The Role of Toll-Like Receptor in the Female Reproductive Tract

M. Moga[1], Maria Mihalache[2], Ioana Manitiu[2], Nicu Bâgiu[2]

1. University Transilvania Braşov, Hospital of Obstetrics and Gynecology "I.A. Sbircea" Braşov
2. Hospital of Obstetrics and Gynecology "I.A. Sbircea" Braşov

Abstract

The aim of this review was to discuss the role of toll like receptors in the female reproductive tract. Toll-like receptors (TLRs) are considered a link between innate (non-specific) and adaptive (specific) immunity and TLRs contribute to the immune system's capacity to efficiently combat pathogens. This is done by means of the induction of signaling cascades resulting in the induction of type I interferons (IFNs I) and other cytokines. Recognition of pathogen associated molecular patterns (PAMPs) by TLRs results in the activation of signaling events that induce the expression of effector molecules such as cytokines and chemokines. A normal immune response leads to an adequate clearance of the infection, but the key element of the immune response activation is recognition of the pathogen trough TLRs receptors. The immune response in endometriosis is characterized by a small number of TLR 3 and 4 in the endometrial ectopic cells. The naturally powerful immunostimulatory property of TLR agonists can be exploited for active immunotherapy against cancer. The expression levels of TLR3, TLR4 and TLR9 have clinical interest as indicators of tumor aggressiveness in breast cancer. TLRs may represent also therapeutic targets in breast cancer. Dampened TLR expression in the cervical mucosa is a type-specific mechanism by which HPV 16 interferes with innate immune responses, contributing to viral persistence. TLR up-regulation and resultant cytokine induction are important in subsequent viral clearance.
Keywords: *toll-like receptors, immunity, infection, carcinogenesis*

Introduction

The immune response is classified in primary (innate) and acquired. The innate immune response is the first line of defense. It differentiates between "self" and "non-self", between pathogen and non-pathogen, and it triggers the adaptative immune response. Neutrophils, macrophages and dendritic cells play an important role. The adaptative immune response appears after the first contact with the antigen, and it develops a memory responsible for the fast immune response in case of a reinfection. The immune response is humoral (lymphocytes B) and cellular (lymphocytes T)[18]. Lymphocytes B produce antibodies that neutralize the antigens. Among LyT, lymphocytes T-helper 1 produce proinflamatory cytokines (IL12, INFα) and lymphocytes T-helper 2 produce IL 4,5,6,10[6].

Toll-like receptors (TLRs) are pattern-recognition receptors related to the *Drosophila* Toll protein. TLR activation alerts the immune system to microbial products and initiates innate and adaptive immune responses. Human TLRs comprise a large family of 10 proteins with member-specific activators and a complex downstream signaling. TLRs are expressed on various immune cells but are also present on mucosal surfaces of the respiratory, gastrointestinal and urinary tract. These receptors play an important role in secretion of various pro- and anti-inflammatory cytokines such as interferons, tumor necrosis factor α (TNFα) and interleukins IL4, IL8, and IL12[1].

A large amount of studies describe the existence of TLRs in the immune tissues: spleen, leucocytes, but also in the lung, gastrointestinal tract, epithelial cells of the female reproductive tract[29]. The expression of TLR 1-6 was detected in the uterine tubes epithelium, endometrium and uterine cervix[2,10]. Endometrium is an important contact interface between the host and the pathogens that end up in the reproductive tract. TLR activation alerts the immune system to recognize and bind the microbial products and stimulates the secretion of cytokines and chemokines at the endometrium level[14,32].

Constitutive expression of TLR2 has been reported in the epithelial cells of the fallopian tubes, endometrium, cervix, and vagina, smooth muscle cells of the cervix and vagina, endometrial stromal cells, and uterine NK cells[20,28]. TLR2 recognize various microbial components, such as peptidoglycans of gram-negative bacteria, lopoproteins of bacteria, mycobacteria structures[4,30], *Trepanosoma cruzi* glycosides, phenol-soluble medulin from Staphylococcus epidermidis, zymosan and phospholipomannan from fungi, glycolipids from *Treponema maltophilum* and *Leptospira interrogans*, *Porphyromonas gingivalis*, *Helicobacter pylori* liposaccharides[26].

