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THE INTERNATIONAL ARABIC JOURNAL OF ANTIMICROBIAL AGENTS

2013
Vol. 3 No. 1:1
doi: 10.3823/722

Antibacterial agents and innate immunity

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Abstract

On their own, antibacterial agents cannot cure an infectious disease. They need help from the innate immune response followed by the adaptive immune response and inflammatory response. An overview of Toll-Like Receptors (TLR) as key players in the innate immune response is given followed by a review of published results obtained in the authors laboratory related to the effect of several antibacterial agents on the action of bacterial lipopolysaccharide (LPS), a ligand for TLR-4. The results indicated that the antibacterial agents tested were anti-inflammatory. Inflammation is a two edged sword; in moderation it is beneficial, but deleterious if in excess. It is suggested that infectious disease specialists monitor serum pro-inflammatory cytokine and/or nitric oxide levels of their patients on antibacterial therapy and when needed, treat with a cytokine, a TLR agonist or a TLR antagonist where indicated.

Antibacterial agents on their own are not capable of eradicating infections efficiently. Help coming from the patient's innate immune response followed by the adaptive immune response and inflammatory response is needed. This has been observed in patients with immunodeficiency diseases such as X-Linked Agammaglobulinemia, Chronic Granulomatous Disease and CD40 Ligand Deficiency (1). Treatment of these patients with antibacterial agents might temporarily control the infection. However, recurrences' always occur.

One of the major players in the innate immune response is the Toll-Like Receptors (TLR). Toll Receptors were first discovered in the fruit fly, *Drosophilia melanogaster* (2). Their human homologues were discovered soon after and were called Toll-Like Receptors (3).

Toll-Like Receptors (TLR) are expressed by a number of cell types including dendritic cells and macrophages. So far 13 TLRs have been identified (TLR-1 to 13). Some are expressed on the cell surface (TLR-1, 2, 4, 5, 6, 10, 11, 12) others are expressed on the cell endosome (TLR-3, 7, 8, 9, 13). Not all TLR are necessarily expressed by the same cell (4). TLR belong to a group of receptors called Pattern Recognition Receptors (PRR). They are called so because they serve as receptors for conserved molecular patterns found in infectious agents. These patterns serve as ligands for TLR. The ligands are called Pathogen Associated Molecular Patterns (PAMPS) (5, 6). Ligands and their receptors are given in **Table 1**.

What happens when a ligand engages its receptor? One or 2 signal pathways are activated; the MyD88 dependent and the MyD88 independent pathways. These results in the activation of the nuclear factors Nf-Kb, IRF-3 and/or IRF-7 that enter the nucleus and promote the transcription of genes that code for the production of cytokines needed for the generation of the adaptive immune response and inflammatory response. Some of the cytokines produced are IL-1 beta, TNF, IL-4, IL-6, IL-8, IL-12, and alfa and beta interferon (7,8) This is schematically shown in **Figure 1** (9) and the functions of the cytokine produced are given in **Table 2**.

Table 1. TLR and their ligands.

TLR	Ligands
1+2	bacterial lipoproteins
2+6	bacterial lipoproteins, lipoteichoic acid, mannans
3	dsRNA
4	bacterial lipopolysaccharide (LPS)
5	bacterial flagellin
7	ssRNA
8	ssRNA
9	unmethylated CpG bacterial DNA
10	unknown
11	profilin (Toxoplasma gondii)
12	unknown
13	23S ribosomal RNA

In addition, it has been reported that nitric oxide is generated when LPS engages TLR-4 and can be considered as a marker for inflammation. Panaro et al. (10) reported that TLR-4 mediated LPS-induced release of nitric oxide and TNF-α by chick embryonal cardiomyocytes. He et al. (11) compared a number of TLR ligands in their ability to induce the production of nitric oxide by chick monocytes and reported that LPS (ligand for TLR-4) and CpG ODN (ligand for TLR-9) were the most potent inducers.

