the conceptus.

Healthy pregnant women produce significantly higher levels of the anti-inflammatory Th2 cytokines IL-4, IL-5 and IL-10, while women with unexplained RSM produce significantly elevated levels of the pro-inflammatory cytokines IL-2, IFNγ, and TNFα. The expression of pro-inflammatory cytokines is upregulated in the endometrium, while that of anti-inflammatory cytokines is downregulated in women with unexplained recurrent miscarriage

Th2 and T reg cells are responsible for maternal tolerance toward foetal alloantigens, while Th1 and Th17 cells are accountable for spontaneous abortion. However, Th17-type IL-17 and IL-22 cytokines together and in association with IL-4 could have both a positive and a negative impact on pregnancy and could thus represent a double-edged sword.

IL -17 has a role in early-stage acute allograft rejection and seems to be a central player in spontaneous abortion. In patients with recurrent miscarriages, the number of IL-17-producingCD4+Tcell are high, whereas that of T reg cells are less compared to a healthy pregnancy. Th17/Treg ratio at the maternal-fetal interface in women with recurrent miscarriages suggests the contribution of Th17 in loss of maternal-foetal immune tolerance.

Higher levels of circulating Th17 cells, which induce strong systemic inflammatory changes and vascular endothelial dysfunction, were observed in preeclamptic women when compared to women with normal pregnancies. However, secretion of IL-4 along with IL-17 & IL-22 provides a protective effect and helps in trophoblast invasion and proliferation.

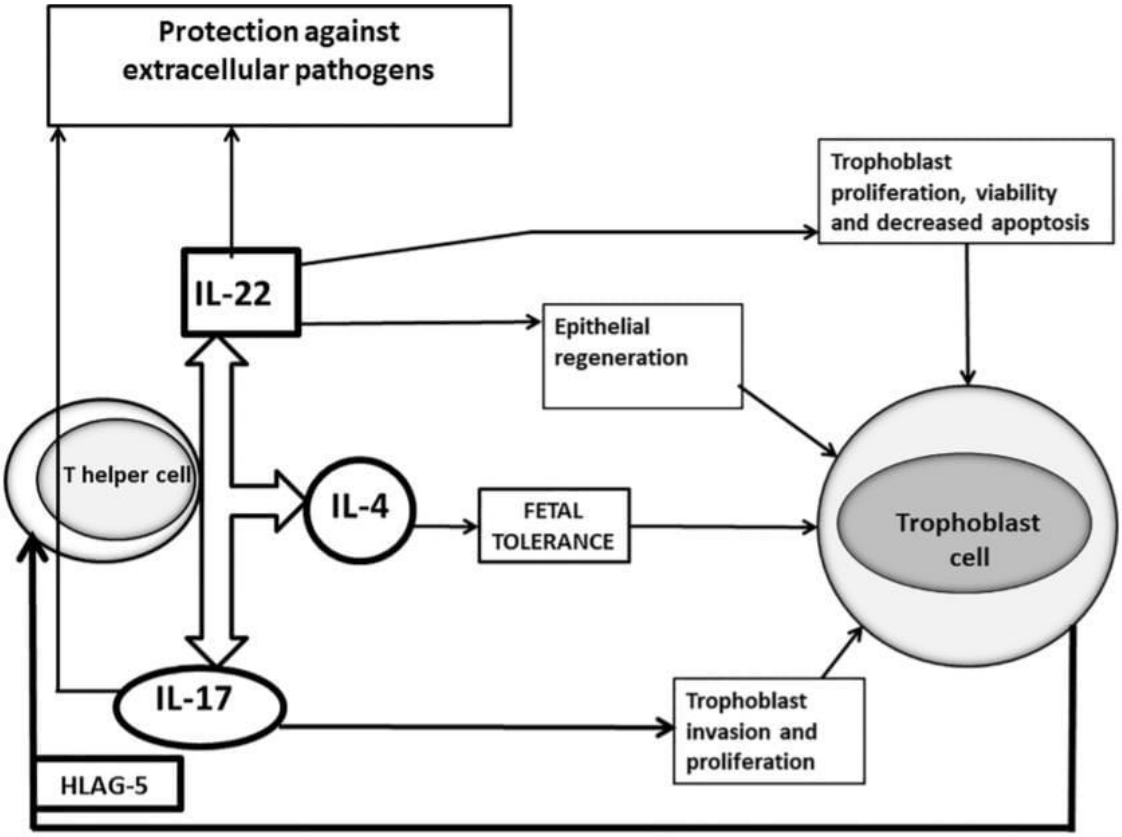

Fig 3 *Role of IL-17 and IL-22 at the maternal-foetal interface(Reference diagram taken from Frontiers in Immunology | www.frontiersin.org).*

γδT cells

The γδT cells are present in the endometrium of all mammals throughout pregnancy. Decidual γδT cells are large granular lymphocytes rich in intracytoplasmic granules and comprise a heterogeneous population of double positive TCRγδ+/CD56 + dim cells and TCRγδ single positive cells. They possess cytotoxic potency and express five major cytolytic molecules: perforin (Pf), granzyme A, granzyme B, granulysin and Fas ligand (FasL), and store them in microvesicles in intracytoplasmic cytolytic granules.

Decidual γδT cells do not express FasL on their surface but they store preformed FasL in the granules, and can rapidly mobilize it to the cell surface upon stimulation.The two major cytotoxic mechanisms – Pf- and FasL-mediated – are performed by one common secretory pathway based on cytolytic granule exocytosis. However, the absence of Pf and FasL effector molecules induces infertility.

