CHAPTER 2

Toll-Like Receptors and Pregnancy

Vikki M. Abrahams and Gil Mor

Abstract

The maternal-fetal interface represents an immunologically unique site that must promote tolerance to the allogenic fetus, whilst maintaining host defense against a diverse array of possible pathogens. Clinical studies have shown a strong association between certain pregnancy complications and intrauterine infections. Therefore, innate immune responses to microorganisms at the maternal-fetal interface may have a significant impact on the success of a pregnancy. There is growing evidence that trophoblast cells are able to recognize and respond to pathogens through the expression of Toll-like receptors, a system characteristic of innate immune cells. This review will discuss the role of Toll-like receptors at the maternal-fetal interface, the potential for trophoblast cells to function as components of the innate immune system and the impact TLR-mediated trophoblast responses may have on a pregnancy.

Introduction

During pregnancy there is a strong immunological presence at the maternal-fetal interface, particularly by cells of the innate immune system. The role of the immune system at the maternal-fetal interface is thought to facilitate implantation and placental development, whilst promoting fetal tolerance. However, a certain level of host defense at this site is also required. As a consequence, either an inefficient clearance of an infectious agent, or an overzealous immune response may have a significant impact on the pregnancy. Clinical studies have shown a strong association between certain pregnancy complications and intrauterine infections, suggesting that the innate immune response can affect the outcome of a pregnancy. Preeclampsia and intrauterine growth restriction (IUGR) are both thought to be associated with infection, and a link between preterm labor and intrauterine infections is now well established. Indeed, infections have been reported as responsible for up to 40% of preterm labor cases. Furthermore, 80% of preterm deliveries occurring at less than 30 weeks of gestation have evidence of infection, suggesting that an intrauterine infection may occur early in pregnancy, preceding such pregnancy complications. Infection, therefore, represents an important and frequent mechanism of disease, yet, the precise molecular mechanisms by which infection can affect a pregnancy remains undefined. While immune cells such as macrophages and NK cells are present the maternal-fetal interface, they may not be the only cells able to respond to infectious agents. In addition to the classical immune cells, placental cells may also have the potential to function as a component of the innate immune system. This review will discuss how trophoblast cells may respond to a pathogen through the system of evolutionary conserved proteins known as Toll-like receptors, and how such responses might impact a pregnancy.

Infections and the Innate Immune

The innate immune system represents the immunological first line of defense against invading pathogens through its ability to distinguish between what is non-infectious self and...
infectious nonself. One way in which the innate immune system achieves this is through an evolutionary conserved system of pattern recognition. Cells of the innate immune system express a series of receptors known as pattern recognition receptors (PRR) which recognize and bind to highly conserved sequences known as pathogen-associated molecular patterns (PAMPs). Pathogen-associated molecular patterns are unique to, and expressed on, the surface of microorganisms. Examples of PAMPs include lipopolysaccharide (LPS), the major component of gram-negative bacterial outer membranes, and peptidoglycan, the major component of gram-positive bacterial cell walls. The ligation of PRR by PAMPs results in an inflammatory response generated against the invading pathogen. Furthermore, activation of TLR expressed by antigen presenting cells, such as dendritic cells, may facilitate the initiation of adaptive immune responses.

There are a number of different PRR including the mannose-binding receptor and the scavenger receptor, however, this review will focus on the major family of PRR, the Toll-like receptors.

**Toll-Like Receptors**

Originally discovered in Drosophila, the Toll gene was found to be critical for dorso-ventricular polarization during embryonic development. However, later studies revealed that Toll also have anti-fungal and anti-bacterial properties in the adult fly. Subsequently, mammalian Toll was identified and to date, 11 Toll homologues have been identified and designated, Toll-like receptor (TLR) 1-11. Ligation of TLR by microbial products results in an inflammatory immune response characterized by the production of cytokines and anti-microbial factors. Furthermore, through the regulation of co-stimulatory molecules, TLR may also facilitate the development of adaptive immune responses.

Toll-like receptors are transmembrane proteins which have an extracellular domain containing leucine-rich repeat motifs. Each receptor differs in their ligand specificity. So while individually, TLR respond to limited ligands, collectively the family of TLR can respond to a wide range of proteins associated with bacteria, viruses, fungi and parasites (Fig. 1). TLR-4 was the first human Toll-like receptor to be identified and was subsequently found to be the specific receptor for recognition of LPS. Early studies showed that overexpression of constitutively active TLR-4 in monocytes resulted in the upregulation of pro-inflammatory cytokines and costimulatory molecules, suggesting that this receptor is involved in both innate and adaptive immune responses. TLR-4 recognition of LPS is thought to be potentiated by additional molecules. Prior to the identification of human TLR, LPS responses were thought to be initiated through CD14 which recognizes the LPS/LBP binding protein (LBP) complex. It is now thought that following the binding of the LPS/LBP complex to CD14, TLR4 becomes either indirectly or directly activated. Another protein that appears to enhance LPS responses is MD-2.

Of all the Toll-like receptors identified, TLR-2 has the widest specificity. TLR-2 binds to gram-positive, gram-negative and mycobacterial associated lipoproteins, gram-positive peptidoglycan and lipoteichoic acid, as well as fungal zymosan. Indeed, TLR-2 deficient mice are highly susceptible to Staphylococcal aureus infections and are unable to respond to either peptidoglycan or lipoproteins. TLR-2 recognition of some microbial products appears to be dependent upon the formation of heterodimers with either TLR-1 or TLR-6. TLR-2/TLR-1 recognize bacterial triacylated lipoproteins, while TLR-2/TLR-6 complexes recognize mycoplasmal diacylated lipoproteins. The natural ligands for human TLR-7 and TLR-10 are, as yet undetermined.

**Toll-Like Receptor Expression**

As expected, TLR are widely expressed throughout the cells of the immune system, specifically those of the innate. Toll-like receptors can also be expressed by non-immune cells, particularly if such a cell can contribute to an inflammatory response, and most tissues express at least one TLR. Toll-like receptor expression by mucosal systems is important for host defense.