LPS and cytokines produced induce macrophages, hepatocytes, vascular smooth muscle and cardiacmyocytes to produce nitric oxide . Nitric oxide is produced when L-arginine is converted to L-citrulline. The enzyme needed for this conversion is nitric oxide oxidase that exists in 3 different isoforms, namely, neuronal, endothelial and inducible nitric oxide synthetase. Nitric oxide is involved in killing invading infectious agents. (12)

Toll-Like Receptors and the generation of nitric oxide.

Considering the production of nitric oxide as a marker for inflammation we studied the effects of gentamicin, tobramycin, imipenem, tigecycline and isoniazid on serum physiological levels of nitric oxide and levels induced by LPS in mice. All 5 antibacterial agents reduced serum physiological levels and levels induced by LPS of nitric oxide in mice (13,14),

With the exception of gentamicin and tobramycin, the antibacterial agents tested had different mechanisms of antibacterial action, yet they all suppressed nitric oxide production. Hence it can be speculated that the 5 antibacterial agents tested are anti-inflammatory agents.

Effect of vancomycin and tobramycin on the production of cytokines

At about the same time that human TLR were discovered (3) we reported that vancomycin and tobramycin suppressed the levels of TNF-α and gamma-interferon induced by LPS (15). It has been mentioned earlier that TNF-α and IL-12 are cytokines produced upon the engagement of LPS with TLR-4. IL-12 is needed for the activation of Th1 lymphocytes. Once activated Th1 lymphocytes will produce gamma-interferon. Later, we studied the effect of two TLR ligands, LPS and bacterial DNA on the production of IL-1β, IL-10 and IL-12 by mouse peritoneal macrophages *in vitro.* The levels of all 3 cytokines were elevated when macrophages were treated with either LPS or bacterial DNA. However, the potency of the two TLR ligands differed. LPS was more potent in inducing the production of IL-1β and IL-12 and bacterial DNA was more potent in inducing the production of IL-10 (16). From these 2 studies it can be suggested that vancomycin and tobramycin interfered with the LPS- TLR-4 signaling resulting in suppressed production of cytokines and thus can be considered anti-inflammatory agents.

Modified bacterial ligands and TLR

An interesting article recently appeared in Science that dealt with 23S rRNA and TLR-13 (53). They reported that the ligand for TLR-13 is the 23S rRNA. Additionally, they showed that the methylated 23S ribosomal RNA, isolated from erythromycin-resistant *Staphylococcus aureus* failed to stimulate TLR-13. The target site for lincosamide, streptogamins and erythromycin is the 23S ribosomal RNA. These antibacterial agents bind to it and inhibit bacterial protein synthesis. The primary means of resistance to these antibacterial agents is by post-transcriptional methylation of 23S ribosomal RNA. So we have the 23S ribosomal RNA as a target site for the antibacterial agents and are a ligand for TLR-13. It can be assumed that the methylated form was not capable of inducing the production of cytokines, and adaptive immunity and the inflammatory response were suppressed. There are questions that need to be answered with respect to the Odenburg et al. report (5). Such as, will other ligand-TLR interactions compensate for this defect? Will other innate immune responses such as activation of the complement system be affected by resistant strains?

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Table 2. Some properties of cytokines that are generated.

Cytokine	Properties
IL-1	mediator of acute inflammation. Stimulates endothelial cells to produce adhesion molecules and chemokines, produces fever, promotes production of acute phase reactants, helps in activating T-lymphocytes.
TNF-α	mediator of acute inflammation. Stimulates endothelial cells to produce adhesion molecules and chemokines, produces fever, promotes production of acute phase reactants,
IL-4	stimulates development of Th2 lymphocytes, stimulates immunoglobulin class switching to the IgE isotype.
IL-6	stimulates production of acute phase reactants
IL-8	attracts (chemokine) and activates neutrophils
IL-12	stimulates production of ϒ-interferon, promotes production of Th1 lymphocytes, enhances cytototoxic effect of T-cytotoxic and NK cells
α and β interferon	antiviral agents

