Active and Passive Vaccines Against Multiresistant Gram-positive Pathogens

Technology

Enterococci and staphylococci are among the leading causes of hospital-acquired infections. Some of these bacteria have developed antimicrobial resistances against most antibiotics used in hospitals. Alternative approaches are desperately needed to prevent and/or treat these multiresistant bacteria that are often also the source of antimicrobial resistances in other species. We have identified and chemically characterized several surface antigens in *E. faecalis* that are targets of opsonic and protective antibodies. Some of these antigens are cross-reactive and cross-protective against other gram-positive pathogens (such as staphylococci). Using a novel approach we constructed a set of human monoclonal antibodies that are highly opsonic and protective against gram-positive pathogens (such as *S. aureus*, enterococci, and streptococci). Together, these active or passive vaccines may be able to protect and treat patients at risk for sepsis or foreign body infections due to nosocomial gram-positive pathogens.

Innovation

- New therapeutic approach for otherwise often untreatable infections
- Possibility of prophylactic administration to patients at risk
- Broad coverage of clinically relevant nosocomial pathogens
- Synergistic to established treatment options (such as antibiotics)
- Not affected by resistance determinants against antimicrobials

Application

- Use as antigens for active immunization
- Development of novel diagnostics
- Highly active monoclonal antibodies may be used as therapeutic to protect and/or treat nosocomial infections

Market Potential

- Enterococci are the third most common pathogen isolated from the blood of hospitalized patients and second most common nosocomial isolate. Certain patient populations (such as patients after organ or bone marrow transplant, or neonates) are at high risk to develop nosocomial enterococcal bacteremia and these populations would benefit from an enterococcal vaccine.

Responsible Scientist

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Patent Status

Patent portfolio:
DE 10 2006 053 385 (Nov. 13th, 2006)
EP 2525800 (Jan.19th, 2010)
EP 2248533 (May 5th, 2009)
EP 2 450 053 (Nov. 5th, 2010)
EP 2 500 349 (Mar. 11th, 2011)
EP 256 951 (May 27th, 2011)
EP 2 644 613 (Mar. 30th, 2012)
EP 2 889 311 (Dec.30th, 2013)
and international patents/patent applications based on those patents/patent applications listed above
(at least in EP (HK), US, CA, JP)

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Vaccine Antigens Against Multiresistant Gram-positive Pathogens

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Background

The development of a vaccine to prevent and/or treat infections due to multi-resistant nosocomial pathogens is currently one of the top priorities of modern health care. Several promising targets have been examined *in vitro* and *in vivo*, but up to now no successful vaccine has been introduced for gram-positive bacteria, such as staphylococcus or enterococcus. Our group has worked on several different antigens that may be used as a basis for a broadly active vaccine targeting nosocomial and community-acquired multi-resistant enterococci and other multiresistant gram-positive bacteria.

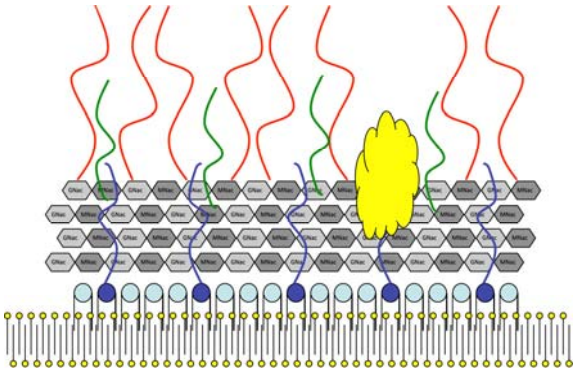

Fig. 1. Schematic representation of the gram-positive cell wall: Capsular polysaccharides (red), lipoteichoic acids (dark blue), wall teichoic acids (green) and cell surface proteins (yellow).

Capsular Polysaccharides

A capsular polysaccharide was isolated from *E. faecalis* type 5, a CPS-D strain (8) and from *E. faecium* E155 (VRE, unpublished). Structures were obtained by one-dimensional and two-dimensional homonuclear and heteronuclear ^{1}H and ^{13}C NMR spectroscopy. Binding of rabbit antiserum to capsular polysaccharide was assessed by ELISA and the specificity of opsonic antibodies was determined in an inhibition opsonophagocytic killing assay (*E. faecalis* type 5 and *E. faecium* E155). Protective efficacy was determined in mouse bacteremia and rat endocarditis models (*E. faecalis* type 5).

