ImmunoTools *special* Award 2014

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Decoding the immune signature of an emerging pathogen: Patient-guided discovery and integrated evaluation of antibody-based vaccine candidates against *Staphylococcus aureus*

Staphylococcus aureus is a widespread human pathogen that has an increasingly negative impact on human health. Every year 10.000.000 individuals around the world visit their doctor presenting symptoms of *S. aureus* infection. The annual cost for the treatment of *S. aureus* reaches 4.5 billion dollars and the rate of mortality due to the pathogen ranges between 11-45%. It is a leading cause of hospital and community-acquired infections of human skin or soft tissue abscesses. In addition, *S. aureus* can cause several potentially fatal conditions (e.g. endocarditis, septicemia) as well as infections associated with implanted medical devices. An effective vaccine that can protect from *S. aureus* infections is not yet available by the scientific community. The emerging multidrug resistance of *S. aureus*, the poor response of hospital-endemic strains to broad-spectrum antibiotics and the diverse repertoire of immune evasion strategies elaborated by this pathogen urge for the consideration of novel therapeutic platforms to neutralize virulence-promoting factors released by such pathogens. In this context, combinatorial monoclonal Ab-based therapies are now gaining momentum as potential means for combating *S. aureus* infections by providing protection through passive immunization of patients.

The challenge of *S. aureus-targeted therapy* has long been to design vaccines that will be used in passive immunization. Several studies have shown that antibodies play a key role in protection against *S. aureus*. To this end it is of profound clinical significance to exploit the humoral immune response after infection and rapidly generate therapeutic hmoAbs (human monoclonal Antibodies).

The proposed research is centred on two Specific Aims.

Aim 1: **Studies on the humoral immune profiles of *S. aureus*-exposed individuals and exploitation of their discrete immune signature for the development of human antibody-based vaccines (hmoAbs) against *S. aureus*.**

We propose to profile the immune response of individuals against *S. aureus* and generate hmoAbs secreted by a distinct population of antibody-secreting cells (ASCs). To this end, patients will be selected based on the *S. aureus* neutralizing activity of their plasma and ASCs from whole blood will will be sorted by flow cytometry using the provided ImmunoTools antibodies. First, the live cell gate will be set and the ASCs will be bulk sorted by first gating on $CD19_{high}/CD20_{low\ to\ neg}/CD3_{neg}$ and then on $CD27_{high}/CD38_{high}$ cells. The appropriate IgG,

IgM and IgD gates will be set to obtain IgG-producing ASCs. Finally, the purified ASCs will be single-cell sorted, their antibody genes will be amplified, cloned into IgG expression vectors and transfected into HEK293A cells. MonoAbs will be expressed, purified and used for the next part of the study.

Aim 2: **Characterization of the neutralizing function of the hmoAbs using *in vitro* approaches**

We propose the characterization of the therapeutic efficacy of the generated hmoAbs, in an *in vitro* whole blood model of *S. aureus* induced bacteremia. The effect of the hmoAbs on the survival of the pathogen will provide a clear picture of their potential therapeutic value as part of a vaccine. In addition, an immediate host response toward bacterial infection is the activation of leukocytes and their subsequent infiltration/migration from the circulation into sites of infection. The impact of hmoAb treatment on a panel of leukocyte activation markers will be determined (i.e. CD11b). Moreover, the effect of hmoAbs on the capacity of leukocytes to respond to a panel of proinflammatory cytokines (IL-6, IL-1β, IL-8, rh MIP-1α/ CCL3, RANTES) will also be assessed.

To determine the neutralizing effect of the antibodies, whole blood will incubated with *S. aureus* in the presence or absence of antibodies. Plasma samples will be collected at various time points and tested using the ImmunoTools ELISA kits (TNF-α, IL-4, and IL-6) for the levels of pro-inflammatory cytokines that have been secreted by. blood cells (neutrophils and monocytes) that mediate key inflammatory reactions to *S. aureus*. Furthermore, the effect of antibody treatment on markers of granulocyte activation (e.g. CD11b upregulation) will be determined by flow cytometry using the available ImmunoTools antibodies.

Overall, the development and therapeutic testing of key *S. aureus* neutralizing hmoAbs will provide a rational framework for the design of novel *S. aureus*-targeted vaccines.