77 An interaction between 14-3-3 and ExoS of *P. aeruginosa* is necessary for ExoS cytoxicity and enzymatic activity

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Aim: To investigate the importance of each amino acid of ExoS at the site of interaction between ExoS and 14–3-3 and analyze the cytotoxicity and enzymatic activity of various ExoS mutants in cell.
P. aeruginosa is an opportunistic pathogen. Using type III secretion system, *P. aeruginosa* secrets 4 toxins, Exoenzyme (Exo) T, U, Y and S, which are translocated into the eukaryotic cell. Our research focus on ExoS, which contains an N-terminal GAP domain, disrupt actin microfilament structure in cells and the C-terminal ADP-ribosylating (ADP-R) domain, which is lethal by blocking receptor-stimulated RAS activation in cells. In addition, a eukaryotic Factor Activating ExoS or FAS (member of the 14–3-3 protein) is required for the ADP-R activity of ExoS and the interaction between 14–3-3 and ExoS is critical for lethality and RAS ADP-ribosylation by ExoS.
Methods: Site directed mutagenesis of ExoS. Infections of cells with bacteria harboring different ExoS mutants, followed by resulting activities assay in cells.
Results: 14–3-3 interacts with ExoS in an unphosphorylated manner which occurs in the most C-terminal part of ExoS. ExoS lacking the 14–3-3 binding site has a reduced capacity for both to ADP ribosylate RAS and to change the morphology of infected cell. Moreover, we are in process to investigate the critical single amino acid important for cytotoxicity and the interaction between 14–3-3 and ExoS.
Conclusion: 14–3-3 is only expressed in eukaryotic cells. It has been suggested that the prokaryotic evolution has created a way to take advantage of 14–3-3, using them as necessary cofactor to activate bacterial toxins. It is apparent that more secrets concerning this intriguingly unphosphorylation dependent interaction needs to be uncovered, which will be discussed.

78 *Pseudomonas aeruginosa* cupA encoded fimbria expression is regulated by a GGDEF and EAL domain dependent modulation of the intracellular level of cyclic diguanylate

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Cyclic-diguanylate (c-di-GMP) is a widespread bacterial signal molecule that has been implicated to play a role in the modulation of cellular surface components, such as exopolysaccharides and fimbria, and in the establishment of a sessile life style. We report here on the positive influence of c-di-GMP on cupA encoded fimbria expression in *Pseudomonas aeruginosa*. CupA is induced by either providing exogenous c-di-GMP or by overexpression of PA1120, which contains a GGDEF domain. In contrast, overexpression of the EAL domain containing phenotypic variance regulator (PvrR) resulted in a decreased intracellular level of c-di-GMP, in a reduced cupA encoded fimbria expression and – as described previously – in a switch from an auto-aggregative SCV to a non-auto-aggregative wild-type phenotype. Thus, both protein domains, the GGDEF and the EAL domain, are involved in c-di-GMP turnover in *P. aeruginosa* in vivo and control CupA expression and thus biofilm formation. The elucidation of this regulation will significantly increase our understanding of bacterial adaptation and might be the basis to initiate the development of new antimicrobial treatment strategies.

79 Fighting *Pseudomonas aeruginosa* by exploiting its communication – quorum sensing

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Conventional antibiotics have afforded a remarkable increase in the life expectancy of cystic fibrosis patients. However, antibiotics target the growth and the basal life processes of bacteria, leading to growth arrest and cell death. This selective force eventually creates antibiotic resistant bacteria. The most obvious alternative to antibiotic-mediated killing would be to attenuate the bacteria with respect to pathogenicity. We have previously demonstrated that the cell-cell communication, Quorum Sensing (QS) renders *P. aeruginosa* highly tolerant to otherwise lethal doses of antibiotics and the bactericidal activity of polymorphonuclear leukocytes (PMNs).
Using a pulmonary mouse model we investigated the impact of blocking the QS systems of *P. aeruginosa* in vivo, either by genetically mutating the bacteria or QS inhibitory (QSI) drugs. The attenuation of QS initially provoked a higher degree of inflammation; however the health status and survival of the mice were significantly higher. We detected a larger amount of PMNs in BAL fluids, an increased endobronchial influx of PMNs, though the production of the PMN chemoattractant MIP-2 decreased, as a result of QS blockage. Consequently, blockage of QS led to significantly faster clearing of the bacteria and reduced mortality of the mice. These results indicate that *P. aeruginosa*, by means of a QS controlled mechanism, modulate the early immune response. We hypothesize that the administration of QSIs, antibiotics and adequate amounts of PMNs, might very well result in only the destruction of the bacteria, and no further lung damage.

80 The development of an octavalent conjugate *P. aeruginosa* (Pa) vaccine for Cystic Fibrosis (CF) patients: Results from two Phase II studies

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Aims: To demonstrate safety and immunogenicity of the octavalent conjugate Pa vaccine in CF patients.
Methods: During 2003 through 2005, 63 CF patients, between 18 months and 18 years of age (study 01), and 23 infants with CF between the age of 6 and 18 months at time of inclusion (study 02) were enrolled in two open, uncontrolled clinical trials. All patients were immunized twice (days 1 and 60, "primary immunization"). Serum samples were taken at days 1, 60 and 180. Humoral immune responses against the 8 serotypes were tested by ELISA. High affinity antibodies having an $aK > 1\times10^6$ mol/L for IgG antibodies against Pa IATS-6 LPS were determined.
Results: There were no deaths during the studies and no withdrawals due to AEs. There were a total of 7 SAEs in 01 and 1 SAE in 02, all of which were considered unrelated to study medication and resolved without sequelae. Solicited injection site reactions occurred in 48%/22% of patients in studies 01/02; while solicited systemic reactions were observed in 3%/52% of patients, respectively. In study 02, there were statistically significant increases over baseline in IgG for every serotype in the vaccine both at day 60 ($p = 0.0003$) and at day 180 ($p = 0.0004$). At days 60 and 180, 87% and 91% of patients had high affinity antibodies, respectively. Results for study 01 are similar.
Conclusions: For the first time, immunogenicity and induction of high affinity antibodies after vaccination of infants with the Pa vaccine has been demonstrated. The octavalent Pa vaccine is safe and immunogenic in the studies presented.