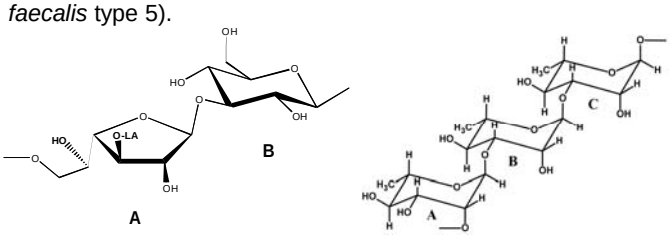

Fig. 2. Chemical structures of the repeating units of *E. faecalis* strain type 5 capsular polysaccharide (left) and *E. faecium* E155 (right). LA, lactic acid. The D-configuration of the Gal*f* residue is only suggested.

Wall Teichoic Acids

Wall teichoic acids (WTA) are a major component of the gram-positive cell wall. Several WTAs from *E. faecalis* and *E. faecium* have been identified and structurally characterized (1,3,5). Our data show that these molecules prevent complement deposition and are therefore important for virulence. These molecules are possible

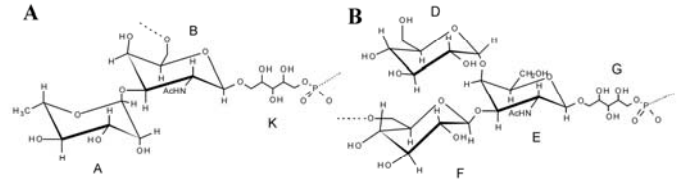

Fig. 3. Chemical structures of the two wall teichoic acids present in E. faecalis V583 (1)

Cell Wall Proteins

Several cell wall proteins have been identified that are targets of opsonic and protective antibodies. A surface protein (SagA) present in all tested *E. faecium* strains was shown to induce opsonic and protective antibodies against different *E. feacium* strains (6).
A prototypical gram-positive type 4 secretion system is present on plasmid pIP501 and rabbit sera were raised against two proteins, i.e. ORF10 and ORF13, that code for an ATPase and a putative channel component. The rabbit sera were used in an opsonophagocytic assay and sera raised against ORF13 showed a killing of 99.2% at a dilution of 1:10 against the homologous strain. Using absorption of the sera with increasing amounts of purified protein this killing could be inhibited by up to 44.5%. Testing of a larger collection of strains from different species revealed that 2/2 (100%) *E. faecalis*, 2/4 (50%) *E. faecium*, and 5/5 (100%) *S. aureus* were effectively killed by anti-ORF13 at a dilution of 1:10, including one vancomycin-resistant *S. aureus* strain and several USA300 CA-MRSA. Using a mouse bacteremia model, significant reductions in colony counts were seen in animals that had received anti-ORF13 as compared to animals that had received antisera against ORF10 and were challenged with the homologous *E. faecalis* as well as with an *E. faecium* and a CA-MRSA strain (Fig. 4). Animals infected with the homologous *E. faecalis* not carrying plasmid pIP501 were not protected. These data demonstrate that ORF13 is the target of opsonic and protective antibodies and may therefore be a promising and broadly cross-protective vaccine candidate targeting multi-resistant gram-positive pathogens (unpublished).

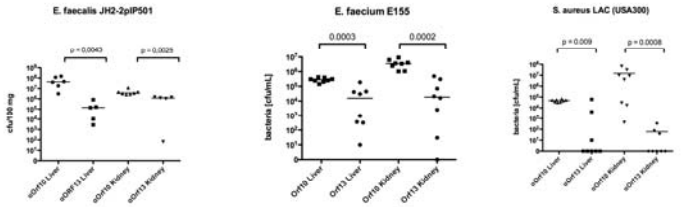

Fig. 4. Mouse sepsis model using sera raised against ORF13 and ORF10 (control): Statistically significant protection was seen in animals infected with *E. faecalis*, *E. faecium* and *S. aureus* (unpublished results).

Lipoteichoic Acid and Synthetic LTAs

Lipoteichoic acid (LTA) is produced by many clinically important Gram-positive bacteria, including enterococci, streptococci and staphylococci. Rabbit sera against LTA of *E. faecalis* opsonize *E. faecalis* and protect mice against bacteremia. We e could demonstrate that antibodies against *E. faecalis* LTA also bind to LTA from other Gram-positive species and efficiently kill *S. epidermidis* and *S. aureus* strains as well as group B streptococci (4). The effectiveness of rabbit antibody against LTA suggests that this conserved bacterial structure could function as a single vaccine antigen that targets multiple Gram-positive pathogens. We described the first automated solid phase synthesis of teichoic acids (TAs) and the preparation by this method of a number of well-defined TA structures, which were assessed for their antigenicity and protective efficacy (2,7). A candidate for vaccine development was identified consisting of a polyglycerol phosphate molecule that had been substituted with one glucose (see Figure 5) and this molecule was shown to be effective in a rat endocarditis model (unpublished results).

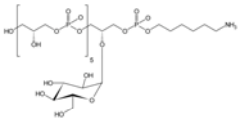

Fig. 5. Chemical structure of a lipo-teichoic acid mimetic

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