Review Article

Toll-like Receptors in Innate Immunity: Role of Bacterial Endotoxin and Toll-like Receptor 4 (TLR4) in Endometrium and Endometriosis

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Abstract

Macrophages, dendritic cells, and toll-like receptors (TLRs) are integral components of the innate immune system. This rapidly reactive system responds immediately to infectious or other non-self agents, thereby inducing inflammatory response to protect the host until the generation of slower adaptive immune system. The fundamentals of innate immune system, functional characteristics of TLRs, and signaling pathways of TLR4 are discussed for easy understanding by the readers. Studies showed that the growth and progression of endometriosis continue even in ovariectomized animal. This indicates that besides ovarian steroid hormones, the growth of endometriosis can be regulated by innate immune system in pelvic environment. As a component of innate immune system, increased infiltration of macrophages has been described in the intact tissue and peritoneal fluid of women with endometriosis. In this review article, we discussed the role of bacterial endotoxin and TLR4 in endometrium and endometriosis and outlined the involvement of endotoxin in causing adverse reproductive outcome.

Introduction

Innate (natural or constitutive) immunity in our body depends on toll-like receptors. From flies to mammals, these proteins provide a first line defense and are implicated in infectious and autoimmune diseases. While scientists have been studying the adaptive (acquired) immune response for several decades, the recognition of the importance of innate immunity was established only during the past few years to understand the association between adaptive and innate immune system. Why is innate immunity necessary for our body? There was always a question of how adaptive immune system could defend us if it were alone, because adaptive immunity depends on the multiplication of host cells with a generation time of at least 12 hours, whereas microbes can divide every 20 minutes. To cover this lag, the rapidly reactive innate immune system responds immediately to infectious agents, protecting the host until slower adaptive system kicks in and eventually makes memory cells for long-term response [1, 2]. Therefore, innate immune responses are, in many cases, necessary for triggering an adaptive immune response just as adjuvant is necessary for a significant vaccine response.

Functional characterization of Toll-like receptors (TLRs) has established that

innate immunity is a skillful system that detects invasion of microbial pathogens.

Recognition of microbial components by TLRs initiates signal transduction pathways,

and triggers expression of genes. These genes control innate immune responses and

further instruct development of antigen-specific adaptive immunity. In adaptive immunity,

B and T lymphocytes utilize antigen receptors such as receptor for immunoglobulins and

T cell receptors to recognize non-self such as foreign antigens. However, these receptors

are present only in vertebrates. In contrast, innate immune system operates in both

vertebrates and non-vertebrates. Mammalian innate immune cells such as macrophages

and dendritic cells can be activated by microbial components (non-self) such as

endotoxin or lipopolysaccharide (LPS) from Gram-negative bacteria. However, a

receptor responsible for the recognition of LPS remained unknown until the end of 20th

century [3].

Analysis of the female reproductive tract indicates that the key cells of the

innate and adaptive immune systems are present and functionally responsive to antigens

[4]. The innate immune system has evolved to recognize foreign structures that are not

normally found in the host. It relies on conserved germ-line-encoded receptors that

recognize conserved pathogen-associated molecular patterns (PAMP) found in groups of microorganism [5]. The pattern recognition receptors (PRR) of the host that recognize PAMP in female reproductive tract are expressed on the cells of the innate immune system. Toll-like receptors are one group of PRRs that are expressed on macrophages (M φ), dendritic cells, and as more recently shown, on neutrophils, natural killer cells, and on epithelial cells [4-6].

At the end of the 20th century, Toll was shown to be an essential receptor for host defense against fungal infection in *Drosophila* (fly), which has only innate immunity [7]. One year later, a mammalian homolog of the Toll receptor (now termed TLR4) was shown to induce expression of genes involved in inflammatory responses [8]. In addition, a point mutation in the Tlr4 gene has been identified in a mouse strain that is unresponsive to LPS [9]. These studies have made rapid progress in our understanding that innate immune system senses invasion of microbial pathogens by TLRs. Furthermore, activation of the innate immunity is a critical step to the development of antigen-specific acquired immunity.

Now it is well recognized that innate and adaptive immune system are the two

key branches that determine host protection throughout the female reproductive tract and

at other mucosal surfaces, including the respiratory, gastrointestinal and urinary tracts.

Our understanding of the innate immune system is a result, in large part, of the pioneering

studies of Charles Janeway, who demonstrated that innate immunity covers many areas of

host defense against pathogenic microbes [10]. During the last decade, investigations of

the innate immune system have shown that microbial pathogens are recognized by TLRs

that, in turn, regulate the activation of both innate and adaptive immunity [11].

(1) Identification of the TLR Family:

After the characterization of the first mammalian TLR (TLR4), several proteins

that are structurally related to TLR4 were identified and named Toll-like receptors [12].

Mammalian TLRs comprise a large family consisting of at least 11 members. TLRs1-9

were found to be conserved between human and mouse. TLR10 is presumably functional

in the human but non-functional in mouse. Similarly, mouse TLR11 is functional, but

there is a stop codon in the human *TLR11* gene, which results in a lack of production of

human TLR11 [13].

The cytoplasmic portion of TLRs shows high similarity to that of the

interleukin (IL)-1 receptor family, and is termed a Toll/IL-1 receptor (TIR) domain.

Despite this similarity, the extracellular portions of both types of receptors are structurally

unrelated. The IL-1 receptors possess an immunoglobulin-like domain, whereas TLRs

bear leucin-rich repeats (LRRs) in the extracellular domain. Functionally, a critical role of

TLR4 in the recognition of microbial component was initially characterized [9].

Subsequently, it has been established that individual TLRs play important roles in

recognizing specific microbial components derived from pathogens including bacteria,

fungi, protozoa and viruses (**Figure 1**).

Toll-like receptor 2 (TLR2) is essential in the recognition of microbial

lipopeptides and peptidoglycan derived from Gram-positive bacteria. TLR1 and TLR6

cooperate with TLR2 to discriminate subtle differences between triacyl and diacyl

lipopeptides, respectively. TLR2 forms heterophilic dimers with TLR1 and TLR6, both

of which are structurally related to TLR2 [14]. TLR4 is the receptor for LPS derived from

the outer membrane of Gram-negative bacteria. TLR5 recognizes flagellin. TLR3 is

implicated in the recognition of viral dsRNA associated with viral replication, whereas

TLR7 and TLR8 are implicated in viral-derived ssRNA recognition. Thus,

polyriboinosinic:polyribocytidylic acid [poly (I:C)], which is a synthetic mimetic for dsRNA, can induce TLR3 signaling [15]. TLR9 is essential in unmethylated (CpG) DNA recognition [3]. As shown in **Figure 1**, the TLR family members recognize specific patterns of microbial components. TLR1, TLR2, TLR4, TLR5 and TLR6 are expressed on cell surfaces and recognize microbial components in different body fluids. In contrast, TLR3, TLR7, TLR8 and TLR9 are present in endosomes within the cytoplasm [16, 17].