T-reg cells

The term 'regulatory T-cells' (Tregs) refers to a family of T-lymphocyte populations with suppressive/regulatory properties that are devoted to maintaining antigen-specific T-cell tolerance. Tregs are produced in the thymus as a functionally mature subpopulation of T cells and can also be induced from naive T cells in the periphery. Treg are CD4+CD25+. Expression of the nuclear transcription factor Forkhead box P3 (FoxP3) is the defining property that determines natural Treg development and function. Tregs suppress activation, proliferation, and cytokine production of CD4+ T cells and CD8+ T cells. Tregs can produce soluble messengers including TGF-beta, IL-10, and adenosine which have a suppressive function. Treg can also modulate the activation state and function of APC and other immune effector cells. Paternal antigen-specific Treg cells accumulate in the uterine draining lymph nodes before implantation and increase in number in the uterus after implantation. Seminal plasma induces the expansion of paternal antigen-specific Treg cells and induces tolerance to paternal alloantigens. Fetal Tregs specific for maternal antigens suppress anti-maternal immunity of the foetus.

THE ROLE OF MAJOR PLACENTAL HORMONES IN IMMUNOMODULATION:

1. PROGESTERONE

Progesterone (P4) is a steroid hormone secreted by the ovary at specific times of the menstrual cycle, as well as the corpus luteum and the placenta. It is also an intermediate in the pathways for the synthesis of adrenal steroids, estrogens, and androgens. During pregnancy, progesterone exerts endocrine as well as immune-modulatory effects.

Endocrine Effects of Progesterone:

Progesterone has a wide range of endocrine functions throughout the pregnancy. Progesterone secreted by the ovaries stimulates the production of Activin A by the endometrium. Thus, it helps in preparing the endometrium for implantation and also to sustain it, in case fertilisation takes place. It stimulates the secretion of nutrients by the endometrium to nourish the early embryo, is pro-angiogenic in nature i.e., it stimulates the development of the blood vessels supplying the endometrium, can control the differentiation of decidual stromal cells and hence, impact decidualization. The disruption of this process can lead to complications in the pregnancy such as, recurrent

miscarriage and pre-eclampsia. It stimulates mammary gland development by stimulating Progesterone Receptor B (PR-B).

Immunomodulatory Effects of Progesterone:

Apart from playing a major endocrine role throughout pregnancy, progesterone also acts as an immune-modulator, to help sustain the pregnancy. Most of its actions are immunosuppressive in nature, however, it also has some immune-tolerogenic functions. Through a number of recent studies, it was shown that Progesterone inhibits the activation of murine dendritic cells, macrophages, and NK cells. Another study conducted using Lipopolysaccharide-stimulated rat dendritic cells showed that progesterone suppresses the production of TNF-α and IL-1β, which are pro-inflammatory cytokines. Progesterone suppresses the secretion of IL-12, a Th1-inducing cytokine, and the NF-κB pathway. The production of chemokines such as macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, and RANTES by $CD8^+$ T lymphocytes is suppressed by progesterone. A study conducted in Mice showed that Progesterone stimulates the development of tolerogenic dendritic cells. A disruption in the progesterone-dendritic cell interaction results in a poor generation of $CD4^+$ Treg cells, which is associated with poor placentation and intrauterine growth restriction. Progesterone helps in enhancing the phagocytic ability of trophoblasts by increasing trophoblast expression of anti-inflammatory mediators such as transforming growth factor (TGF).

Progesterone Receptors and signalling

Progesterone signalling can take place with the help of various receptors present on the plasma membrane or the nuclear membrane.

a. Nuclear PR (Progesterone receptor)

Its defining function is intra-cytoplasmic signalling and modulation of cascades to affect the transcriptional activity. Mediation by 2 isoforms PR-A and PR-B is seen .PR-A plays a role in implantation and decidualization while PR-B is needed for the development of the mammary gland.

In the inactive state, both the receptor isoforms are bound to chaperone proteins. Upon P4 Binding they dissociate to either:

(i) interact with P4 response element (PRE) sequences at gene promoter regions by forming dimers before translocating into the nucleus or,

(ii) activate extracellular signal-related kinases (ERK-1/2) by forming a monomer that interacts with SRC-kinase complexes and therefore modulate the transcription cascade via the mitogen-activated protein kinase (MAPK) pathway.

PRA can modulate transcriptional activity without the involvement of PRB and has the ability to repress the PRB activity. Also, the appropriate PRA/PRB ratio is maintained during pregnancy because labor conditions are stimulated with an increase in this ratio.

b. Membrane-associated PR

It is a non-classical PR and takes part in extracellular signalling. They are mediated by G protein-coupled progesterone receptors (GPCR) on cell membranes and P4 receptor membrane components (PGRMCs).

Every GPCR has a seven-transmembrane domain structure that includes an extracellular P4-binding domain. This domain has high affinity but limited capacity for P4 binding.

Activation of these PRs has been shown to suppress adenylyl cyclase activity and enhance the phosphorylation of myosin light chain protein in human myometrial cells.

When Progesterone binds with the Membrane-associated PR, activation of MAPK pathway is seen. This alters the phosphorylation status of transcription factors. These kinases of the MAPK pathway play important roles in T lymphocyte activation.

c. Nuclear GR

Glucocorticoids have anti-inflammatory effects in immune cells, such as inhibiting the transcription of pro-inflammatory cytokines and chemokines in macrophages and increasing regulatory T cell (Treg) proportions. Its excess in early pregnancy is associated with effects on placental function i.e it has the potential to convert myometrium from a quiescent state to an estrogen-primed contractile state in late pregnancy. Which is done by upregulating COX-2 expression in the amnion and placenta to induce parturition.

