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Review Article

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The Role of Cytokines and Immune System in Unexplained Infertility

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Abstract

Many factors contribute to infertility, including immune conditions which may lead to immune infertility (immunologic infertility). The aim of this review is to evaluate the peer-reviewed studies reported so far regarding the immune cell sub-populations of T helper (Th) 1, Th2, and Th17 cells, regulatory T cells (Tregs), and cytokines produced by these cells, and their possible interplay in relation to unexplained infertility and to provide a potentially new diagnostic approach to immunologic infertility by investigating the correlation of Th1, Th2, Th17, and Treg and their secreted cytokines. Because the association of the immune system with almost all pathologies is relatively new, albeit remarkable, which opens to research and exciting development, hence we aimed to write this review article. Moreover, this review focuses on the potential roles of cytokines during pregnancy, which we believe will contribute to the understanding of the roles and mechanisms behind the cytokines that affect physiological and pathological conditions during pregnancy.

Key words: Unexplained infertility, T helper cells, cytokines, immunity, pregnancy

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Introduction

According to the World Health Organization (WHO) and International Auxiliary Reproductive Techniques Monitoring Committee (IARTMC), infertility is a disease of reproductive system, which is expressed as the inability to form a clinical pregnancy after 12 months or more despite unprotected and/or regular relationship (1). Infertility has been reported to be an increasing problem that affects approximately 15% of the reproductive age couples (2). When the factors that may cause infertility are listed, it is stated that male factor is 35%, tubal and pelvic pathologies are 35%, ovulatory dysfunction is 15%, and the remaining 15% is unexplained infertility (3).

The human endometrium produces a variety of cytokines during the proliferative and secretory stages of the menstrual cycle. It has been observed that these cytokines undertake many important roles during the pregnancy, such as the arrangement of the uterine environment, preparation of the uterus for the implantation of the developing concept, and the formation of functional placenta (4). T helper (Th) 1 cells mainly produce interleukins (IL), such as IL-1, IL-2, IL-12, IL-15, IL-18, interferon (IFN)-γ and tumor necrosis factor (TNF)-α. IL-4 produces IL-5, IL-6, IL-13, and granulocyte-macrophage colony stimulating factor (GM-CSF) (5,6). Th17 cells are the source of IL-17A and IL-17E (7). IL-10 and transforming growth factor (TGF)-β as an example of cytokines secreted by T regulatory (Treg) cells. During pregnancy, cytokine sources such as Th1 and Th2 consist of decidual epithelium and stroma, cyto- and syncytiotrophoblast, chorion, amnion and Haufbauer cells. Cytokines originating from these tissues are involved in the initiation of the maternal tolerance against fetal allografts, regulation of local immunity against infective factors, and tissue regeneration with placental hormonal production during trophoblast invasion (5).

For the unexplained infertility, many possible underlying causes have been proposed. When standard studies such as sperm analysis, ovulation test, and tubal potency do not give specific results or any abnormalities cannot be detected, unexplained infertility diagnosis is performed several times. In some studies, it has been suggested that the diagnosis of unexplained infertility is subjective and often misdiagnosed for endometriosis, tubal infertility, premature ovarian aging, and infertility (8).

Our bodies are constantly attacked by foreign pathogens since the birth. In order to remain healthy, our immune system has developed strategies that will continue to tolerate foreign antigens. To date, a large number of T helper cells' sub-groups have been identified based on the expression of the main transcriptional regulators and cytokine production profiles (9).

T cells send signals to cytokines in response to the antigenic stimulation. The stimulating signals stimulate other signaling pathways to facilitate the proliferation and differentiation of T cells, B cells, and macrophages (10). Cytotoxic T lymphocytes kill the cells that are infected with viruses or other pathogens that may live within the cell. Th and cytotoxic T lymphocytes as well as the suppressing immune responses have suppressive T

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lymphocytes that eventually control them. These cells are called regulatory T cells (Reg.T, regulator T lymphocytes). The most common reported Th cells are Th1, Th2, Th9, Th17, and Th22 (11; 12).