(2) Ligands of Toll-like Receptor 4 (TLR4):

There are two types of ligands, exogenous and endogenous, for TLR4 [18]. As described above, TLR4 is an essential receptor for bacterial endotoxin or LPS recognition. In addition to LPS, other exogenous ligands are F protein from respiratory syncytial virus, chlamydial heat shock protein 60 and taxol, a plant derived anticancer reagent that mimics the action of LPS in mice but not in humans. Endogenous ligands of TLR4 comprise fibronogen, fibronectin, heparan sulphate, hyaluronic acid and heat shock proteins (Hsp) 60 and 70. However, all of these endogenous ligands require very high concentration to activate TLR4. It has been shown that contamination of LPS in Hsp70 preparation confers ability to activate TLR4. LPS is a very potent immuno-activator and

accordingly, TLR4 can be activated by a very small amount of LPS, contaminating these endogenous ligand preparations [3, 17-20]. Therefore, we need careful attention in biological research using these endogenous ligands. The different TLRs and their corresponding ligands are described in **Table 1**.

(3) Signaling Pathways Triggered by TLR4 (Figure 2):

Several lines of evidence indicate that all TLR signaling pathways are similar and elicit similar biological responses except TLR3 [21, 22]. Lipopolysaccharide (LPS) is a potent activator of Mφ and other dendritic cells. After being released into the blood stream or other body fluids, LPS is immediately captured by LPS-binding protein that delivers LPS to TLR4 or CD14. CD14 lacks a trans-membrane domain and so is incapable of transducing signals [16]. Both the positional cloning of the locus responsible for LPS hypo-responsiveness in C3H/HeJ mice and the generation of TLR4 knockout mice have shown that TLR4 is essential for LPS signaling [9, 19]. In addition, the interaction of LPS with TLR4 requires another molecule, MD-2, which associates with the extracellular domain of TLR4. MD-2 is also involved in the intracellular transport of TLR4 and subsequent activation of a number of intracellular adaptor molecules [23].

Once TLRs are activated, the intracellular signaling pathways are very similar between insects and mammals. In mammals, TLR4 signaling involves activation of one or more of the adaptor proteins. The adaptors relevant to TLR4 signaling are known as MyD88 (myeloid differentiation factor 88), TIRAP (TIR domain-containing adaptor protein), TRIF (TIR-domain containing-adaptor inducing IFN- β), and TRAM (TRIF-related adaptor molecule) [1, 3]. Most TLRs act through MyD88 alone or through both MyD88 and TIRAP, which leads to the production of different pro-inflammatory cytokines. MyD88 is an adaptor molecule that recruits the kinase IRAK (IL-1 receptor-associated kinase) to the TLR4 receptor complexes after stimulation with LPS. The lipopeptide activation of nuclear factor (NF)- к B and MAP (mitogen-activated protein) kinases, as mediated by TLR2, is completely abolished in TLR2-depleted or MyD88-deficient M ф . By contract, LPS activation of MAP kinases and NF- к B remains intact in MyD88-deficient M ф . This indicates that LPS response is mediated by both MyD88-dependent and MyD88-independent pathways, each of which leads to the activation of MAP kinases and NF- к B (**Figure 2**).

The MyD88-dependent pathway is essential, however, for the inflammatory

response mediated by LPS. The TIRAP has a crucial role in the MyD88-dependent signaling pathway shared by TLR2 and TLR4. Recent studies have shown that MyD88-independent pathway for TLR4 operates through different adaptor molecules, TRIF and TRAM, activates interferon (IFN) regulatory factor 3 (IRF-3), up-regulates co-stimulatory molecules and leads to the subsequent induction of type I interferon such as IFN- β , nitric oxide synthase (iNOS) and IFN-inducible protein (IP-10) [1,3]. It is important to remember that in addition to activation of IRF3, MyD88-independent pathway also elicits delayed activation of NF- κ B. Studies are still limited with MyD88-independent pathway. TLR4 signaling pathways are shown in **Figure 2**.

Unlike other TLRs, TLR3 uses only one adaptor protein, TRIF, whose activation leads to IRF3 translocation to the nucleus. IRF3 dimerizes and enters the nucleus where it binds to interferon-sensitive response element (ISRE) motifs and induces the expression of type I interferons, IFN- α and IFN- β [21, 22]. Female reproductive tract and placenta may become exposed to viruses in addition to bacterial or fungal infection, which may pose a substantial threat to reproductive outcome or embryo/fetus well-being. Although studies are limited, it is important to determine the

type of virus and whether the engagement of TLR3 with viral dsRNA could induce production of factors necessary to generate an antiviral response. In fact, TLR3 expression has been demonstrated in the epithelial cells of vagina, uterine cervix, endometrium, fallopian tubes and also in placenta [24, 25].

(4) Links between Innate and Adaptive Immune System (Figure 3):

The TRIF/TRAM pathway provides a direct link between TLR4 activation and adaptive (acquired) immunity. Although purified LPS acts like a strong adjuvant, its effects are abolished in the mutant mouse strains. This suggests that both inflammatory and adjuvant effects of LPS flow through TLR4. Combination of MyD88/TIRAP and TRIF/TRAM pathways provides a biochemical basis for how adjuvants work. Activation of adaptive immune system requires antigen-presenting cells such as macrophages and dendritic cells to express co-stimulatory molecules such as CD40, CD80 and CD86, and to produce pro-inflammatory cytokines. When TLR4 recognizes LPS on the surface of macrophages or dendritic cells, it leads to the production of cytokines via MyD88/TIRAP pathway and co-stimulatory molecules via TRIF/TRAM pathway, providing both components to activate T helper lymphocytes of the adaptive immune system [1, 3].

Some evidence suggests that the initial innate immune process influences the type of acquired immune response that is generated [1, 14]. When naïve T helper cells are presented with antigens by antigen-presenting cells, they differentiate into two subsets, T helper 1 (Th1) and T helper 2 (Th2) cells. Th1 cells secrete interferon-γ , which promote mainly cellular immunity whereas Th2 cells produce IL-4, IL-5, IL-10, and IL-13, and promote mainly humoral immunity [1, 14]. It has been demonstrated that autoimmune diseases such as Crohn's disease and multiple sclerosis are associated with an abnormally strong Th1 response, whereas allergic diseases seem to involve an abnormally strong Th2 response.

