

## INTERVIEW about

### LPS antagonist with

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#### Why is it important to look for LPS antagonist?

**Angela Ianaro:** Lipopolysaccharide (LPS) or endotoxin, the major constituent of the outer membrane of Gram negative bacteria, has been implicated as the bacterial product responsible for the clinical syndrome of sepsis. Sepsis and its *sequelae*, septic shock, acute respiratory distress syndrome, and multiple organ dysfunction/injury, represent a continuum of a syndrome characterised by systemic inflammation and widespread tissue injury, caused by an overwhelming body's systemic response to an infection (1). LPS binding to the host receptor Toll like receptor 4 (TLR4) triggers an inflammatory response with the release of large number of inflammatory mediators that allow the host to respond to the invading pathogen. When this production becomes uncontrolled and excessive leads to the develop-

ment of septic shock. Despite decades of efforts in supporting therapies, sepsis remain the leading cause of death amongst critically ill patients. Unfortunately, the major factor contributing to the high morbidity and mortality of sepsis is the lack of the effective treatment. Consequently, over 30 pharmaceutical products are in development for this condition: many of these target specific inflammatory mediators and have thus been, in general, unsuccessful since the process of sepsis involves many mediators (2). More successful strategies modulating entire inflammatory pathways include those that prevent the binding of LPS to host cells and the subsequent cascade of detrimental events. In this light, effective LPS antagonists would represent invaluable tools to efficaciously manage of sepsis.

#### How many molecules are known to antagonize LPS?

**Angela Ianaro:** LPS structurally consists of three distinct regions: the O-specific polysaccharide, the core oligosaccharide and the unique fatty-acylated diphosphorylated diglucosamine portion termed lipid A (LIP A), which represent the main toxicophore. Thus, LIP A is an attractive target for the development of effective drugs for sepsis and septic shock treatment. Interestingly, the toxicity level of LIP A strongly depends on its acylation pattern and it is the highest when LIP A is hexa-acylated, such as in *Escherichia coli* (*E.coli*) LPS. Recent findings demonstrate that structurally altered LIP A are less or

not agonistically active, rather acting as antagonist of *E.coli* LPS and reducing its deleterious effects. In fact, penta-acylated LIP A from two phototropic species, *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* have shown antagonistic effect (3,4). More recently, we have also demonstrated that a structurally novel LIP A derived from *Halomonas magadiensis*, a Gram-negative extremophilic and alkaliophilic bacterium, consisting of a heterogeneous mixture of penta- and tetra-acylated LIP A, has antagonistic properties in human cells, likely due to its capability to interfere with TLR4-mediated immune activa-

tion (5). Consequently, two generations of synthetic analogs of antagonistic LIP A have been designed to antagonize the effect of endotoxin. The first generation exponent E5531, an analog of the LIP A from *Rhodobacter capsulatus*, demonstrated potent inhibition of LPS *in vitro* and *in vivo*

but its activity decreased as a function of time for the interaction with plasma lipoproteins (6,7). Superior activity and pharmacological characteristics have been shown by E5564 (8), a second-generation LPS antagonist derived from the structure of *Rhodobacter sphaeroides*.

#### Do LPS antagonists interfere with TLR4?

**Angela Ianaro:** TLR4 is the long sought after LPS receptor (9). Infusion of LPS alone can induce a systemic inflammatory response that results in hypotension, multiorgan system failure and death. Blocking the TLR4 receptor or its downstream pathway should attenuate this response and may possibly induce LPS tolerance. The specific TLR4 antagonist, E5564, brand name Eritoran, is now in clinical trials to determine the potential efficacy of TLR4 inhibitors in septic shock. Eritoran has recently been tested in a double-blind, placebo-controlled human study, and did indeed block the toxic effects of an endotoxin infusion in healthy volunteers (11). Fever, tachycardia, leukocytosis, rise in C-reactive protein, and measurements of TNF-alpha and IL-6 were significantly different in individuals receiving Eritoran than in those receiving placebo. Subjec-

tive symptoms such as nausea, headache and myalgia were also minimised. No agonist effect has been observed with this compound. This TLR4 inhibitor has successfully completed phase II clinical trials in septic patients and has now entered a phase III clinical program, results are expected for the end of 2006 (12). Another potential therapeutic agent that affects TLR4 signalling is a molecule known as TAK-242 (13). TAK-242 is a specific, small molecule inhibitor of the intracellular signalling pathways of TLR4 and limits nuclear factor-kB-mediated proinflammatory cytokine generation. The molecule is effective in protection against LPS-mediated pathologies in both small and large animal preclinical studies (14). This molecule has now (August 2005) entered a phase III clinical program (12).

#### Do you believe that LPS antagonists can be used for therapeutical applications?

**Angela Ianaro:** Despite advances in diagnostic techniques, surgical therapy, intensive care treatment, and use of potent antibiotics, serious gram-negative bacterial infection in conjunction with the sepsis syndrome is associated with significant risk of mortality (about 30-50%) (15). LPS is a major toxin in patients undergoing cytoreductive chemotherapy for cancer, as is invasion by Gram-negative organisms or their cell wall components across a damaged gut mucosa. LPS is also a major stimulus for reactive airways diseases, including asthma and chronic obstructive pulmo-

nary disease (COPD) (16) and obstetric dysfunction in "ripening" of the cervix and preterm labour (17). The ability to block LPS signalling in these situation may prove helpful. The development of new therapies for the treatment of gram-negative bacterial sepsis has been the focus of extensive investigation. Molecular and cellular biologic techniques have led to important advances including identification of naturally occurring LPS-binding protein; generation of novel LPS-binding antibodies, proteins and peptides; and characterization of the molecular determinants of LPS

binding. Taken together these advances can be expected to further the development of the next generation of novel, adjuvant therapies for the treatment of sepsis syndrome caused by gram-negative bacterial infection and endotoxemia.

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