T Cells

Auxiliary T Cells (T Helper, or Th)

Cluster differentiation (CD)4+ Th cells, one of the basic building blocks of the immune system, start to secrete the cytokines that affect the function of almost all cells of the immune system after activation (13). These cytokines activate and regulate B cells, T cells, natural killer (NK) cells, macrophages, and other immune system cells (14). These cytokines are produced by trophoblast cells, stromal cells, epithelial cells, maternal T lymphocytes, macrophages, NK cells, and other maternal leukocytes (15). This suggests that the development and maintenance of the fetal-placental unit depends on these cytokines. The presence of these cytokines in the maternal-fetal range influences the provision of appropriate environment by regulating the processes such as implantation, placental development, cytotrophoblast proliferation, angiogenesis, extravillous trophoblast cell invasion, spiral artery reconstruction, cell growth, and apoptosis (4). As shown in Figure 1, antigen presentation in the presence of IL-12 induces expression of T-beta and IFN-γ production. As a result, naïve T cell differentiation is in the Th1 direction. On the other hand, IL-4 induces GATA3 (GATA binding protein 3) expression and IL-4 production, and this is necessary to differentiate into the Th2 cell direction. IL-6 and TGF-β retinoic acid-related orphan receptor (ROR)det expression induce IL-17 production in Th17 cells. TGF-β is required for the expression of FOXP3 (Forkhead Box P3) and Treg cell differentiation (10).

Th1 Cells

The production of Th1 cells with basic cytokine IFN-γ is controlled by the tissue-specific transcription factor T-bet (16). The task of IFN-γ is to develop NK cells in the Th1 cell direction (17). IFN-γ enhances Th1 differentiation by increasing IL-12 release from the antigen presenting cells (APC). Th1 cells fight with intracellular pathogens by activating macrophages, NK cells, and CD8+ T cells (18). IFN-γ regulates T-bet and stimulates Th1 differentiation by signal transducer and activator of transcription (STAT)1 by secretion from natural immune system cells. T-bet is a member of the T-box family, which is the key transcription factor associated with Th1 differentiation and functions. T-bet-deficient T cells have been reported not to differentiate into Th1 cells (11). The Th cells are responsible for the secretion of cytokines that direct immune cells to attack the infectious or abnormal targets. Increased Th-1 cytokines were seen in patients with recurrent pregnancy losses, and in vitro recurrent implantation failure Th-1 cells inhibit trophoblast growth and differentiation to produce cell-mediated inflammation and pro-inflammatory cytokines (18).

Th2 Cells

Th2 cells have a critical role in immune response against the extracellular parasites and have the effect in producing IL-4, IL-5, and IL-13. The host cells of skin, lungs and intestines, and parasite products of the natural immune system, recognize the parasite products and produce Th2 cytokines including IL-4, IL-25, and IL-33 (12;19). It also affects the cells of the

natural immune system, such as basophil and dendritic cells, and/or allows direct differentiation of naïve T cells into the Th2 direction. IL-4 activates STAT6, which plays a role in Th2 differentiation by interacting with its receptor. Activation of STAT6 enhances the induction of the GATA3 transcription factor, which is the main regulator of Th2 differentiation. When the function of GATA3 is disrupted, Th2 differentiation is not realized. IL-4 produced from the mature Th2 cells promotes the differentiation of more naïve T cells into the Th2 cell after encountering the antigen (12;20). It is thought that the recognition of the mother during the pregnancy is caused by the signals sent by the fertilized ovum, which leads to the predominance of intrauterine Th2 cells (21).

Differentiation of Th2 cells requires in the absence of IL-4 stimulation (22). STAT6-deficient mice have been reported to cause limited Th2 cell differentiation (23); both in response to Th2 polarization conditions in vitro and in Th2 cytokine production. IL-4 signal through STAT6 induces GATA3 expression (24) and directs the expression of GATA3, IL-4, respectively. IL-2 signals from STAT5-deficient mice have been shown to be less in Th2 cells, indicating the importance of this pathway in Th2 differentiation. STAT5 has been shown to bind to IL-4 gene and induce IL-4 receptor (R) α expression. IL-2 induces initial IL-4 production by naive T cells, which stimulates GATA3 expression via STAT6. Thus, this activation leads to positive feedback between GATA3 and IL-4, thereby enhancing its commitment to the Th2 sub-group. However, in Th2 cells, Stat5 activation is important to maintain Gata3 expression (25).