Which limb of acquired immunity predominates may be modulated by innate immune response. For instance, MyD88-deficient mice are skewed toward a Th2 response and activation of TLR4 by LPS stimulates Th1 activity. This suggests that the default pathway for acquired immune development with absent MyD88 signaling is the Th2 pathway. This integrated knowledge of innate and adaptive immunity may give future therapeutic possibilities for infectious diseases, allergic diseases and autoimmune diseases.

(5) Bacterial Endotoxin and TLR4 in Endometrium:

For most of the reproductive cycle in humans and animals, the uterus is thought to be sterile or at least clear of pathogenic bacteria, but it is readily contaminated with bacteria during sexual intercourse and around the time of parturition. In fact, the upper genital tract is vulnerable to the spread of microorganisms from the lower genital tract, resulting in the development of infectious diseases such as endometritis and salpingitis. These diseases, commonly termed as pelvic inflammatory disease, deteriorate women's health, posing risks of infertility and ectopic pregnancy. In addition, these infections perturb normal ovarian cycles by suppressing follicular growth and disrupting luteolysis as exemplified in the cattle [26]. In fact, an enormous number of Gram-negative and Gram-positive microbes are present in vaginal cavity (**Table 2**). All these microbes reside in vaginal cavity as normal vaginal flora and may cause genitourinary infections upon ascending migration [27].

In recent years increasing attention has been paid to innate immunity, the primary defense system against pathogens. *Escherichia coli (E.coli)* are the most commonly isolated pathogenic bacteria from clinical uterine diseases in cattle [26] and

also in human vaginal cavity [27]. The ascending migration of *E.coli* towards endometrial cavity is possible that may cause contamination of endometrium. In bovine uterine lumen, there are high concentrations of the pathogenic ligand of *E.coli* known as bacterial endotoxin or LPS. The endometrium provides a barrier against infection and an opportunity to detect these bacteria by innate immune receptors. Toll-like receptors were first identified on immune cells but have since been identified on other cell types including endometrium [28]. In the human endometrium, nine TLRs are identified at the protein and mRNA level including TLR4 [29-32]. Engagement of these receptors initiates a signaling cascade stimulating the production of immune mediators that orchestrate the immune response to clear the infection. It is the principal role of TLR4 to detect LPS, although signaling through TLR4 also requires accessory molecules such as LBP, CD14 and MD2.

As a component of innate immune system, an increase in the infiltration of Mφ was found in normal endometrium and also in the endometrium of women with different reproductive diseases such as endometriosis, adenomyosis and uterine leiomyoma [33-35]. The expression of TLR4 mRNA and protein was detected in Mφ,

endometrial epithelial cells and stromal cells [29, 30, 36]. RT-PCR analysis also demonstrated the expression of CD14, MD2 and MyD88 mRNA in both endometrial epithelial cells (EECs) and endometrial stromal cells (ESCs) [30]. The expression levels of TLR4, CD14, and MD2 appeared to be higher in ESCs compared with that in EECs. However, the expression levels of MyD88 were similar between ESCs and EECs.

Treatment of endometrial stromal cells with LPS significantly increased the production of hepatocyte growth factor (HGF), vascular endothelial cell growth factor (VEGF), interleukin (IL)-6, IL-8 and tumor necrosis factor alpha (TNF α) in a dose-dependent fashion [30, 37, 38]. A significantly more growth promoting effect of LPS was observed on endometrial cells derived from women with endometriosis when compared with similar cells derived from control women [37, 38]. The stimulatory effect of LPS was inhibited by the addition of neutralizing antibodies for TLR4 and also by an LPS antagonist, polymyxin B [39]. This indicates that M φ , ESCs and EECs express TLR4 and respond to LPS through TLR4. In fact, we recently demonstrated that both ESCs and EECs were able to significantly proliferate in response to LPS and this growth promoting effect of LPS was abrogated after pretreatment of cells with anti-TLR4

antibody (40, 41). Since, there are other exogenous and endogenous ligands for TLR4 in addition to LPS, we presume that blocking of TLR4 alone is more effective in order to suppress inflammatory response in pelvic environment and cell growth.

A recent study [30] demonstrated that LPS was able to stimulate TLR4- and CD14-mediated increased production IL-8 by ESCs. This effect of LPS was associated with the activation of NF-kB as examined by nuclear translocation of NF-kB in ESCs. On the other hand, LPS alone did not stimulate IL-8 secretion in EECs. However, LPS did stimulate IL-8 secretion from EECs in the presence of soluble CD14. These findings indicate that TLR4 system might represent local immunity in the human endometrium with different modes of TLR4 actions between ESCs and EECs. The role of soluble CD14 in LPS-stimulated proliferation of ESCs and EECs is yet to be determined.

Human endometrium is regulated by changing concentration of female sex hormones, estradiol (E2) and progesterone (P), during the ovarian cycle [42]. These ovarian steroids also have a profound effect on infections. For example, in humans, rodents and cattle, P suppresses uterine immune function by decreasing the proliferative capacity of lymphocytes, thereby increasing the susceptibility to bacterial infection [42,

43]. Conversely, E2 may play a role in the recruitment of immune cells as more Mφ

infiltrate in the endometrium when E2 concentrations are higher in rodents [42, 43].

In addition to producing different macromolecules as mentioned above,

endometrial explants also produce prostaglandins in response to LPS, with an increasing

ratio of PGE2 to PGF2α [43]. A recent report by Herath et al. [43] demonstrated that

addition of LPS or *E.coli* to bovine endometrial stromal and epithelial cells stimulated

production of PGE2 and PGF2α with a parallel increase in the expression of

cyclooxygenase (COX) 2 mRNA. Polymyxin B, an LPS antagonist, was able to abrogate

the production of prostaglandins. In addition, E2 and P were found to inhibit

LPS-mediated production of PGE2 and PGF2α, indicating a role of steroid hormones in

bacterial infections. TLR4 mRNA, CD14 mRNA and proteins were also detected in

bovine endometrial cells. This study indicates that endometrial cells detect and respond to

bacteria, which modulate their endocrine function.