Less apparent is the reduced TLR2 expression observed in the endometrium. It is possible that this expression pattern is related to the role of the endometrium in the maintenance of an environment hospitable to fetal implantation. Thus, TLR2 expression may be maintained at a low level in the healthy uterus but be up-regulated in response to pathogenic challenge as a defense mechanism that can be mobilized to protect the fetus from infection during pregnancy and labor[3].

TLR3 recognizes double-stranded RNA (dsRNA) and mRNA of the viruses and stimulation of TLR 3 is followed by the secretion of cytokines: TNFα, IL6, GM-CSF, G-CSF and chemokines: CXCL8/IL8, CCL2/MCP-1, CCL4/MIP-1ß[19]. Constitutive expression of TLR3 has been reported in female genital tissue samples from fallopian tubes, endometrium, cervix and vagina, in the vagina and cervix stromal fibroblast, and it is more frequent in the endometrial epithelial cells than in the stromal ones[13]. The expression of TLR3 in the endometrium is significantly higher during the secretory phase than in other phases of the menstrual cycle[28].

TLR4 is expressed in the epithelium of fallopian tubes and endometrium[31]. It is implicated in the initiation of the immune response against bacteria endotoxins[39] and recognizing endogenous ligands[3] such as "heat shock proteins" (HSP60 and HSP70), fibronectin extradomain A, hialuronic acid oligosaccharides, heparin sulphate and fibrinogen[26]. TLR4, in association with accessory molecules MD-2 and CD14, is the signal transduction receptor for gram-negative bacterial lipopolysaccharide[3].

Moleculary mechanism of TLR action

The presence of a pathogen triggers activation of TLRs as well as NOD molecules with receptor role, stimulating the secretion of chemokines, INF 1 and proinflamatory cytokines at the site of the epithelial cells. When immunity circulating cells get to the site of infection, their receptors bind to the pathogen. Consecutively the macrophages, neutrophiles and dendritic cells destroy the pathogen[37,38].

The extracellular part of the TLRs contains a leucine-rich repeat (LRR) motif. The LRR domains are directly involved in the recognition of a variety of pathogens. TLRs and members of the IL-1R family share a domain in their cytoplasmic region known as the Toll/IL-1R (TIR) domain. The IL-1R and TLR family signal via shared downstream signaling molecules. Triggering of the IL-1R or TLR causes the adaptor protein MyD88 to be recruited to the receptor complex, which in turn promotes association with the IL-1R-associated kinases IRAK4 and IRAK1[25]. During the formation of this complex, IRAK4 is activated, leading to the hyperphosphorylation of IRAK-1, which then induces the interaction of TRAF6 with the complex. The association of IRAK-4·IRAK-1·TRAF6 causes some conformational change in one or more of these factors, leading to their disengagement from the receptor complex. The IRAK-4·IRAK-1·TRAF6 complex then interacts at the membrane with another preformed complex consisting of TAK1, TAB1, and TAB2. This interaction induces phosphorylation of TAB2 and TAK1, which then translocate together with TRAF6 and TAB1 to the cytosol. TAK1 is subsequently activated in the cytoplasm, leading to the activation of IKK. Inactive IKK sequesters NF-κβ in the cytoplasm, but activation leads to phosphorylation and degradation of Iκβ and consequent release of NF-κβ. Activation of TAK1 also results in the activation of MAP kinases and c-Jun NH2-terminal kinase (JNK)[27].

References data collection - present insights

Tesuya Hirata et al. (2005) investigated the expression of TLR4 mRNA in endometrial epithelial cells (EECs) and stromal cells (ESCs) by RT-PCR and *in situ* hybridization. Western blotting analysis revealed the TLR4 protein expression in both cell populations. Treatment of lipopolysaccharide (LPS), the actions of which are mediated through TLR4, significantly increased IL-8 secretion from cultured ESCs in the presence of soluble CD14 and stimulates nuclear translocation of NFkB, but does not stimulate the secretion of IL8 in the EECs. A high level of INFγ (mediated trough TLR) in the deciduas[39] in the first pregnancy trimester was also observed, presumably having an antiinfectious role throughout the pregnancy[12,15].

Intrauterine infections have been associated with pregnancy complications that are also linked with increased trophoblast apoptosis. Vikki M. Abrahams et al. demonstrated the presence of TLR 2 and 4 in the gestational trophoblast[16]. Apparently intrauterine infections can cause TLR-2 activation induced apoptosis which in time can lead to pregnancy complications, such as preterm labor and delivery, intrauterine growth restriction (IUGR), and preeclampsia[17]. Studies in term placenta have demonstrated the expression of TLR-2 and TLR-4. TLR-2 recognizes bacterial lipoproteins, peptidoglycan (PDG) and lipoteichoic acid (LTA)[35], while TLR-4 recognizes Gram-negative bacterial LPS. Explants from term placenta have been shown to produce IL-6 and IL-8 following ligation of TLR-2 or TLR-4[17].