Regulator T Cells (Treg Cells)

The main function of the regulator T cells is to suppress the immune system in cases where an immune response is not required. This mechanism is useful when suppressing the autoimmune responses and clearing the pathogenic microorganism after infection. At implantation, the embryo expresses paternally derived alloantigens and evokes inflammation that can threaten the reproductive success. To ensure a robust placenta and sustainable pregnancy, an active state of maternal immune tolerance mediated by CD4+ regulatory T cells is essential (26). The role of Treg lymphocytes in pregnancy was first shown in studies on mice in 2004 and have been reported to be detected in lymph nodes draining the uterus after early mating (27). It is known that IL-10 and TGF-β levels increase during immunosuppression and play important roles during pregnancy (28), because Treg cells proliferate during pregnancy in humans and animals (29). In studies conducted in mice, neither estrogen nor progesterone have been shown to have any role in Treg cell expansion (30;12). The differentiation of Tregs is induced by stimulation with IL-2 and TGF-β (31). While STAT5, IL-2Rα deficient mice and STAT5 deficient mice showed a decrease in Treg populations, STAT5 rescued Treg population in the IL-2Rα deficient mice. STAT5 is directly linked to FoxP3 to induce the Treg differentiation program (32). In their study, Laurance et al. reported that IL-2 signaling through STAT5 blocked Th17 differentiation and the loss of IL-2 signaling resulted in a decrease in Treg

populations while increasing Th17 populations (33).

Th17 Cells

Significant findings related to a new helper T cell group different from the Th1 and Th2 cells were demonstrated in 2005, and these cells have been shown to play an important role in autoimmune tissue damage (34). In 2006, a group of researchers working on autoimmunity at Harvard described the differentiation mechanism of these cells (35). At the end of 2006, with the introduction of the transcription factor retinoic acid-related orphan receptor gamma t (ROR-γt) involved in the differentiation of these cells, it was contributed to the recognition of the classification of these cells as a separate helper T cell group (36). Th17 differentiation is induced by stimulation with IL-6 and TGF-β (37). It has been reported that a blockade of Th17 differentiation occurs in mice that lack these cytokines or their associated receptors (38). This indicates the necessity of both cytokines for Th17 differentiation. Since TGF-β also initiates Treg differentiation, IL-6 should direct cells to make T regulatory cells. A decrease in the production of Th17 populations and ROR-γt has been shown in patients mutated in STAT3 (39, 40). The loss of IL-6 and IL-21 (41) or STAT3 (37) reveals the mutual regulation not only by the reduction of Th17 populations but also by the increase of Treg populations. IL17 and Th17 cells produced by the prominent cytokine, although they stated that the Th17 differentiation can not sustain (42). The T-helper cells play a central role in modulating immune responses, while only the Th1⁄Th2 paradigm has been mentioned in the regulation of pregnancy, this paradigm has now turned into Th1/Th2 ⁄ Th17 paradigm. In addition to the effector cells, the Th cells are also regulated by Tregs (42).

B Lymphocytes

The only group of cells that produce immunoglobulins (Ig), called antibodies, is B lymphocytes. The antigen receptors of B lymphocytes are also transmembrane immunoglobulins that are bound to the cell membrane. These antigen receptors are indicated by Ig. B cells are activated by binding to their antigens with these Ig molecules that play a receptor role and differentiate into plasma cells to produce antibodies (13).

NK Cells

The natural killer (NK) cells are the third important class of lymphocytes, constituting 5-20% of mononuclear cells in the blood and spleen systems. The phenotypic feature that differentiates NK cells from the T and B lymphocytes is the absence of molecules specific for T and B lymphocytes such as CD3 and CD19 on their surface. Therefore, NK cells are defined as null cells (i.e. cells without markers).