(6) Bacterial Endotoxin and TLR4 in Endometriosis:

Endometriosis, the presence of functional endometrium outside of the uterine

cavity, is a common disease, causing abdominal pain, dysmenorrhea, dyspareunia and

infertility in 6-10% of the female population. The pathogenesis of endometriosis is still

controversial. A number of papers have already demonstrated the potential role of ovarian

steroid hormones in the growth of endometriosis. However, as a non-self lesion in the

pelvic environment, the growth or persistence of endometriosis can also be regulated by

the innate immune system. We already came to learn that an innate immune system and

ovarian steroid hormones participate either alone or in an orchestrated fashion in the

regulation of endometriosis. In fact, as a cell component of innate immune system,

increased infiltration of M φ has been demonstrated in the blood-filled opaque red

endometriotic lesions, their corresponding eutopic endometria and also in the peritoneal

fluid [33, 44].

The different macromolecules as secreted by M φ in the pelvic environment

are believed to enhance the growth of endometriosis. However, the initial inflammatory

mediator that stimulates M φ for the production of different cytokines and growth factors

was poorly described. We reported that bacterial endotoxin, lipopolysaccharide (LPS),

could be a potential inflammatory mediator of M φ stimulation and consequent

production of different cytokines and growth factors, such as HGF, VEGF, IL-6 and TNF

α in pelvic environment [37]. This LPS and together with LPS-induced macromolecules are possibly involved in the growth of endometriosis in an autocrine or paracrine mechanism [37].

We recently demonstrated that activation of basal Mφ further enhanced the response of these cells to ovarian steroids. Exogenous treatment with E2 was able to further increase the amount of both HGF and VEGF secretion by peritoneal fluid Mφ when these cells were activated with LPS [44]. These results from our laboratory confirmed that irrespective of activation status, CD68-immunoreactive Mφ were independently stimulated to produce HGF and VEGF by estrogen. This indicates that an inflammatory response and ovarian steroid hormones may function either alone or in combination to regulate the production of different macromolecules in the pelvic environment. The enhanced cell proliferation in response to IL-6, TNFα, LPS, and estrogen suggested that a combined effect among steroid hormone, initial inflammatory mediator (LPS) and other secondary inflammatory mediators (IL-6, TNFα) may be involved in the growth of endometriosis [44, 45].

There was no information until now about the presence of bacterial endotoxin

in pelvic environment. We examined endotoxin concentration for the first time in the peritoneal fluid of women with or without endometriosis. We found that endotoxin concentration in PF was significantly higher in women with endometriosis than that in non-endometriosis. The expression pattern of TLR4 in Mφ, endometrial cells and endometriotic cells was identical between women with endometriosis and non-endometriosis in the proliferative phase but this expression pattern was higher in the secretory phase of the menstrual cycle [31, 32]. The production of HGF, VEGF, IL-6 and TNFα by LPS-treated peritoneal Mφ was significantly higher in women with endometriosis than that in women without endometriosis. This was evident at both protein and mRNA level. The blocking of TLR4 after pretreatment of Mφ with anti-TLR4 antibody significantly reduced the production of all these cytokines [39-41]. The addition of culture media from TLR4-blocked macrophages caused significant suppression in the growth of endometrial and endometriotic cells comparing to TLR4 non-blocking macrophages. The direct application of LPS also promoted the growth of endometrial cells and was suppressed after pretreatment of cells with anti-TLR4 antibody [39-41].

These results suggested that a substantial amount of endotoxin in PF is involved in pelvic inflammation and may promote TLR4-mediated growth of endometriosis. Therefore, targeting TLR4 could be a new therapeutic strategy to reduce inflammatory reaction in pelvic environment and prevent consequent growth of endometriosis. We recently reported that an internal stress reaction and an inflammatory reaction in peritoneal cavity cooperate with each other and are involved in TLR4-mediated growth of endometriosis [39].

The higher concentration of bacterial endotoxin in the pelvic environment added further evidence that endometriosis induces an inflammatory reaction. But we don't know the exact source of this endotoxin in pelvic environment. There might be two mechanisms for the residual accumulation of bacterial endotoxin in pelvic environment: (a) translocation of *E.coli* or endotoxin from the gut through enterocytes and their entry into the pelvic cavity as demonstrated by Alexander et al. [46], (b) contamination of menstrual blood by *E.coli* after ascending migration from vagina.

Since endometriosis is a product of retrograde menstruation and LPS is a cell wall extract of *E.coli,* we speculated that menstrual blood of women with endometriosis

could be contaminated with *E.coli*. We collected menstrual blood with strict aseptic measure on day 1 to day 3 of menstrual cycle from women with and without endometriosis. We cultured menstrual blood on eosin methylene blue (EMB) agar plate and examined the colony formation of *E.coli*. We detected colony formation of *E.coli* in menstrual blood and this was significantly higher in women with endometriosis than that in non-endometriosis [40, 41, 47]. The contamination of menstrual blood with *E.coli* was associated with a parallel increase in the level of endotoxin in the menstrual blood. The level of endotoxin in menstrual blood was significantly higher in women with endometriosis than that of women with non-endometriosis [41,47]. Our findings suggested that contamination of menstrual blood with *E.coli* in women with endometriosis could be a constant source of bacterial endotoxin in peritoneal fluid due to periodic retrograde menstrual reflux and this cyclic event may initiate TLR4-mediated growth of endometriosis.

(7) Endotoxin-mediated Reproductive Dysfunction and Infertility (Figure 4):

The ultimate fates of women who suffer from endometriosis are impairment in quality of life and reduction in the rate of fertilization, implantation and finally failure to

achieve pregnancy [48, 49]. The purpose of current medical and surgical therapy for the women with endometriosis is to remove endometriotic lesions, to relieve periodic or non-periodic pain experience and to establish pregnancy. Endometriosis-associated pain can be successfully alleviated either transiently or permanently by the application of recent therapeutic modalities. However, the problem of infertility still remains a major issue to be resolved. Endometriosis-associated infertility can be explained by one of the several mechanisms as shown in **Figure 4**.