In case of human female genital tract infection with *Chlamydia trachomatis*, a normal immune response leads to a clearance of the pathogen. The key element of the normal immune response is the adequate signaling trough toll-like receptors. Genetic variations of TLRs such as abnormal density of the receptors and dysfunctional molecules, can affect the receptor function and induces inadequate recognition of *Chlamydia trachomatis* and persistence of infection with long term sequelae. TLR 2 and TLR 4 play an important role in triggering the immune response in *Chlamydia* infection. TLR 2 is the receptor for the peptidoglycan component and TLR 4 is the receptor for the lipopolysaccharid component and for *Chlamydia* heat shock proteins, elements found by Poltrak, Ohashi and col.

Clett Eridge et al. found that *Chlamydia* lipopolysaccharid component is recognized by TLR 2 instead of TLR 4 like other authors revealed. In their study, signalling in response to LPS was measured using an NF-κβ-responsive region (the IL8 promoter) linked to firefly luciferase. The HeLa cells used in the study have been shown by RT-PCR to express TLR2. Signalling through TLR2 was therefore examined by introduction of a TLR2 expression plasmid and through TLR4 by introduction of a plasmid expressing MD-2, which combines with endogenous TLR4 to produce a functional receptor. Clett Eridge et al. have shown that the LPS of *C. Trachomatis* signals via TLR2 and not TLR4[6].

Catherine M. O'Connell et al. showed that expression of Toll-like receptor (TLR)-2 was required for IL-8 secretion from *Chlamydia Trachomatis* infected cells, whereas the effect of TLR4 expression was minimal. IL 8 secretion is dependent on the pathogen status. Cell activation was dependent on infection with live, replicating bacteria, because infection with UV-irradiated bacteria and treatment of infected cells with chloramphenicol, but not ampicillin, abrogated the induction of IL-8 secretion[8].

Darville T. et al. studied the expression of TLR 2 and 4 on the secretion of TNFα and IL 6 in the macrophages from mice infected with *C. Trachomatis*. They showed that macrophages lacking TLR 2 secrete less TNFα and IL 6 in response to active infection, concluding that TLR2 is

an important mediator in the innate immune response to *C. Trachomatis* infection[9].

Svejna Allhorn et al.[1] describe the expression pattern of TLR3 and TLR4 in the ectopic endometrial cells and their role in endometriosis. The immune response in endometriosis is characterised by increased number of activated macrophages and their secreted products, such as growth factors, cytokines, and angiogenic factors. Young et al.[29] reported an increase in interleukin-8 (IL-8) production after stimulating TLR3 and TLR4 in endometrial cell lines with appropriate ligands. IL-8 is a chemotactic activating cytokine for leukocytes and it has been hypothesized to play a role in the growth and maintenance of ectopic endometrial tissue. Recent studies consider endometriosis as a process of sterile inflammation in the pelvis, which is accompanied by elevated levels of inflammatory key regulators such as TNFα or NF-κβ. Both are known downstream targets of TLRs[1]. Studies of RT-PCR, immunohistochemistry, immunofluorescence at the University of Duisburg-Essen Germany found a small number of TLR 3 and 4 in ectopic endometrial tissues[1].

Members of the Hsp family are candidate molecules that potentially signal tissue damage or cellular stress to the immune system. Hsp60, Hsp70 and Hsp90 have been implicated in a variety of autoimmune and inflammatory conditions. Human Hsps (Hsp60, Hsp70 and Hsp90) are reported to be produced by macrophages, vascular endothelial cells, smooth muscle cells, endometrial cells and other dendritic cells (Wallin and co. - 2002). A great variety of stimuli such as UV light, bacteria and viral infections, physical stress, chemical factors, pelvic inflammation, induce the synthesis of intracellular heat shock proteins.

