In Search of the Holy Grail: The Quest for Universal Lipid A Binding and Neutralizing Ligands for Treatment of Severe Gram-negative Infections

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Introduction

Adehesion of microorganisms, including Gram-negative bacteria, to host epithelial cells may be followed by penetration through the epithelial lining, invasion into the bloodstream and finally, dissemination to lungs, liver, brain tissue and other organs. This process may have severe consequences for the host, since bacterial lipopolysaccharide (LPS, endotoxin) is a very potent inducer of cytokines like tumour necrosis factor (TNF) and interleukins (IL-1, -6, -8). The overinduction of these cytokines causes the clinical symptoms of sepsis and septic shock and leads to inadequate tissue perfusion, multiple organ failure, and ultimately, death. Mortality due to Gram-negative sepsis and shock remains high despite the availability of potent antibiotics: the bacteria may be killed by antibiotics but the endotoxin remains active! Hence, the novel strategies to lower mortality of Gram-negative infections have focused on ways of inactivating endotoxin. As lipid A (LA) is the toxic part of LPS these attempts have focused on preparing LA-binding ligands. LA is structurally the most conserved part of LPS and thus an additional advantage of the anti-LA approach is that potentially, a single ligand (monoclonal antibody or peptide) may be prepared active against all or the majority of strains belonging to the species most often involved in Gram-negative sepsis and shock, i.e. *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae.*

Subject

The immunochemical properties of four currently known groups of LA ligands will be described, i.e., three groups of LA binding monoclonal antibodies (Mabs), and a non-antibody LA ligand: recombinant/bactericidal permeability increasing protein ($rBPI_{23}$).

Results

The **first** group of anti-LA Mabs recognizes the hydrophilic part of LA. Binding requires a non-substituted C'60H in LA and affinity for Re- and other R-and S- LPS was very low. These Mabs (IgG and IgM) do not display hydrophobic interactions and do not bind to non-lipid A antigens, i.e. they are monoreactive.

The **second** group of Mabs (IgM) displays hydrophobic properties, binds also to single-stranded (ss) DNA, phospholipids and proteins, i.e. Mabs are polyreactive. Binding of these Mabs to bacteria involved interaction with outer membrane proteins; affinity for LPS was very low. The **third** group of LA Mabs (IgM) are anti-Kdo Mabs in disguise. They bind to LA, Kdo, ssDNA and phospholipids, i.e., they are also polyspecific. Due to their Kdo (a common epitope) specificity, these Mabs bind with high affinities to some but not all LPS of *E. coli*, *Klebsiella* and *Psuedomonas*. They are functionally active in passive hemolysis.

The **fourth** group comprises antibacterial peptides like $rBPI_{23}$, which binds well to all lipid As ($n = 8$) and all Re-, R- and S- LPS tested ($n = 200$). Thus, unique for BPI is its ability to bind to lipid A even when the C'60H group is substituted with Kdo. Other peptides have been reported in the literature that have binding- and neutralizing properties similar to $rBPI_{23}$, for example polymyxin B and derivatives thereof and a LA-binding polypeptide present in amoebocytes of the horseshoe crab *Limulus polyphemus* and oligopeptide derivatives thereof.

Summary

LA Mabs are a heterogenous group of antibodies. Immunochemically speaking, none of the various LA Mabs looks promising as an immunotherapeutic agent. In contrast, $rBPI_{23}$ is a universal LA/LPS ligand.

Future

For the future is will be crucial to understand on a molecular scale, i.e. three-dimensionally, the manner in which LPS-neutralizing peptides interact with LA and to define what structural element(s) in a peptide are crucial for neutralization. To this end we have synthesized a variety of small (less than 30 amino acids) LA-binding peptides. These novel peptides, that bind to and neutralize LA, are currently under investigation in a variety of *in vitro* and *in vivo* assay systems.

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