A strong inflammatory reaction of the endometrial bed elicited by the infiltration of inflammatory cells especially Mɸ constitutes the central feature of impaired fertility of affected women. These scavenger cells may have two-fold effects. One, direct phagocytosis of implanting embryos; secondly, indirect impairment in the process of implanted blastocyst. These hazardous effects of Mɸ can be contributed by producing some biological mediators such as reactive oxygen species (ROS) or by inducing humoral immune response [49-51]. A moderate to severe inflammatory reaction in pelvic environment leads to the formation of tubo-ovarian adhesion or peri-tubal adhesion finally resulting in narrowing or occlusion of the Fallopian tube [52]. On the

other hand, endotoxin derived from Gram-negative bacteria may directly cause endometrial or tubal damage. Endotoxins have been found to be deleterious on pre-implantation stage embryos [53]. The presence of endotoxin in IVF culture media results in high rate of polyspermy, decreased embryo cleavage rate and blastocyst formation in human and bovine species. Endotoxins also possess the capacity to induce apoptosis of cells impairing sperm motility and induce spermicidal activity [50-53]. A recent ART clinical trial has demonstrated that pregnancy rate after IVF-ET was significantly higher in women with an endotoxin level of <200 pg/ml in menstrual fluid, than that in women with an endotoxin level of >200 pg/ml [54].

Many of the biological effects of bacterial endotoxin are mediated by pro-inflammatory cytokines such as IL-1, IL-6, and TNF α . One recent study demonstrated that adding recombinant IL-6 to culture media suppressed the rate of blastocyst formation in mouse embryos and reduced the percentage of motile human spermatozoa [55]. Higher concentrations of TNF α possess apoptosis- and necrosis-inducing activity on a variable type of cells including sperm, ova and endometrial cells [53, 56]. T-helper 2 (Th2) type cytokines (IL-4, IL-5, IL-10, IL-13) are

also produced at the tissue level in response to endotoxin [50, 53]. These Th2 cytokines in turn could induce an autoimmune response resulting in limitation of fertilization or implantation [26, 53]. Several of these cytokines have been implicated in the delicate balance of immune system that exists within the maternal-fetal interface. Any disturbance of this delicate immune balance within the maternal-fetal interface may result in pregnancy loss or other perinatal complications.

Endotoxin also produces higher levels of prostaglandin (PG) F2 α and PGE2 by M φ and endometrial cells. PGE2 causes immunosuppression and may promote growth of endometriotic cells either indirectly or directly by stimulating local aromatase activity resulting in the elevation of estrogen synthesis at the tissue level of endometriotic lesions [57, 58]. The higher local estrogen levels may recruit immune cells and induce inflammatory reaction in pelvic cavity and culminate in the impairment of reproductive outcome. PGF2 α causes uterine contraction as well as vasoconstriction, leads to ischemic or hypoxic change in endometrial bed and may result in abnormal sperm motility or implantation failure [35, 59].

Macrophages also produce increased concentrations of ROS (OH^-, O^{2-} and

H$_2$O$_2$) in response to bacterial endotoxin. The increased generation of ROS at the tissue level induces a wide range of biological activity such as lipid peroxidation, protein denaturation, inactivation of enzymes and decomposition of cellular DNA [60]. In this way, ROS may cause cellular and tissue damage. These unwanted effects of ROS may cause impairment of ova or sperm function. Bacterial endotoxin-induced increase in ROS production may also cause caspase-mediated apoptosis [56]. This apoptosis-inducing effect of ROS may result in endometrial or tubal epithelial damage, impairment in fertilization and sperm motility [50, 51].

Conclusions

We now know that innate immunity plays an important role in the initiation of an immune response that follows the activation of antigen-specific adaptive immunity. The association between innate immunity and adaptive immunity is as important as an adjuvant is necessary for an effective vaccine response. A number of mechanisms are

involved in the development or pathogenesis of endometriosis. The production of pro-inflammatory cytokines and growth of endometriosis in pelvic environment can be regulated by innate immune system. We proposed a novel concept in the cross-talk between bacterial endotoxin and TLR4 in the pathogenesis of endometriosis. Targeting bacterial endotoxin or TLR4 could be useful as a therapeutic strategy to suppress pelvic inflammation and growth of endometriosis with consequent improvement in the quality of life and fertility rate of women who suffer from this enigmatic disease. A complete understanding of the mechanisms of innate immunity and its association with adaptive immunity will be helpful for the future development of innovative therapies for the manipulation of endometriosis and other reproductive diseases, infectious diseases, cancer and allergies.

Acknowledgments

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Figure Legends

Figure 1. TLRs and their ligands and is reproduced with the permission of Dr.

Shizuo Akira of Osaka University (Int. Immunol. 17:1-14,2005). TLR2 is essential in the

recognition of microbial lipopeptides. TLR1 and TLR6 cooperate with TLR2 to

discriminate subtle differences between triacyl and diacyl lipopeptides, respectively.

TLR4 is the receptor for LPS. TLR5 recognizes flagellin. TLR3 is implicated in the

recognition of viral dsRNA, whereas TLR7 and TLR8 are implicated in viral-derived

ssRNA recognition. TLR9 is essential in unmethylated (CpG) DNA recognition. Thus,

the TLR family members recognize specific pattern of microbial components.

Figure 2. TLR4 signaling pathway and is reproduced with the permission of Dr.

Shizuo Akira of Osaka University (Int. Immunol. 17:1-14,2005). TLR4 signaling

pathways originate from the cytoplasmic TIR domain. A TIR domain-containing adaptor,

MyD88, associates with the cytoplasmic TIR domain of TLR, and recruits IRAK to the

receptor upon ligand binding. IRAK then activates TRAF 6, leading to the activation of I

к B kinase (IKK) complex. The IKK complex phosphorylates I к B, resulting in nuclear

translocation of NF- к B which induces expression of inflammatory cytokines. TIRAP, a

second TIR domain-containing adaptor, is involved in the MyD88-dependent signaling

pathway via TLR2 and TLR4. In TLR3 and TLR4-mediated signaling pathways,

activation of IRF-3 and induction of IFN- β are observed in a MyD88-independent

manner. A third TIR domain-containing adaptor, TRIF, is essential for the

MyD88-independent pathway. Atypical IKKs mediate activation of IRF-3 downstream of

TRIF. A fourth TIR domain-containing adaptor, TRAM, is specific to the TLR4-mediated

MyD88-independent/TRIF-dependent pathway.

Figure 3. Link between innate and adaptive immunity and is reproduced with the permission of Dr. Shizuo Akira of Osaka University (Int. Immunol. 17:1-14,2005). Innate immune cells, such as macrophages and dendritic cells, engulf pathogens by phagocytosis, and present pathogen-derived peptide antigens to naïve T cells. In addition, TLRs recognize pathogen-derived components and induce expression of genes, such as co-stimulatory molecules and inflammatory cytokines. Phagocytosis-mediated antigen presentation, together with TLR-mediated expression of co-stimulatory molecules and inflammatory cytokines, instruct development of antigen specific adaptive immunity, especially Th1 cells.