Khaleque Newaz Khan et al. investigated the role of human heat-shock protein 70 (Hsp70) in Toll-like receptor 4 (TLR4)-mediated growth of endometriosis. TLR4 expression was examined in macrophages isolated in primary culture from the peritoneal fluid of women with and without endometriosis. The level of Hsp70 in ectopic and eutopic endometrium was examined by enzyme linked immunosorbent assay (ELISA). The results of the study showed that Hsp70 stimulates the secretion of hepatocyte growth factors, vascular growth factors, IL 6, TNFα in women with endometriosis comparing to healthy group. They also demonstrated that locally produced Hsp70 might be responsible for TLR4-mediated induction of inflammatory reaction and direct promotion in the growth of endometriosis[21].

Endometrium carcinoma is one of the most frequent gynecological diseases in Europe and North America, affecting postmenopausal women[22,23]. TNFα and NF-kβ play an important role in endometrium cancer pathogenesis[1]. Recent studies talk about bacterian infection as being a bridge between inflammation and carcinogenesis. Michael G.Nelly et al.(2006) found an inflammation activation path in carcinogenesis trough toll-like receptors[24].

Svejna Allhorn and co. (2008) demonstrated trough RT-PCR and, immunohistochemistry techniques that TLR 3 and 4 mARN expression is decreased in endometrial hyperplasia and adenocarcinoma when compared with postmenopausal controls. The lowest TLR expression levels were determined in poor differentiated carcinoma (grade 3)[1]. Recent studies demonstrate that TLRs are expressed in some tumor cells, and that the expression of TLRs in these cells is associated with tumorigenesis. Cervical intraepithelial neoplasia (CIN) is a key stage in the development of cervical cancer and human papillomavirus (HPV) infection is an essential factor in cervical carcinogenesis. The cervix is in constant contact with bacteria, especially Gram-negative bacteria and bacteria mediated TLR stimulation is involved in cervical carcinogenesis. Yu L., Wang L., Li M. et al. in their study published in February 2010, analyze the expression and distribution of TLR4 in CIN and cervical squamous carcinoma by immunohistochemistry. They observed a decrease in the expression of TLR4 during the progression of cervical neoplasia and this down-regulation of TLR4 appeared to be associated with the expression of P (16INK4A), which is a crucial marker of HPV integration into host cells. These data offer further insight regarding the association of HPV infection and TLR signaling during the carcinogenesis of cervical cancer[41,42].

In November 2009, Sylvia Adams publishes a paper in which she discusses the role of TLR agonists in cancer therapy. Toll-like receptor agonists used as single agents especially when applied locally can effectively eradicate tumors due to their potent stimulation of innate and adaptive immunity as well as their effects on the tumor microenvironment. Two TLR agonists, bacillus Calmette-Guerin (BCG) and imiquimod are US FDA approved for clinical use as monotherapy for cancer[40].

Cervical cancer development is linked to the persistent infection by high-risk mucosal human papillomaviruses (HPVs) types. The E6 and E7 major oncoproteins from this dsDNA virus play a key role in the deregulation of the cell cycle, apoptosis, and adaptive immune surveillance. Uzma A. Hasan et al.[45] conducted a study in 2007 in which they showed that HPV type 16 (HPV16), the most carcinogenic type among the high-risk subgroup, interferes with innate immunity by affecting the expression of TLRs. Infection of human primary keratinocytes with HPV16 inhibits TLR9 transcription and hence functional loss of TLR9-regulated pathways[33,34]. Interestingly, E6 and E7 from the low-risk HPV type 6 are unable to down-regulate the TLR9 promoter. In addition, E6 and E7 from the high-risk HPV type 18, which are known to persist less competently in the host than HPV16, have reduced efficiency compared with HPV16 in inhibiting TLR9 transcription. Furthermore, a CpG motif derived from the HPV16 E6 DNA sequence activated TLR9, indicating this virus is able to initiate innate responses via the receptor it later down-regulates. Their study reveals a novel mechanism used by HPV16 to suppress the host immune response by deregulating the TLR9 transcript, providing evidence that abolishing innate responses may be a crucial step involved in the carcinogenic events mediated by HPVs[36,45].

Daud II, Scott M.E. et al. also investigated the association between TLR expression and viral persistence or

clearance in young women with incident infections with oncogenic HPV types 16 and they concluded that dampened TLR expression in the cervical mucosa is a type-specific mechanism by which HPV 16 interferes with innate immune responses, contributing to viral persistence, and that TLR up-regulation and resultant cytokine induction is important in subsequent viral clearance[46].