The nGR family comprises a number of isoforms, but the two principal variants are GRα and GRβ, which share a similar relationship to that between PRA and PRB.GRβ is thought to be a negative regulator of GRα.nGRs are ligand-dependent transcription factors. The Unliganded nGRs reside in the cytoplasm bound to heat shock proteins (HSPs) to form an inactive complex. This interaction between nGR and HSPs is disrupted by glucocorticoid binding to nGR, which then allows nGR to interact with its DNA-binding sites within the nucleus.

d. Membrane-associated GR

These are similar to membrane-bound PR receptors. The rapid effects of glucocorticoids are thought to occur *via* membrane GR (mGR). These receptors were first reported in a mouse lymphoma cell line and found to be coupled to G proteins.

Both nGR and mGR appear to share components of the NR3C1 gene.

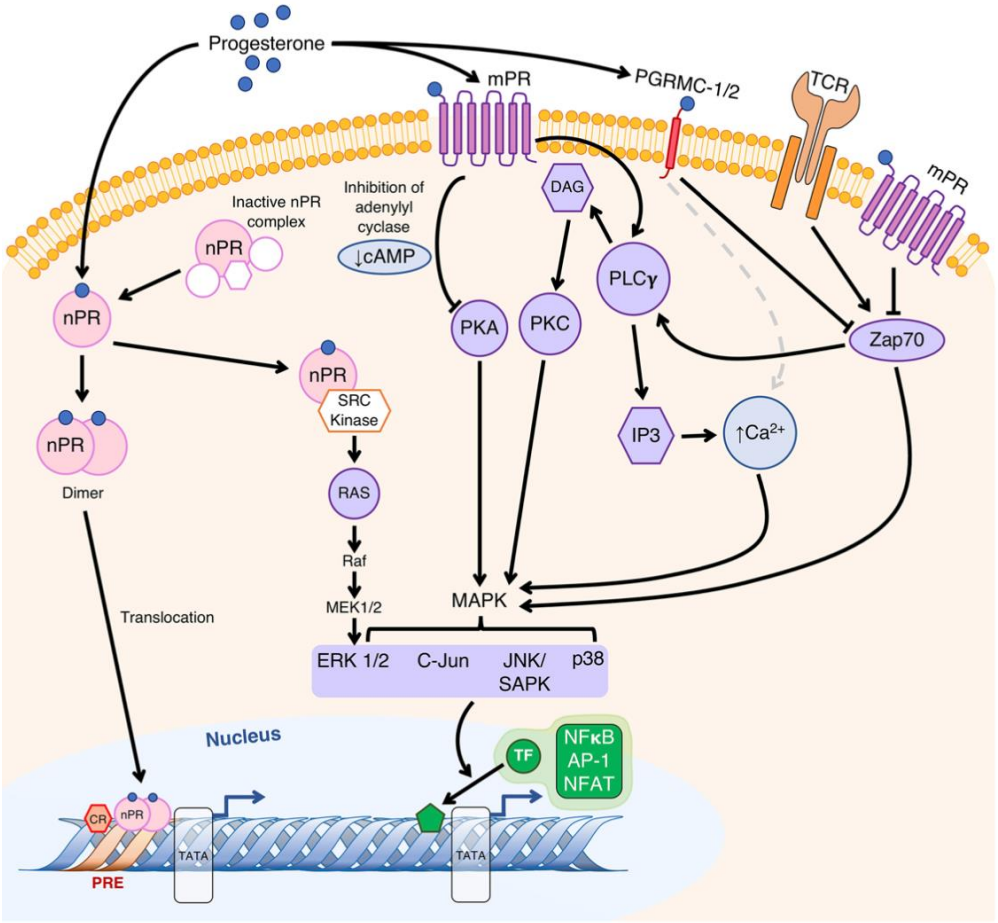

Fig 4: *Signal Transduction via Progesterone Receptors (Source: Shal et al.)*

Indirect action of progerstrone through P4-Induced blocking factor (PIBF)

PIBF is a PR-regulated gene and potent immune-modulator. Its expression is hormone-dependent, which increases during pregnancy. PIBF mediates a large proportion of P4-regulated effects in lymphocytes during pregnancy. Many functions of T cells are modulated by PIBF which include Th2- type cytokine synthesis, suppression of cytotoxic T and NK cell activity, and arachidonic acid synthesis.

P4-Dependent Regulation of PIBF Expression:

PR+ T cells in the peripheral circulation in pregnancy are predominantly γδ+ or CD8+ T cells which express PRs once activated after immune recognition of fetal and placental derived antigens, which occurs in a hormone-independent manner.

In pregnancy, the trophoblast, which forms the interface between maternal and fetal compartments, expresses a number of nonclassical MHC class I molecules, including HLA-C, HLA-E, and HLA-G proteins. γδ T cells interact with unprocessed foreign antigens in an MHC non-restricted manner and with non-polymorphic Class I or Class II-like molecules.

Placenta-derived nonclassical HLA molecules, such as HLA-C, serve to present fetal/paternal antigens to maternal lymphocytes. The expression of HLA-G, in particular, is enhanced in the presence of P4 and cytokines IL-10 and IFN-γ.

PIBF expression on CD8+TCR-γδ+ T cells is increased during healthy pregnancy and that is why the quantity of γδ+ PR-expressing T cells in peripheral blood is higher in pregnant women than in non-pregnant women.