Figure 4. Shows diagrammatic representation of different mechanisms of infertility in women suffering from endometriosis that may be directly or indirectly associated with bacterial endotoxin. LPS, lipopolysaccharide; ROS, reactive oxygen species. Other abbreviations as shown in this figure are described in the text.
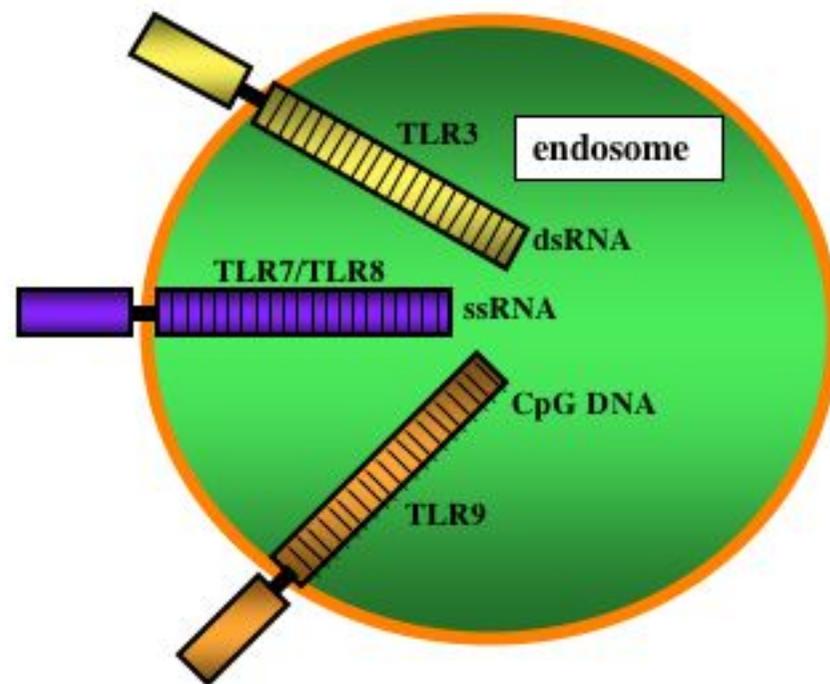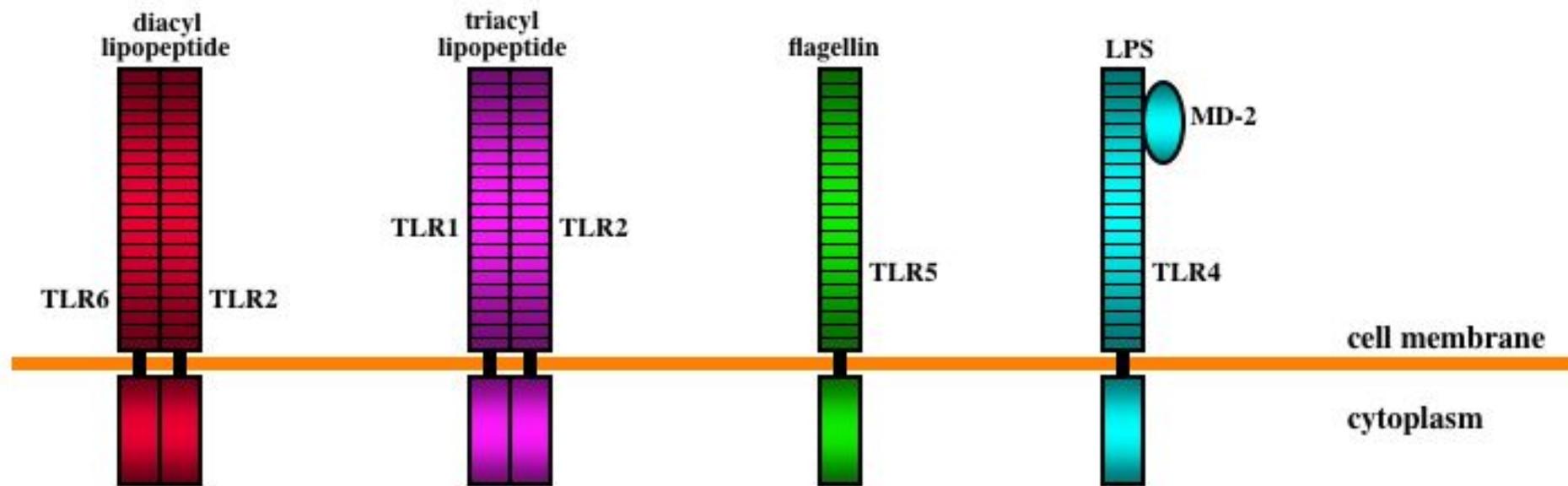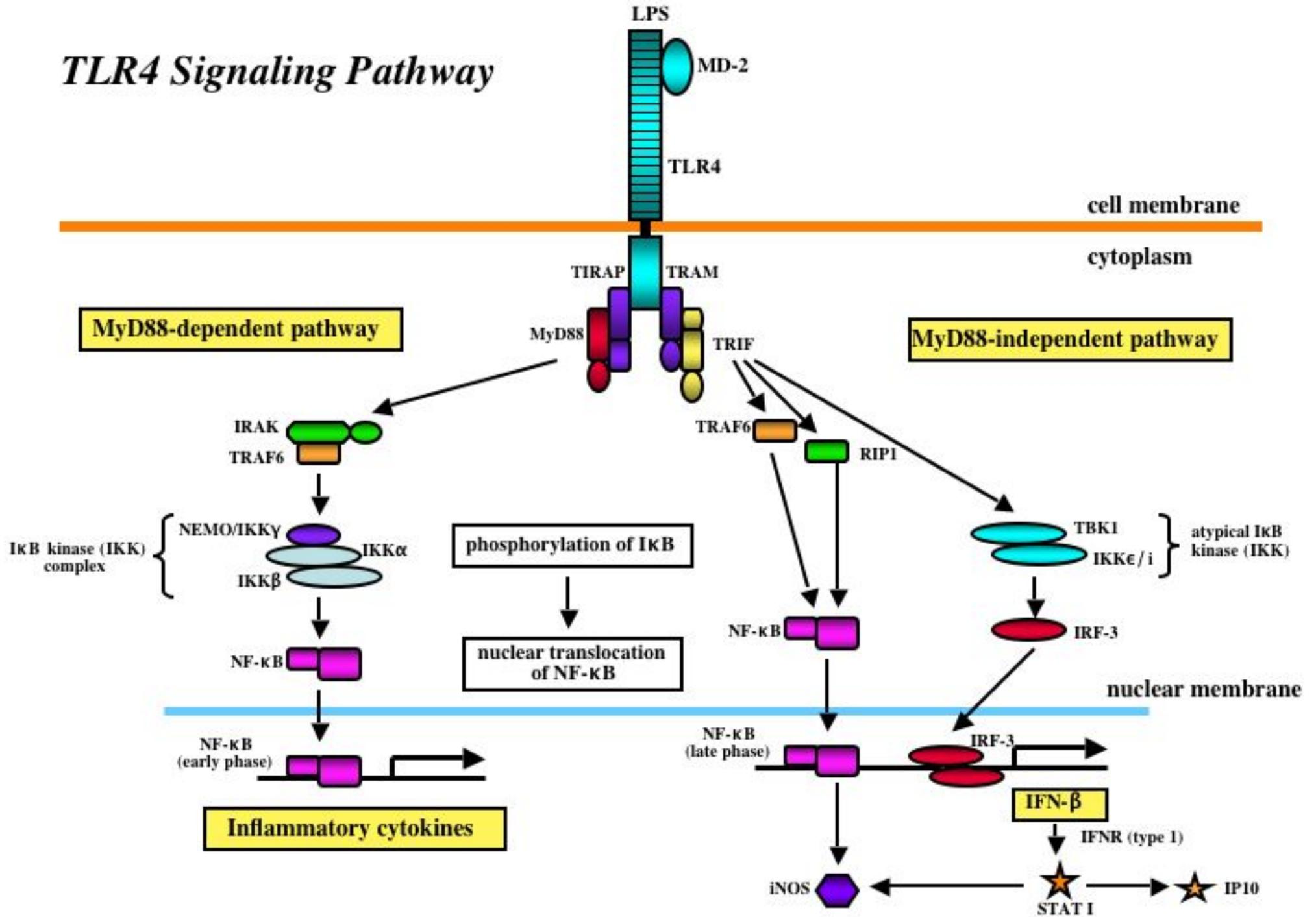
diacyl
lipopeptide