Langerhans cells (LC) are the resident APCs in the cervical epithelium and are responsible for initiating an immune response against HPV16. On the contrary, LC exposed to HPV16 do not induce a specific T cell immune response, which leads to the immune evasion of HPV16. Demonstrating that TLR7 and TLR8 are expressed on human LC, Laura M. Fahey, Adam B. Raff et al. hypothesized that imiquimod (TLR 7 agonist) would activate LC exposed to HPV16, leading to the induction of an HPV16-specific cell-mediated immune response. Surprisingly, both phenotypic and functional hallmarks of activation are not observed when LC are exposed to HPV16 virus-like particles and treated with imiquimod. However, we found that LC are activated by 3M-002 (TLR8 agonist) and resiquimod (TLR8/7 agonist). LC exposed to HPV16 virus-like particles and subsequently treated with 3M-002 or resiquimod highly up-regulate surface activation markers, secrete proinflammatory cytokines and chemokines, and initiate an HPV16-specific $CD8^+$ T cell response. These data strongly indicate that 3M-002 and resiquimod are promising therapeutics for treatment of HPV infections and HPV-induced cervical lesions[47].

S.G. Reyes, L. Marin et al. investigated the expression and clinical relevance of TLR3, 4 and 9 in breast cancer[49]. The results of the study showed that the expression levels of TLR3, TLR4 and TLR9 have clinical interest as indicators of tumor aggressiveness in breast cancer. Cancer cells activated by TLR signals may release cytokines and chemokines that in turn may recruit immune cells and stimulate them to release further cytokines and chemokines. This process results in a cytokine profile that is associated with immune tolerance, cancer progression and propagation of the tumor microenvironment. Recent evidences also show that functional TLRs may play an important role in tumor progression by activating the production of interleukins, TNF-α, NF- kβ) and metalloproteases[43]. Activation of tumor cell TLRs promotes tumor cell proliferation, resistance to apoptosis and enhances tumor cell invasion and metastasis by regulating metalloproteases and integrins[48]. Results showed that TLR3 expression is associated with high probability of metastasis, which is in agreement with previous studies indicating that TLR3 expression is related to tumoral aggressiveness[44,48]. TLR expression have prognostic significance and suggest that these markers may represent new therapeutic targets in breast cancer[49]. Mononuclear TLR 4 expression was associated with a high incidence of metastasis, so using TLR 4 agonists could become a useful anticancer therapy. Fibroblast TLR 9 expression is associated with a low incidence of metastasis and stimulating TLR 9 activated dendritic cells and B cells inducing a potent immune response[49].

Discussions - conclusions

Infections can have serious consequences on the human female reproductive tract such as chronic inflammation and infertility. One of six couples are affected by infertility and the tubal cause stands for 25% of cases. *Chlamydia trachomatis* infection is considered to be the first cause of tubar infertility5. Up to 30% of *Chlamydia trachomatis* infections of the reproductive tract remit spontaneously between one week and one month, 50% in the first year, 80% in the first two years and 94% in the first four years from the infection (Golden et al., 2000; Joyner et al., 2002; Morré et al., 2002; Molano et al., 2005)[6].

The superior genital reproductive tract is susceptible to the microorganisms from the inferior genital tract, leading to infections such as endometritis or metroanexites. These infections enhance the risk of developing infertility or ectopic pregnancies. The epithelial cells of the female genital tract are the first line of defense against sexually transmitted diseases and pathogen invasion. Establishing the role of those cells in local defense opens new insights in producing vaccines and protocols of antiinfectious treatment. *Chlamydia* infection remains in most of cases asymptomatic and untreated. More important in eliminating the pathogen is an adequate immune response, rather than antibiotic treatment[6].

Genetic variations of TLRs, such as abnormal density of the receptors and dysfunctional molecules, can affect the receptor function and induces inadequate recognition of *Chlamydia trachomatis* and persistence of infection with long term sequelae. TLR2 expression is needed for the stimulation of IL8 secretion in the *C. Trachomatis* infected cells, while the effect of TLR4 expression is minimum. Healthy endometrial tissue needs an adequate expression of TLR 2, 3, 4[29,31]. TLRs are present in the decidua in the first trimester and, also, in the trophoblast. Intrauterine infections have been associated with pregnancy complications that are also linked with increased trophoblast apoptosis. TLR-2 activation induced apoptosis can lead to pregnancy complications, such as preterm labor and delivery, intrauterine growth restriction (IUGR), and preeclampsia.