PIBF binds to PIBF receptors after which the receptor forms a heterodimer with the IL-4 receptor and activates the Jak1/Stat6 pathway, leading to increased production of Th2 type cytokines. PIBF produces significantly higher levels of IL-4 and IL-10. The blocking of PIBF with anti-PIBF antibodies, or the inhibition of PIBF synthesis, results in Th1-dominant cytokine production which significantly increases NK activity and fetal loss which is corrected by the treatment with anti-NK antibodies.

2. hCG - HUMAN CHORIONIC GONADOTROPIN

Best known for its function of maintenance of corpus luteum for progesterone secretion during the early days of pregnancy, Human Chorionic gonadotropin is secreted by the trophoblastic cells.

It is a glycoprotein hormone weighing 36-40kDa and is made of 2 subunits α and β linked with a covalent bond. The α subunit is composed of 92 amino acids and is encoded in chromosome 6. This subunit is common in various hormones of the glycoprotein family including luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and thyroid-stimulating hormone (TSH).

The β subunit is different for each hormone and is encoded on different genes on chromosome 19 (LH, hCG, and TSH) or on chromosome 11 (FSH). The β subunit of hCG is encoded in six different genes located on chromosome 19. The β subunit contains the highest number of amino acids ie 145 & also the largest glycosylated domain. This gives the hCG greater stability and facilitates its rapid secretion. hCG contains four N-linked oligosaccharides and four O-linked oligosaccharides.

Isoforms of hCG

hCG occurs in various isoforms such as:-

1. Classical hCG
2. Hyperglycosylated hCG
3. Free β unit of hyperglycosylated hCG
4. Sulfated hCG

Hyperglycosylated hCG (hCG-H)

Hyperglycosylated hCG's β subunit has four oligosaccharide-linked Os instead of two like in classical hCG. This variant is massively produced during the first trimester of pregnancy by the extravillous cytotrophoblasts and represents 87% of the total hCG in the third week of gestation and 51% during the fourth week. Then, it decreases rapidly until it completely disappears from the maternal blood circulation at the end of the first trimester. hCG-H has an autocrine action rather than an endocrine action.
hCG-H monitoring is useful for predicting Down's syndrome, preeclampsia, therapeutic response to trophoblastic diseases, and pregnancy predictions performed in *in vitro* fertilization.

Functions of hCG-H

It Induces the implantation of the embryo by increasing the trophoblastic invasion and decreases the apoptosis of trophoblast cells. It is also massively secreted by choriocarcinomas and germ cell tumors. Recently, it was found that hCG-H is functionally similar to hCG, although it has lower potency for luteinizing hormone/choriogonadotropin receptor (LHCGR) activation.

Free β unit of hyperglycosylated hCG

The free β subunit of hCG acts like an antagonist through the transforming growth factor beta (TGF-β) receptor. It also activates LHCGR.

Sulfated hCG

The sulfated hCG produced by the pituitary gland is hardly detectable during the menstrual cycle. It is secreted in parallel with LH during the cycle and is at approximately one-fifth of the LH concentration. While its levels are low, sulfated hCG is exactly 50 times more potent than LH. Thus, sulfated hCG could perform comparable work with LH in stimulating androstenedione production during the follicular phase of the cycle as well as stimulating ovulation and corpus luteum formation. During the luteal phase, it may help stimulate progesterone production.

Endocrine effects of hCG:

Just like Progesterone, hCG also has a dual action i.e., it has both endocrine and immunomodulatory effects on pregnancy. Both are equally important for maternal tolerance of the embryo and hence, a successful pregnancy. Some of the endocrine effects are as follows: The primary function of hCG is to stimulate the secretion of progesterone by the corpus luteum, in the initial stages of pregnancy before the placenta is established. It also plays a role in the production of estradiol in human granulosa lutein cells by inducing the upregulation of aromatase expression. It helps prepare the decidua for implantation. hCG is secreted by the pre-implantation blastocyst into the uterine space, which binds to the LHCGR resulting in decidualization of stromal cells and secretion of prolactin. hCG promotes trophoblastic invasion by promoting the differentiation of cytotrophoblasts into invasive syncytiotrophoblasts. This can happen independently of the classical hCG receptor, LHCGR, and by different forms of hCG. However, it has been observed that Hyperglycosylated hCG (hCG-H) is more beneficial for implantation. It can regulate the synthesis of prostaglandins and cAMP formation.

Both hCG and hCG-H display a number of angiogenic effects. hCG promotes the secretion of VEGF through the NF-κB pathway, thus increasing blood vessel formation and migration and maturation of pericytes. The angiogenic effect of hCG-H was seen by studying its interaction with TGFbRII (Berndt et al.). It is not necessarily dependent on the LHCGR pathways. Further, Gallardo et al. found a relation in the functions of hCG and Heme oxygenase-I (HO-I). It is known that HO-1 regulates angiogenesis, vasculogenesis, trophoblast proliferation, migration, and invasion.