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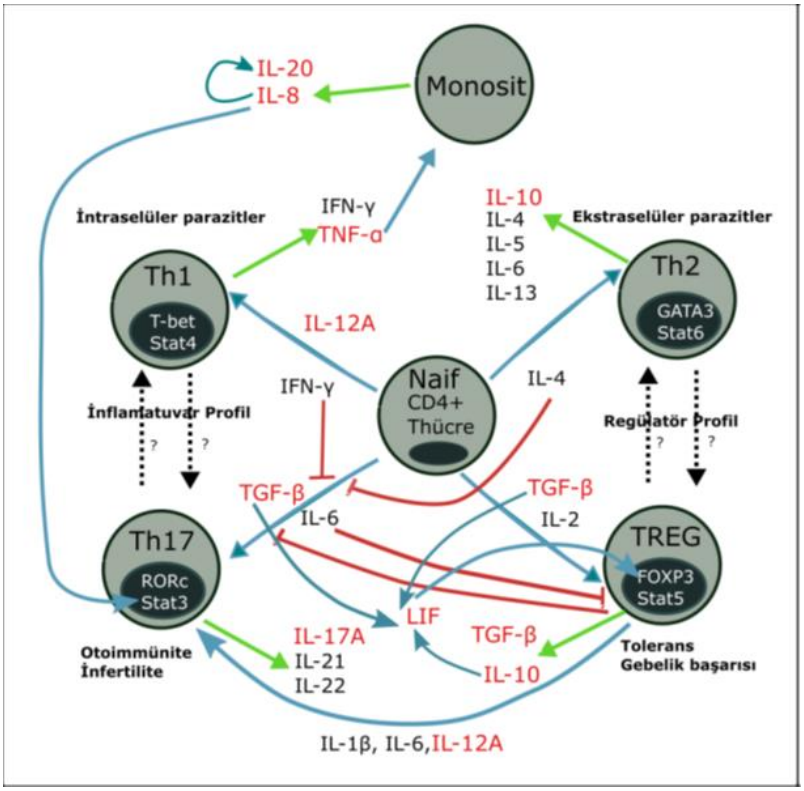

Figure 1: Sample pattern of naïve T cell differentiation to Th1, Th2, Th17 or Treg cells depending on cytokine profile.

As shown in Figure 1, IL-12 and IFN-γ stimulated naive T cells differentiate into Th1 cells. These cells express IFN-γ and TNF-α and are responsible for the intracellular parasite clearance and allergy conditions. IL-4-induced naïve T cells differentiate into Th2 cell, which are responsible for the extracellular parasite removal by expressing IL-4, IL-5, IL-6, and IL-13. TGF-β-induced naïve T cells are transformed into Treg cells, which express TGF-β and IL-10 and are responsible for tolerance and pregnancy success. TGF-β and IL-6-stimulated naive T cells differentiate into Th17 cells, which express IL-17, IL-21 and IL-22 and are responsible for the autoimmunity and pregnancy loss. IFN-γ or IL-4 may inhibit differentiation from naïve T cells to Th17. IL-6 and IL-12, respectively, and inhibit Treg and Th2 cells (43,78).

Cytokines

Cytokines, also known as immune system hormones, have important roles in the regulation of the immune system (44;45). These macromolecules are synthesized from the immune and non-immune cells by the action of a stimulus and are usually linked to their specific target cell receptors, often affecting more than one mechanisms (46,47). Low molecular weight proteins, cytokines; are involved in the protection of cyclic corpus luteum, fetal adhesion and

invasion, implantation, fetal and placental growth and differentiation, and some modulatory mechanisms. Cytokines involved in the cell-mediated communication are not only secreted by the embryo, but also by the peripheral blood lymphocytes, macrophages, oviductal and endometrial cells (45). Some of these cytokines have anti-inflammatory properties, while others have pro-inflammatory properties.

General Properties of Cytokines

A regulated environment is needed to prevent the rejection of the fetus by the mother. This environment should occur in the maternal-fetal range and uterine tissue. Pure CD4 T cells are the main producers of cytokines (4). Among the functions of lymphocyte CD4 cells are: the immune response to antigens, regulation of antibody production by B cells, and the role of cytotoxic T cells (7). Cytokines can be classified, for example, as Th1, Th2, Th17 and Treg cells associated with pro- and anti-inflammatory cytokines or different helper T cells (4). Th1 is mainly responsible for the production of interleukins such as IL-1, IL-2, IL-12, IL-15, and IL-18, IFN-γ and TNF-α, whereas Th2 cells produces IL-4, IL-5, IL-6, IL-13, and granulocyte-macrophage colony stimulating factor (GMCSF) (5). Th17 cells are the source of IL-17A and IL-17E (48). Examples of cytokines secreted by Treg cells are IL-10 and TGF-β. Treg cells were reported to be detected at the earliest mating lymph nodes after uterine drainage. It is known that IL-10 and TGF-β increases during immunosuppression and play important roles in the pregnancy process. Th17 cells, a subset of helper T cells, produce pro-inflammatory IL-17A, which play important roles in the initiation of inflammation and acute transplant rejection. In recent studies, it was observed that there was an increase in Th17 cells in peripheral blood and decidua when pregnant women who had idiopathic recurrent miscarriages compared with the normal pregnant women (49).