triacyl
lipopeptide

flagellin

LPS

MD-2

TLR1   TLR2

TLR6   TLR2

TLR5

TLR4

cell membrane

cytoplasm

TLR3

endosome

dsRNA

TLR7/TLR8

ssRNA

CpG DNA

TLR9

TLRs and Their Ligands
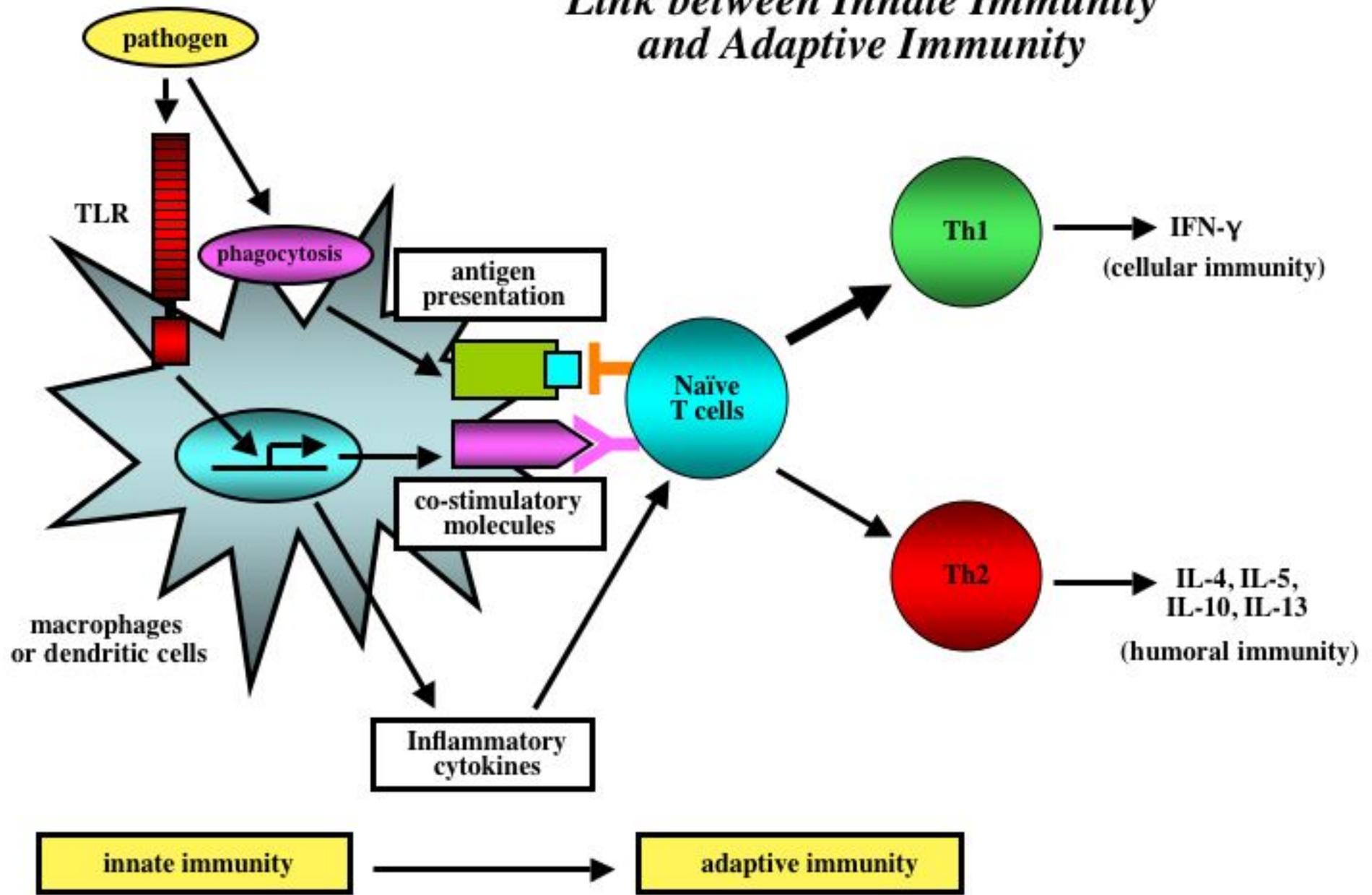
Link between Innate Immunity and Adaptive Immunity
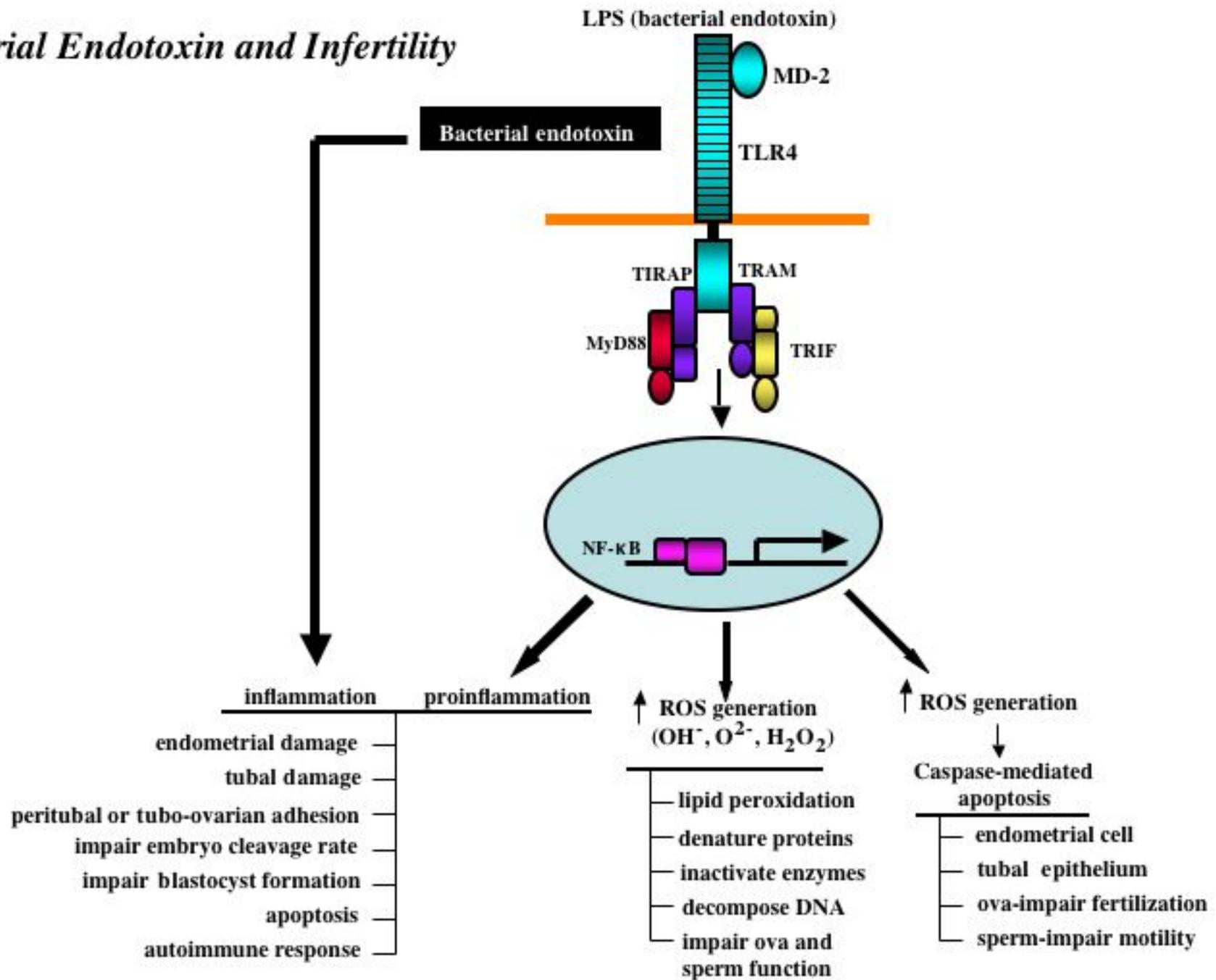
Table 1. Toll-like receptors (TLRs) and their ligands

