

Development of peptides that inhibit the cellular invasiveness *S. pyogenes* and their testing in a new high-throughput-assay

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The human pathogen *Streptococcus pyogenes*, a gram-positive, spherical bacterial species, belongs to the group A streptococci (GAS). It causes several diseases that range from pharyngitis, scarlet fever, rheumatic fever to severe infections like toxic shock syndrome and necrotizing fasciitis [Molinari, G. & Chhatwal, G.S. (1999) *Curr. Opin. Microbiol.* (2) 56ff]. *Streptococcus pyogenes* possesses several virulence factors [Mitchell, T.J. (2003) *Nat. Rev. Microbiol.* (1) 219ff]: on the one hand binding proteins like M protein, SfbI- and SfbII-protein, or IgA Binding protein; on the other hand several enzymes that seem to be involved in virulence (e.g. Streptokinase, Streptolysin O, Streptolysin S).

In this project, the focus is on the SfbI-protein, a fibronectin binding protein consisting of several structural domains. During infection it is known that two of this domains bind to the 70 kDa fraction of human fibronectin [Tomasini-Johansson, B.T. et al. (2001) *J. Biol. Chem.* (276) 23430ff] which itself binds to the $\alpha 5\beta 1$ -integrin of the host cell. This leads to streptococcal adhesion to and later on to invasion into the host cell.

Aim of this project is to find peptidic inhibitors which prevent the binding of streptococci to fibronectin via the SfbI protein and therefore decrease the invasion into the host cell.

The potential inhibitors are synthesized in form of libraries. For screening these libraries a cell-based high-throughput-assay has been established. It consists of an *in vitro* invasion assay in a 96-well-plate format in which each peptides' ability to inhibit adhesion and invasion of *S. pyogenes* to the host cell (Hep-2) is tested. Adherent and invaded GAS are quantified using fluorescence-labelled antibodies. A special part of the SfbI protein, a 49mer, is used as a positive control, since it is known to inhibit the streptococcal invasion into the host cell [Tomasini-Johansson (2001)].