Hormonal treatments can affect the immune response in the endometrial cells. It has been demonstrated that treatment with 17-β-estradiol suppresses the cytokine and chemokine production therefore it can affect the innate immune response to different pathogens[14]. Several studies demonstrated TLR 3 and TLR 4 implication in endometrial pathology due to their altered expression in endometriosis and adenocarcinoma. The immune response in endometriosis is characterised by increased number of activated macrophages and their secreted products, such as growth factors, cytokines, and angiogenic factors. Recent studies consider endometriosis as a process of sterile inflammation in the pelvis, which is accompanied by elevated levels of inflammatory key regulators such as TNFα or NF-κβ, both molecules activated trough TLRs. Endometrial hyperplasia and adenocarcinoma revealed significantly reduced TLR3 and TLR4 mRNA expression when compared with postmenopausal controls. The lowest TLR expression levels were determined in poor differentiated carcinoma (grade 3). Recent studies

reveal a novel mechanism used by HPV16 to suppress the host immune response by deregulating the TLR9 transcript, providing evidence that abolishing innate responses may be a crucial step involved in the carcinogenic events mediated by HPVs. Dampened TLR expression in the cervical mucosa is a type-specific mechanism by which HPV 16 interferes with innate immune responses, contributing to viral persistence, and that TLR upregulation and resultant cytokine induction is important in subsequent viral clearance.

LC are activated by 3M-002 (TLR8 agonist) and resiquimod (TLR8/7 agonist). LC exposed to HPV16 virus-like particles and subsequently treated with 3M-002 or resiquimod highly up-regulate surface activation markers, secrete proinflammatory cytokines and chemokines, and initiate an HPV16-specific $CD8^+$ T cell response.

TLR4 expression by mononuclear inflammatory cells (MICs) is increased in breast cancer cases with recurrence, the results suggest that the use of TLR4 agonists may become a useful anticancer strategy. Fibroblast TLR 9 expression is associated with a low incidence of metastasis and stimulating TLR 9 activated dendritic cells and B cells inducing a potent immune response. TLR3 expression is associated with high probability of metastasis, which is in agreement with previous studies indicating that TLR3 expression is related to tumoral aggressiveness.

Recent studies demonstrated that uterine epithelial cells are capable of recognizing a variety of pathogens, including bacteria and viruses through TLRs and consecutively secret cytokines and chemokine that alter the immune response in preventing and controlling infections. The fast answer to these antigens suggest that endometrial cells play an important role in maintaining the protection against potential pathogens trough TLRs. ■