Receptor	Ligand	Origin of ligand
TLR1	Triacyl lipopeptide	Bacteria and mytcobacteria
TLR2	Lipoprotein/lipopeptides	Various pathogens
	Peptidoglycan	Gram-positive bacteria
	lipoteichoic acid	Gram-positive bacteria
	Porins	Neisseria
	Atypical lipopolysaccharide	Porphyromonas gingivalis
	Zymosan	Fungi
TLR3	Double-stranded RNA	Viruses
TLR4	Lipopolysaccharide	Gram-negative bacteria
	Taxol	plants (*Taxus brevifolia*)
	Fusion protein	Respiratory cyncytial virus
	Heat-shock protein 60	*Chlamydia trachomatis/pneumoniae*
	Heat-shock protein 70	Host
	Fibronectin	Host
	Hyaluronic acid	Host
	Heparan sulphate	Host
	Fibronogen	Host
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides	Mycoplasma
	Zymosan	Fungi
TLR7	Single-stranded RNA	Viruses
	Imidazoquinoline	Synthetic compounds
TLR8	Single-stranded RNA	Viruses
	Imidazoquinoline	Synthetic compounds
TLR9	CpG-containing DNA	Bacteria and viruses
TLR10	Not determined	Not determined
TLR11	Profilin-like protein	Uropathogenic bacteria (*T.gondii*)

Table 2. Normal vaginal flora and microbes associated with genitourinary tract infections

Type and sub-type	Normal vaginal flora	Microbes associated with genitourinary tract infections
Gram-negative aerobic bacteria	*Escherichia coli* *Proteus vulgaris* *Klebsiella* *Enterobactor*	*Escherichia coli* *Proteus vulgaris* *Klebsiella* *Enterobactor* *Acinetobacter calcoaceitus* *Pseudomonas aeroginosa* *Serratia* *Neisseria gonorrhoeae*
Gram-negative anaerobic bacteria	*Bacteroids fragilis* *Bacteroids urolyticus* *Pervotella* *Mombiluncus spp.*	*Bacteroids fragilis* *Bacteroids urolyticus* *Pervotella* *Mombiluncus spp.* *Porphyromonas* *Mombiluncus spp.* *Chlamydia trachimatis* *Gardenerella vaginalis*
Gram-positive aerobic bacteria	*Enterococcus faecalis*	*Enterococci* *Staphylococcus saprolyticus* *Staphylococcus aureus*
Gram-positive anaerobic bacteria	*Streptococcus faecalis* *Staphylococcus epidermidis* *Lactobacillus acidophilus* *Clostridium*	*Peptostreptococcus* *Mycoplasma hominis*
Protozoa		*Trichomoniasis vaginalis*
Yeast		*Candida albicans* *Blastomyces* *Coccidioides immitis*