Interleukin-17A

IL-17 is a glycoprotein with 155 amino acids and N-terminal signal peptides. When IL-17 binds to its receptors, it supports inflammation, the immune response, and hematopoiesis (50, 51). This potent inflammation-inducing activity leads to the local production of chemokines such as IL-8, monocyte chemoattractant protein-1 (MCP-1) and growth-regulating factor-α (GRF-α), which leads to a rapid increase in monocytes and neutrophils. Studies have shown that IL-17 is associated with various diseases involved within the airway inflammation, tumor growth, and other chronic inflammatory diseases. This pro-inflammatory cytokine (IL-17A), regulates the activity of NF-κB and mitogen-activated protein (MAP) kinases. IL-17A induces the expression of IL-6 and cyclooxygenase-2 [PTGS2 (prostaglandin-endoperoxide synthase 2) / COX-2] as well as the synthesis of nitric oxide (NO). The expression of IL-17A is associated with the pathogenesis of various tumors and has been shown to exhibit both tumor-specific and anti-tumor effects due to the microenvironment (52). IL-17A has been shown to be involved in promoting ovarian cancer growth in mice by upregulation of pro-angiogenic and inflammatory mediators from small peritoneal macrophages (53). Hirata et al. have shown that it induces IL-8 and COX-2 production

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from endometriotic stromal cells and promote the proliferation of these cells (54).

Interleukin-10

IL-10 is an anti-inflammatory cytokine and suppresses the Th1-mediated cellular immunity by inhibiting the production of inflammatory cytokines (e.g. IFN-γ, TNF-α and IL-1). With this feature, IL-10 is an anti-inflammatory, inflammation-controlling cytokine (55). IL-10 is known with different names such as cytokine synthesis inhibitory factor (CSIF) or T-cell growth inhibitory factor. IL-10 was formed by the combination of two identical protein fragments (homodimers) containing 160 amino acids. The IL-10 receptor (CDw210) that detects IL-10, is a protein of 110 kD. IL-10 as well as Th2 cells are produced by regulatory T cells in the materno-placental range (56). This cytokine is not only suppressed by Th1 immunity, but also by introducing certain inflammatory mediators, it is more appropriate to suppress Th2 immunity, and therefore is called anti-inflammatory cytokine (57). During early pregnancy stage, IL-10 and its receptors produced by Treg cells should be present in the endometrium and decidua. IL-10 causes proliferation of decidual cells and secretion of TNF-α (58). It was reported that there was a significant increase in the level of IL-10 during early pregnancy in women and remained high before the onset of labor during the third trimester (59).

Interleukin 12-A

At the beginning of the inflammatory response, phagocytic cells produce IL-12, a cytokine that provides an important functional bridge between the innate resistance and immune responses (60). IL-12 is a heterodimeric molecule consisting of two covalently bound proteins of 40 kD (p40) and 35 kD (p35) (61). The major IL-12-producing cell types in the peripheral blood mononuclear cells (PBMC) population are monocytes, but B cells and other helper cells also produce IL-12 (62). IL-12 signals through IL-12 receptor (IL-12R) containing IL-12Rβ1 and IL-12Rβ2 subunits that are expressed on T cells, NK cells, and dendritic cells (DCs) (10,11). IL-12 stimulates activities of non-receptor Janus kinase 2 (JAK2) and tyrosine kinase 2 (TYK2), leading to the phosphorylation of STATs, particularly STAT4 homodimers (64). Production of the IL-12 heterodimer requires coordinated expression of the p40 and p35 chains (62).

Transforming Growth Factor-β

TGF-β is a cytokine known to have long-term immunosuppressive and anti-inflammatory properties. TGF-β activated T cells are secreted by mononuclear phagocytes and many other cells. TGF-β inhibits proliferation of T cells, secretion of pro-inflammatory cytokines, and activation of macrophages. TGF-β inhibits the proliferation of activated T cells to control inflammation, decreases the secretion of cytokines which cause inflammation to initiate and sustain inflammation. In the absence of IL-6, TGF-β simultaneously induces the retinoic acid-associated receptor βt (RORβt) synthesis of both the FoxP3 and the Th17 cell master switch. FoxP3 then directly interacts with ROR-γt to suppress the transformation of naïve T-cells into Th17 cells (64).