References

1. Allhorn S, Böing C, Koch A, Kimmig R and Gashaw I. TLR3 and TLR4 expression in healthy and diseased human endometrium. Reproductive Biology and Endocrinology 2008; 6:40.
2. Mollen KP, Anand RJ, Tsung A, Prince JM, Levy RM, Billiar TR. Emerging paradigm: toll-like receptor 4-sentinel for the detection of tissue damage. Shock 2006; 26(5):430-437.
3. Pioli P , Amiel E, Schaefer T, Connolly J, Wira C, Guyre P. Differential Expression of Toll-Like Receptors 2 and 4 in Tissues of the Human Female Reproductive Tract. Infection and immunity, 2004; 72(10): 5799-5806.
4. Michelsen KS, Aicher A, Mohaupt M, Hartung T, Dimmeler S, Kirschning CJ, Schumann RR. The role of toll-like receptors (TLRs) in bacteria-induced maturation of murine dendritic cells (DCS). Peptidoglycan and lipoteichoic acid are inducers of DC maturation and require TLR2. J. Biol. Chem., 2001; 76:25680-25686.
5. Horne A, Stock S, King A. Innate immunity and disorders of the female reproductive tract. Reproduction 2008; 135:739-749.
6. Erridge C, Pridmore A, Eley A, Stewart J, Poxton I. Lipopolysaccharides of Bacteroides fragilis, Chlamydia trachomatis and Pseudomonas aeruginosa signal via Toll-like receptor 2. Journal of Medical Microbiology. 2004; 53:735-740.
7. O'Connell C, Ionova I, Quayle A, Visintin A, Ingalls R. Localization of TLR2 and MyD88 to Chlamydia trachomatis Inclusions. The Journal of Biological Chemistry 2006; 281(3):1652-1659.
8. Darville T, O'Neill JM, Andrews CW Jr, Nagarajan UM, Stahl L, Ojcius DM. Toll-like receptor-2, butnot Toll-like receptor-4, is essential for development of oviduct pathology in chlamydialgenital tract infection. J Immunol. 2003; 171(11):6187-97.
9. Schaefer T, Desouza K, Fahey J, Beagley K, Wira C. Toll-likereceptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. Immunology 2004; 112(3): 428-436.
10. Fazeli A, Bruce C, Anumba DO. Characterization of Toll-like receptors in the female reproductive tract in humans. Human Reproduction 2005; 20(5):1372-1378.
11. Eriksson SK, Meadows CR, Wira CR, Sentman CL. Endogenous transforming growth factor-betainhibits toll-like receptor mediated activation of human uterine natural killer cells. American Journal of Reproductive Immunology 2006; 56(5-6):321-8.
12. Herath S, Lilly ST, Santos NR, Gilbert RO et al. Expression of genes associated with immunity in the endometrium of cattle with disparate postpartum uterine disease and fertility. Reprod. Biol. Endocrinol. 2009; 7:55
13. Lesmeister MJ, Jorgenson RL, Young SL, Misfeldt ML. 17Beta-estradiol suppresses TLR3- induced cytokine and chemokine production in endometrial epithelial cells. Reprod Biol Endocrinol. 2005; 3:74.
14. Tetsuya Hirata, Yutaka Osuga, Yasushi Hirota, Kaori Koga, Osamu Yoshino, Miyuki Harada, Chieko Morimoto, Tetsu Yano, Osamu Nishii, Osamu Tsutsumi, and Yuji Taketani. Evidence for the Presence of Toll-Like Receptor 4 System in the Human Endometrium. The Journal of Clinical Endocrinology & Metabolism 2005; 90(1):548-556.
15. Koga K, Mor G. Expression and function of toll-like receptors at the maternal-fetal interface. Reprod. Sci. 2008; 15(3):231-42.
16. Abrahams VK, Bole-Aldo P, Yeon Mee K et al. Trophoblast Responses to Bacterial Products Mediated by TLRs. J. Immunol. 2004; 173:4286-4296.
17. Krikun G, Lockwood CJ, Abrahams VM, Mor G, Paidas M, Guller S. Expression of Toll-like receptors in the human deciduas. Histol. Histopathol. 2007; 22(8): 847-54.
18. Schaefer T, Fahey J, Wright J, Wira C. Innate Immunity in the Human Female Reproductive Tract: Antiviral Response of Uterine Epithelial Cells to the TLR3 Agonist Poly(I:C). J. Immunol. 2005; 174;992-1002.
19. Pamer E. TLR Polymorphisms and the Risk of Invasive Fungal Infections. N. Engl. J. Med. 2008; 359:1836-1838.
20. Aflatoonian R, Tuckerman E, Elliott SL et al. Menstrual cycle-dependent changes of Toll-like receptors in Endometrium. Human Reproduction 2007; 22(2):586-593.
21. Khan KN, Kitajima M et al. Toll-like receptor 4-mediated growth of endometriosis by human heat-shock protein 70. Human Reproduction 2008; 23(10):2210-2219.
22. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet 2005; 366(9484):491-505.
23. Kelly M, Alvero A et al. TLR-4 Signaling Promotes Tumor Growth and Paclitaxel Chemoresistance in Ovarian Cancer. Cancer Res. 2006; 66:3859-3868.