Immunomodulatory effects of hCG:

hCG has effects on substances that influence embryo implantation. The secretion of leukemia inhibitory factor (LIF) is increased and that of interleukin-6 (IL-6) by endometrial cells is decreased. hCG affects the Th1/Th2 ratio, which also plays a role in the implantation of the embryo. Another way by which hCG helps in implantation is that it stimulates the proliferation of CD4+25+ T cells and leads them to the endometrium. Certain effects of hCG on T cells have been observed in animal models. In murine models, the frequency of Treg cells *in vivo* increases and their suppressive activity decreases *in vitro*. In mice models, Treg cells and IL-1β levels increase. There are many other regulatory effects of hCG on T cells. It promotes the differentiation of memory T cells but reduces their functional activity toward fetal antigens through a competitive process. It also promotes IL-2 production by naive and memory T cells. hCG acts on uNK cells through mannose receptors, which are expressed on uNK cells and not the LHCGR. Their proliferation is regulated in a dose-dependent manner *in vitro*.An in vitro study demonstrated an inhibitory effect of hCG on bone-marrow-derived dendritic cells (DCs) as well as on peripheral and local (decidual) DCs, where hCG seems to retain a tolerogenic DC phenotype. Furthermore, hCG influence the differentiation and function of DCs, decreasing their ability to stimulate T-cell proliferation. hCG helps in the maintenance of pregnancy by acting on other immune cells, like monocytes, by promoting their function and secretion of IL-8. And also induces the functions of macrophages by which s, hCG cleans the endometrium by purifying apoptotic cells and fighting possible infections.

hCG could increase the ability of trophoblast cells to invade the extracellular matrix in vitro, which is done by an increase in the expression of matrix metalloproteinase (MMP)-2, MMP-9, and VEGF and a decrease in the expression of tissue inhibitor matrix metalloproteinase (TIMP)-1 and TIMP-

2. hCG enhances the effects of PBMCs (Peripheral blood mononuclear cells) which support in vitro embryo invasion. A study was performed to demonstrate that hCG inhibits the expression of tumour necrosis factor-alpha (TNFα) and interferon-gamma (IFN-γ) in the maternal/fetal interface and decreases the rate of resorption in abortive mouse models. In vitro treatment of PBMCs with different concentrations of hCG showed that hCG significantly inhibits IL-6 and TNFα messenger RNA (mRNA) expression, indicating that hCG could inhibit the production of proinflammatory cytokine

3. Leptin

Circulating leptin levels are increased during pregnancy and decreased after birth revealing its important role in pregnancy. Leptin and leptin receptors are expressed at the blastocyst stage, indicating its role in implantation. Leptin is an important regulator during the first stages of pregnancy which has physiological effects on the placenta, including angiogenesis, growth, and immunomodulation. Leptin induces proliferative activity in many human cell types via mitogen-activated protein kinase (MAPK).

Leptin as an Immunomodulator in Pregnancy

Leptin secreted by the placenta has many immune-modulatory functions.
It stimulates the secretion of IL-6 from trophoblast cells, and TNF-α release from the placenta, which is mediated by the NF-κB and the PPAR-γ pathways. It also induces placental expression of HLA-G thereby helping in providing an environment that is tolerogenic for the foetus. Leptin regulates the generation of matrix metalloproteinases (MMPs), arachidonic acid products, nitric oxide production, and T-cell cytokines. Additionally, the expression of Leptin itself is regulated by some cytokines including Interleukin-1α (IL-1α), IL-1β, IL-6 and interferon-γ (IFN-γ).

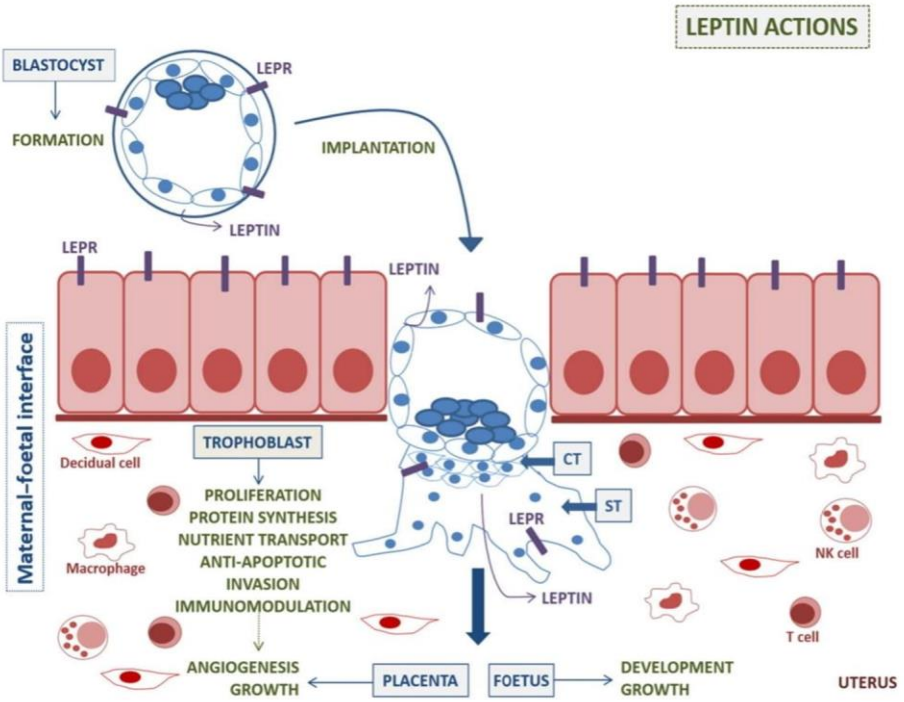

Fig 5: *Action of Leptin during the first stages of pregnancy (Reference diagram taken from Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine).*