Interleukin-20

It is a protein belonging to the family of interleukin 10, which has been shown to

transmit signal via keratinocytes through STAT3. A specific receptor for this cytokine has been found to be expressed in the skin and dramatically upregulated in the psoriatic skin; and this protein plays role in epidermal function and psoriasis (65). IL-20 is produced by activated keratinocytes and monocytes, which transmits an intracellular signal through two separate cell surface receptor complexes in keratinocytes and other epithelial cells. IL-20 regulates the proliferation and differentiation of keratinocytes during inflammation, particularly the inflammation of the skin. In addition, IL-20 also causes cell expansion of multi-potential hematopoietic progenitor cells (66).

Interleukin-8

In recent years, a new family of cytokines with chemotactic activity has been described for leukocytes and fibroblasts. These chemotactic cytokines are called chemokines. The molecular weights of these chemokines range from 8,000 to 16,000 D. It is similar to each other with 20-50% amino acid sequence. IL-8 is a member of chemokine family. Monocytes, macrophages, fibroblasts, keratinocytes, and endothelial cells are the cells of IL8. IL-8 is involved in the migration of cells of specific types to sites with tissue injury and inflammation (67). Neutrophils and T cells are target cells of IL-8., which provides mobilization, activation and degranulation of neutrophils and also has a role in angiogenesis (68).

Tumor Necrosis Factor-α

Tumor Necrosis Factor (TNF) is a multifunctional pro-inflammatory cytokine with two sub-groups, TNF-β, also known as TNF-α and lymphotoxin-α (69,70). TNF has two structurally similar distinct cell surface receptors; TNF receptor-1 (TNFR1) and TNF receptor-2 (TNFR2) (71). These two receptors have different cytoplasmic domains, thus activating different signaling pathways. The majority of the inflammatory effects depend on TNFR1, whereas TNFR2 improves the response given by TNFR1. Interestingly, TNFR1 is expressed by all human tissues and is the most important signal receptor for TNF-α. TNFR2 is often expressed in the immune cells and mediates limited biological responses. TNFR2 binds to both TNF-α and TNF-β (72). TNF-α is a multifunctional pro-inflammatory cytokine with effects on lipid metabolism, coagulation, insulin resistance, and endothelial function. Members of the TNFR superfamily can send survival and death signals to the cells. TNF-α family members play important roles in various physiological and pathological processes such as cell proliferation, differentiation, apoptosis and modulation of immune responses, and induction of inflammation (73).

Leukemia Inhibitory Factor

LIF is a cytokine belonging to the IL-6 family, which is considered to be one of the cytokines required for the successful completion of human pregnancy. Maternal LIF affects trophoblast growth and development. Therefore, it is essential for implantation and is defined as a sign of embryo implantation (74). LIF is expressed in luminal epithelium in the mid-late sequestrant phase of the menstrual cycle (approximately 18-28 days) (75). It has been suggested that recombinant LIF may help in the correction of implantation rate in

the unexplained infertility cases (76). The LIF signals through the common receptor subunit glycoprotein (gp)130 in conjunction with the low affinity LIF receptor (LIFR) and the ligand specific receptor subunit. Binding of the LIF to LIFR induces heterodimerization with gp130. The formation of this complex results in the activation of receptor-linked JAKs, phosphorylation of receptor sites, and consequently activation of STAT3 [collection of Src homology-2 (SH2)] domain containing proteins such as signal transducer) - and when the transcription activator binds to the receptor, STAT3 molecules, tyrosine 705 (Tyr705) is phosphorylated on the residues and dimerized with another phosphorylated STAT3 (77). The clinical importance of cytokines in the diagnosis, treatment and prevention of the disease is increasing. Measurement of some of these cytokines in body fluids or serum is important in the diagnosis of certain diseases.

Conclusions

Pregnancy in mammals is a unique immunological process that requires a balance between the immune tolerance and suppression. The continuation of pregnancy in the early period depends on the interaction between fetal tissues and maternal decidua. A specific leukocyte population and appropriate cytokine(s) expression are needed to achieve this interaction successfully. In this review, we sought to describe that Th1 Th2, Th17, and Treg activities should be in a certain balance during the pregnancy, thus appropriate immunological reactions (balance) could occur leading to a successful pregnancy. In addition, we also tried to clarify the biochemical roles cytokines that are critically involved in the pathobiogy of unexplained infertility.

Conflict of Interest

None of the authors has any potential financial or commercial conflict of interest associated with this research manuscript (review article).

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