24. Soboll G, Shen L, Wira CR. Expression of Toll-Like Receptors (TLR) and Responsiveness to TLR Agonists by Polarized Mouse Uterine Epithelial Cells in Culture. Biology of Reproduction 2006; 75: 131-139.
25. Sawaki J, Tsutsui H et al. Type 1 cytokine/chemokine production by mouse NK cells following activation of their TLR/MyD88- mediated pathways. International Immunology 2007; 19(3):311-320.
26. Kiyoshi T, Shizuo A. Toll-like receptors in innate immunity. International Immunology 2005; 17(1):1-14.
27. Akira S. Toll-like Receptor Signaling. The Journal of Biological Chemistry 2003; 278(40):38105-38108.
28. Nasu K, Narahara H. Pattern Recognition via the Toll-Like Receptor System in the Human Female Genital Tract. Mediators Inflamm. 2010; 2010:976024.
29. Young S, Lyddon T, Jorgenson R, Misfeldt M. Expression of Toll-like Receptors in Human Endometrial Epithelial Cells and Cell Lines. Am. J. Reprod. Immunol. 2004; 52(1):67-73.
30. Herath S, Lilly S, Santos N et al. Expression of genes associated with immunity in the endometrium of cattle with disparate postpartum uterine disease and fertility. Reproductive Biology and Endocrinology 2009; 7:55.
31. Hart K, Murphy A, Barrett K, Wira CR, Guyre P, Pioli P. Functional Expression of Pattern Recognition Receptors in Tissues of the Human Female Reproductive Tract. J. Reprod. Immunol. 2009; 80(1-2): 33-40.
32. Akira S, Yamamoto M, Takeda K. Role of adapters in Toll-like receptor signaling. Biochemical Society Transactions 2003; 31:637-642.
33. Andersen J, Al-Khairy D, Ingalls R. Innate Immunity at the Mucosal Surface: Role of Toll-Like Receptor 3 and Toll-Like Receptor 9 in Cervical Epithelial Cell Responses to Microbial Pathogens. Biology of Reproduction 2006; 74:824-831.
34. Yokota S, Okabayashi T, Fujii N. Review Article - The Battle between Virus and Host:Modulation of Toll-Like Receptor Signaling Pathways by Virus Infection. Mediators of Inflammation 2010; 2010:184328.
35. Kiyoshi T, Kaisho T, Akira S. Toll-like receptors. Annu. Rev. Immunol. 2003; 21:335-76.
36. So E, Ouchi T. The application of Toll like receptors for cancer therapy. Int. J. Biol. Sci. 2010; 6(7):675-681.
37. Ashton K, Proietto A, Otton G et al. Toll-Like Receptor (TLR) and Nucleosome-binding Oligomerization Domain (NOD) gene polymorphisms and endometrial cancer risk. BMC Cancer 2010; 10:382.
38. Bas S, James R, Gabay C. Serum lipoproteins attenuate macrophage activation and Toll-Like Receptor stimulation by bacterial lipoproteins. BMC Immunology 2010; 11:46.
39. Simhan H, Chiao JP, Mattison D, Caritis S. Human decidual cell Toll-like receptor signaling in response to endotoxin: The effect of progestins. Am. J. Obstet. Gynecol. 2008; 198(1): 119.e1–119.e4.
40. Adams S. Toll-like receptor agonists in cancer therapy. Immunotherapy 2009; 1(6):949-964.
41. Xagorari A, Chlichlia K. Toll-Like Receptors and Viruses: Induction of Innate Antiviral Immune Responses. The Open Microbiology Journal 2008; 2:49-59.
42. Yu L, Wang L, Li M et al. Expression of toll-like receptor 4 is down-regulated during progression of cervical neoplasia. Cancer Immunol. Immunother. 2010; 59(7):1021-8.
43. Zeromski J, Mozer-Lisewska I, Kaczmarek M. Significance of Toll-like Receptors Expression in Tumor Growth and Spreading: A Short Review. Cancer Microenvironment 2008; 1:37-42.
44. So E, Ouchi T. The application of Toll like receptors for cancer therapy. Int. J. of Biol. Sci. 2010; 6(7):675-681.
45. Uzma H, Bates E, Takeshita F, Biliato A. TLR9 Expression and Function Is Abolished by the Cervical Cancer-Associated Human Papillomavirus Type 16. The Journal of Immunology 2007; 178: 3186-3197
46. Daud II, Scott ME, Ma Y, Shiboski S, Farhat S, Moscicki AB. Association between toll-like receptor expression and human papillomavirus type 16 persistence. Int. J. Cancer. 2010; 128(4):879-86.
47. Fahey L, Raff A, Da Silva D, Kast M. Reversal of Human Papillomavirus-Specific T Cell Immune Suppression through TLR Agonist Treatment of Langerhans Cells Exposed to Human Papillomavirus Type 16. The Journal of Immunology 2010; 182(5):2919-2928.
48. Scarlett UK, Cubillos-Ruiz JR, Nesbeth YC et al. In situ stimulation of CD40 and Toll-like receptor 3 transforms ovarian cancer-infiltrating dendritic cells from immunosuppressive to immunostimulatory cells. Cancer Res. 2009; 69:7329-7337.
49. González-Reyes S, Marín L, González L et al. Study of TLR3, TLR4 and TLR9 in breast carcinomas and their association with metastasis. BMC Cancer 2010; 10:665.