CONCLUSION

For a successful pregnancy a balance of functions of immune cells and mediators released by them has to be maintained. The Immune cells accumulate in the human endometrium at the time of decidualization and play a major role in trophoblastic invasion, and transformation of spiral arteries cells and maintenance of tolerance. The immune cells present at MFI can be broadly categorized into CD8+ cytotoxic T cells,CD4+ T helper cells, Th-17, Treg and γδT cells, Macrophages and Natural Killer cells. Bsesides this HLA –G and HLA-E play a crucial role in maintenance of pregnancy. Progesterone (P4) is a steroid hormone secreted by the ovary at specific times of the menstrual cycle, as well as the corpus luteum and the placenta. It is intermediate in the pathways for the synthesis of adrenal steroids, estrogens, and androgens. During pregnancy,progesterone exerts endocrine as well as immune-modulatory effects. Progesterone signaling can take place

with the help of various receptors present on the plasma membrane or the nuclear membrane. hCG is another placental hormone showing effect on immune cells. Leptin an adipokine has now been shown as an important immunomodulator and its being discussed in detail in the next unit.

ADIPOKINE LEPTIN AS AN IMMUNOMODULATOR

Adipose tissue is a specialized connective tissue formed from mesenchymal stem cells during embryonic development. It is made of adipocytes, which can be white, beige, or brown. White adipocytes mainly store lipids, brown adipocytes contain lipids, and many mitochondria and beige adipocytes are usually scattered amongst white adipocytes. On the basis of the type of adipocyte present, adipose tissue can be classified as white adipose tissue and brown adipose tissue. Each of these types has its own functions. White adipose tissue consists of white adipocytes and some beige adipocytes. It can be subcutaneous fat (present under the skin), visceral fat (surrounding the organs such as kidneys, liver, intestines, etc.), and bone marrow fat (present in the central cavity of bones). It mainly functions to store fats and acts as a

reservoir of energy. It also secretes a variety of adipokines. Brown adipose tissue, which is mostly present in infants, consists of brown adipocytes and plays an important role in protecting infants from hypothermia, by generating heat (thermogenesis).

Leptin is one of the adipocytokines synthesised by adipose tissues and the placenta during pregnancy. It is an important signalling molecule of the reproductive system, as it regulates the production of gonadotropins, blastocyst formation, implantation, normal placentation, as well as the foeto-placental communication. Leptin or LepR deficiencies cause severe obesity, abnormalities in hematopoiesis, immunity, angiogenesis, bone formation, blood pressure, and reproduction. Mutations in the leptin gene can result in infertility or significant reproductive dysfunction.

The leptin receptor has 7 isoforms, as a result of alternative splicing, which differ in their intracellular domains. These isoforms can be short or long, wherein only the long isoform has complete signalling capabilities and the short isoform is not completely capable of signalling. Amongst the immune cells that express Leptin receptors, T cells express the long form, neutrophils express the short form and NK cells express both long and short form receptors. Leptin receptors can signal through the JAK/STAT Pathway as well as the PI3K/Akt and MAPK Pathways.

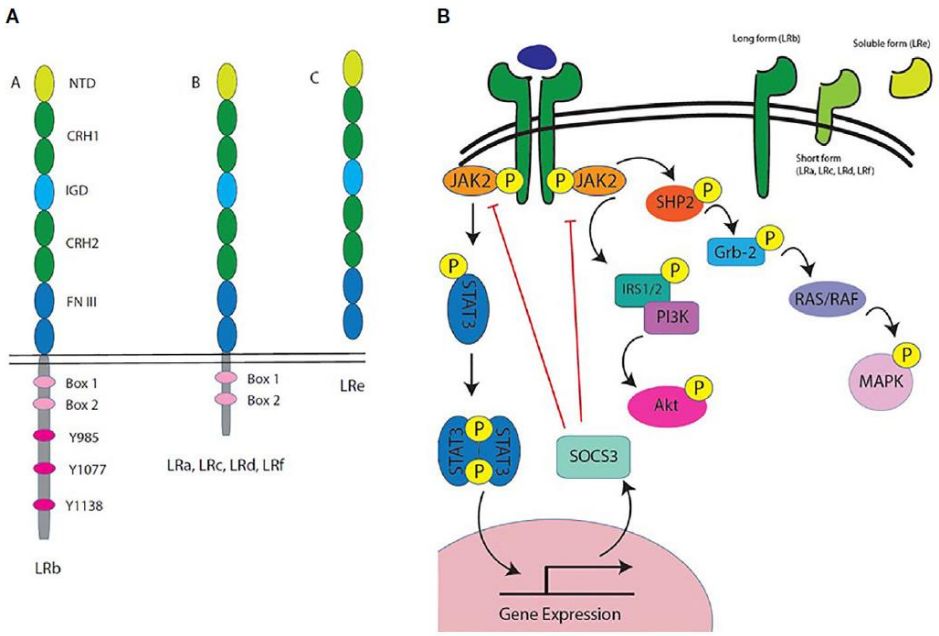

Fig 6: *Leptin receptor isoforms and intracellular signaling (Reference diagram taken from Frontiers in Immunology | www.frontiersin.org).*

Recent studies show that adipocytes have immunological functions capable of recruiting and activating immune cells. Here we would review the role of leptin on various immune cells

Effect of Leptin on T-cells

Leptin has an imperative role in modulating T cell development, function and metabolism. They are also responsible for development of CD4+T cells. Leptin receptors are expressed on double negative, double positive, and CD4 single positive thymocyte subsets.

CD4+ T cells express high levels of leptin receptor (Ob-Rb) because it is the only isoform that can signal through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway.