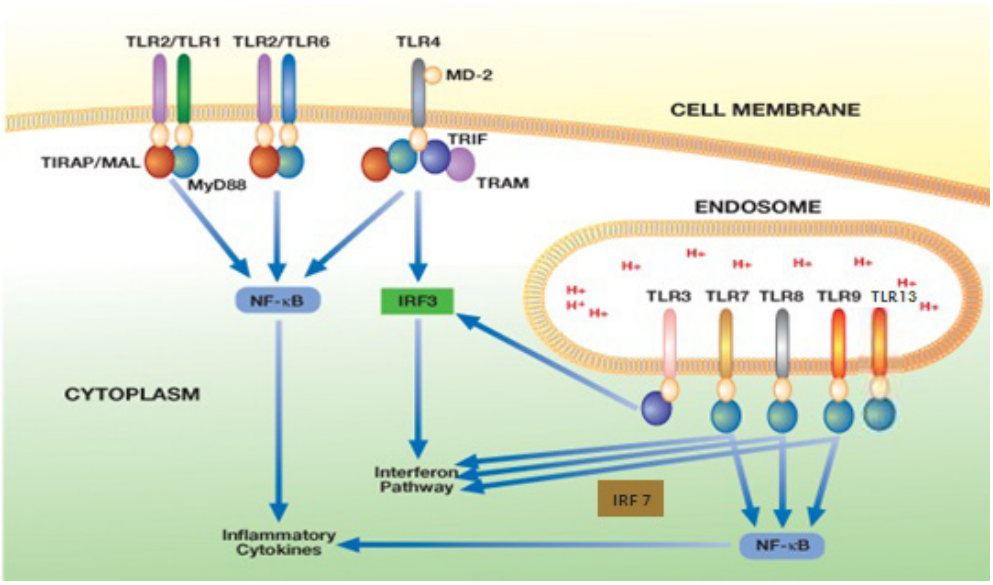

Figure 1. Ligand-TLR engagement and signaling pathways leading to the production of cytokines

* Taken from MacKichan ML (9). Modified to include TLR 13.

Is the production of cytokines and nitric oxide in the innate immune response beneficial or harmful?

It appears that some antibacterial agents suppress the production of pro-inflammatory cytokines and nitric oxide by interfering with ligand-TLR interactions and their signaling pathways. Moreover, methylated 23S rRNA was reported to be unable to activate TLR-13. Production of pro-inflammatory cytokines and nitric oxide in physiological amounts would be an aid to antibacterial agents in controlling an infection. The inability to produce pro-inflammatory cytokines could lead to recurrent, invasive pyogenic infections. This is seen in Inherited Human IRAK-4 deficiency. IRAK-4 is an intermediate in the ligand-TLR signaling pathway leading to the production of pro-inflammatory cytokines. A defect in the

gene that codes for IRAK-4 will block the signaling pathway (17). On the other hand, excessive production of pro-inflammatory cytokines and nitric oxide can be deleterious. Toxic Shock Syndrome that often results in multi-organ failure is due to excessive production of cytokines. Certain strains of *Staphylococcus aureus* and *Streptococcus pyogenes* produce toxins that behave as superantigens. They activate both antigen presenting cells and T-lymphocytes causing these cells to produce excessive amounts of cytokines. (18). In addition, uncontrolled production of pro-inflammatory cytokines, in particular IL-1□ is seen in Hereditary Periodic Fever. These patients suffer from recurrent episodes of fever that may or may not be accompanied by infection (19).

Nitric oxide on one hand is involved in killing microorganisms and on the other hand excess production is thought to contribute to hypotension and septic shock (12).

Perhaps it would be advisable that infectious disease specialists monitor serum cytokine and/or nitric oxide levels of their patients on antibacterial therapy and consider using a cytokine, TLR agonist or TLR antagonist where indicated (5). In the case of excess production of nitric oxide, the use of 1400W, an inhibitor of inducible nitric oxide can be considered if this drug is FDA-approved (20, 21).

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