Leptin receptor signaling in T cells promotes survival, proliferation, cytokine production, and differentiation. In response to leptin treatment, naive CD4+ T cells, show an increase in proliferation in a mixed lymphocyte reaction. JAK-STAT signaling is downstream of many lymphocyte receptors that promote the production of various cytokines, thus leptin treatment of bulk, non-differentiated T cells influence cytokine production by these cells. Leptin treatment of CD4+ T cells increase proinflammatory cytokine production, namely T helper 1 (Th1) cytokines interferon-gamma (IFN-γ), IL-2 and decreasing production of the T helper 2 (Th2) cytokine IL-4.

Treg cells express high amounts of leptin receptor, and are capable of secreting leptin. Leptin inhibits Treg cell proliferation in primary human cells, and blockade of leptin binding to Treg cells using anti-leptin antibodies can lead to increased Treg cell proliferation.

B-Cells

Leptin promotes B cell homeostasis by inhibiting apoptosis and promoting cell cycle entry. It promotes cell cycle entry by increasing the transcription of genes that regulate the cell cycle. Human B cells stimulated with leptin in vitro exhibit more pro-inflammatory phenotype characterised by increased expression of inflammatory cytokines IL-6 and TNF, as well as toll-like

receptor 4 (TLR4). B cells show reduced class switching and IgG production in response to leptin revealing they do not necessarily have increased function.

Human peripheral blood B cells have increased IL-6, TNF, and IL-10 production when treated in vitro with leptin.
Leptin signaling in B cells activates JAK2, STAT3, ERK1/2, and p38 MAPK pathways. On inhibition of these signaling molecules there is decreased production of IL-6, TNF, and IL-10 following leptin treatment, revealing that signaling through JAK2, STAT3, ERK1/2, and p38 MAPK is required to increase cytokine production in response to leptin.

Dendritic Cells:

Dendritic Cells (DCs) are Antigen Presenting Cells i.e., they capture and process antigens and then present them to naïve T cells, triggering an adaptive immune response. Leptin receptors are expressed by DCs. One of the effects of Leptin on DCs, shown *in vitro,* is increasing the expression of anti-apoptotic proteins. Leptin also promotes the maturation of DCs, and mature DCs stimulate a stronger T-Cell response. Production of IL-1β, IL-6, IL-12, TNF, and MIP-1α is also stimulated by Leptin. In *ob/ob* mice, where Leptin is deficient, it was found that Bone Marrow Dendritic Cells (BMDCs) produced less IL-6, IL-12, and TNF after two days of maturation. *ob/ob* BMDCs also had reduced expression of MHC-II, CD80, CD86, and CD40, which have a role in the activation of CD4+ T cells. A comparison of CD4+ T cells stimulated by BMDCs from *ob/ob* or *db/db* mice and from wild-type mice showed that the CD4+ T cells from the former proliferated less and produced lesser IFN-γ.

Neutrophils, Basophils, and Eosinophils:

Leptin receptors are expressed by Neutrophils, Basophils, and Eosinophils. However, only the short-form leptin receptor is expressed by Neutrophils, which lacks the JAK-STAT signalling. Leptin has some common effects on these 3 immune cells:

1. It acts as a survival factor for them. In neutrophils, Leptin inhibits apoptosis.
2. It acts as a chemoattractant for these cells:

Neutrophils from WT mice showed chemotaxis towards leptin but those from mice with a variant of leptin receptor showed less chemotaxis towards leptin. In the case of LPS-induced lung injury in *db/db* mice, who have a deficiency of leptin receptors, lesser neutrophils at the infection site were observed and more neutrophils in the blood. In the case of *Clostridium difficile* colitis in mice with mutant leptin receptor STAT-3, lesser neutrophils were observed in the lamina propria. In in vitro studies on basophils and eosinophils from human blood, it was shown that they migrated towards leptin. Chemotaxis of human basophils and eosinophils towards eotaxin, a chemoattractant is promoted by leptin.

NK cells and ILC's

NK cells and the ILC's or innate lymphoid cells which are part of a complex family of lymphocytes respond to pathogens with rapid cytokine production and killing of infected cells. It was seen that when the leptin receptor was mutated (experiment done on db/db mouse), the NK cell number was found to decrease in the spleen, liver, lung, and blood. This implies that the leptin receptor is required for normal NK cell development. It was further confirmed when the db/db mice were injected with poly I:C, they expressed an early NK cell activation marker-CD69.

Mast cells

Mast cells play an important role in allergic responses and protection against helminth infection and respond to leptin secretion. A decreased percentage of mast cells in inguinal adipose tissue was observed in leptin mutant ob/ob mice but did not show mast cell deficiencies in other tissues. Further studies have shown a role for mast cells in polarization of macrophages by secretion of cytokines. This was observed when IL-33 treatment of mast cells causes the production of IL-6 and IL-13, which are cytokines known to promote activated macrophages that suppress T cell inflammation

- Mast cells derived from WT bone marrow (BMMCs) when co-cultured with bone marrow-derived macrophages (BMDMs) from leptin receptor mutant db/db mice (in the presence or absence of leptin), led to increased macrophage production of IFN-**γ** when leptin was present. This indicates that leptin promotes mast cell phenotype that drives inflammatory M1-like macrophage cell phenotype. It is also seen that leptin inhibits the anti-inflammatory M2-like

macrophage phenotype by decreasing arginase-1 and IL-10 expression.

- Mast cells from leptin mutant ob/ob mice promoted the maturation of WT macrophages to an M2- like anti-inflammatory phenotype which is the case when leptin is not present in sufficient amounts.

Macrophages and Monocytes:

The effects of leptin on macrophages are highly relevant in the setting of diet-induced obesity since macrophages are key regulators of adipose tissue inflammation in obesity. Leptin acts specifically on macrophages via the leptin receptor to promote both phagocytosis and cytokine production

1. Bone marrow-derived macrophages from leptin receptor mutant db/db mice and ob/ob mice were shown to have decreased phagocytic ability and decreased inflammatory cytokine production in response to LPS treatment in vitro, and ob/ ob mice failed to clear infections such as Escherichia Coli and Klebsiella pneumonia in vivo. Also, obese Zucker (fa/fa) rats with a leptin receptor mutation, had a reduced ability to clear the fungal infection Candida albicans in vivo.

2. Macrophage-specific leptin receptor knockout mice have elevated pulmonary IL-13 and TNF compared to WT mice 48 h after infection with S. pneumoniae, the same mice with macrophage-specific deletion of the leptin receptor have impaired clearance of Streptococcus pneumoniae in the lungs and spleen. But in vitro studies of alveolar macrophages from macrophage-specific leptin receptor knockout mice showed decreased macrophage killing and phagocytosis.

Monocytes are innate immune cells that can differentiate into tissue-specific macrophages and myeloid-derived dendritic cells.

In response to leptin treatment primary human monocytes from PBMCs and THP-1 monocytes, have been shown to increase toll-like receptor 2 (TLR2) expression in vitro. TLR2 is a pattern recognition receptor by which innate immune cells recognize pathogens and by promoting TLR2 expression on monocytes, leptin is able to promote the innate immune response to pathogens such as E. coli.

Leptin treatment of monocytes isolated from PBMCs increased the production of type 1 cytokines, including IL-1b, IL-6, and TNF, and resistin in humans.

CONCLUSION

Leptin is a pleiotropic adipokine predominantly synthesised by the Adipose tissue. It is now considered an important signalling molecule during pregnancy as it regulates the blastocyst formation and implantation During pregnancy, it is also synthesised by the placenta. Leptin, is responsible for satiety signals and decreased leptin sensitivity or lepR deficiency can lead to obesity. Leptin shows an immunomodulatory role by acting as a chemoattractant, promoting the maturation & proliferation of various immune cells and stimulates cytokine production . Leptin uses various signalling pathways such as MAPK and JAK-STAT. The concentration of leptin increases during pregnancy. Leptin seems to play a crucial role during the first stages of pregnancy as it modulates critical processes such as proliferation, protein synthesis, invasion and apoptosis in placental cells. The level of this hormone decrease after child birth.

REFERENCES:

1. Gridelet, V., Perrier D'Hauterive, S., Polese, B., Foidart, J. M., Nisolle, M., & Geenen, V. (2020). Human Chorionic Gonadotrophin: New Pleiotropic Functions for an "Old" Hormone During Pregnancy. Frontiers in Immunology, 11.https://doi.org/10.3389/fimmu.2020.00343

2. Huppertz, B. (2008b, July 19). The anatomy of the normal placenta. Journal of Clinical Pathology, 61(12), 1296–1302. https://doi.org/10.1136/jcp.2008.055277

3. Jørgensen, N., Persson, G., & Hviid, T. V. F. (2019, May 8). The Tolerogenic Function of Regulatory T Cells in Pregnancy and Cancer. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.00911

4. Kiernan, K., & MacIver, N. J. (2021b, January 29). The Role of the Adipokine Leptin in Immune Cell Function in Health and Disease. Frontiers in Immunology, 11. https://doi.org/10.3389/fimmu.2020.622468

5. Mincheva-Nilsson, L. (2003). Pregnancy and gamma/delta T cells: Taking on the hard questions. Reproductive Biology and Endocrinology, 1(1), 120. https://doi.org/10.1186/1477-7827-1-120

6. Pérez-Pérez, A., Toro, A., Vilariño-García, T., Maymó, J., Guadix, P., Dueñas, J. L., Fernández-Sánchez, M., Varone, C., & Sánchez-Margalet, V. (2017b, November 21). Leptin action in normal and pathological pregnancies. Journal of Cellular and Molecular Medicine. https://doi.org/10.1111/jcmm.13369

7. Piccinni, M. P., Raghupathy, R., Saito, S., & Szekeres-Bartho, J. (2021, July 28). Cytokines, Hormones and Cellular Regulatory Mechanisms Favoring Successful Reproduction. Frontiers in Immunology, 12. https://doi.org/10.3389/fimmu.2021.717808

8. Raghupathy, R., & Szekeres-Bartho, J. (2022). Progesterone: A Unique Hormone with Immunomodulatory Roles in Pregnancy. International Journal of Molecular Sciences, 23(3), 1333. https://doi.org/10.3390/ijms23031333

9. Shah, N. M., Lai, P. F., Imami, N., & Johnson, M. R. (2019). Progesterone-Related Immune Modulation of Pregnancy and Labor. Frontiers in Endocrinology, 10. https://doi.org/10.3389/fendo.2019.00198

10. Woods, L., Perez-Garcia, V., & Hemberger, M. (2018, September 27). Regulation of Placental Development and Its Impact on Fetal Growth—New Insights From Mouse Models. Frontiers in Endocrinology, 9. https://doi.org/10.3389/fendo.2018.00570

11. Xu, X., Zhou, Y., & Wei, H. (2020). Roles of HLA-G in the Maternal-Fetal Immune Microenvironment. Frontiers in Immunology, 11.https://doi.org/10.3389/fimmu.2020.592010