170 Pseudomonas aeruginosa quorum sensing (QS) impact on host defences: QS-inhibitors and treatment of lung infections in cystic fibrosis (CF)

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Objectives: Discovery of Quorum Sensing (QS) offers attractive strategies in Pseudomonas aeruginosa infection control, interfering with this bacteria-to-bacteria communication system. Molecules, such as natural furanone, have been described as QS-inhibitors given their similarity to the natural QS-autoinducers called N-acyl-homoserine lactones (HSL) C4-HSL and 3-oxo-C12-HSL. Besides their role in bacterial virulence regulation, HSL also have an immuno-modulatory activity. These properties have been especially demonstrated with 3-oxo-C12-HSL, but never with C4-HSL. The goal of this study was to examine the ability of 3-oxo-C12-HSL and C4-HSL to induce pro-inflammatory cytokine production in human monocyte derived macrophages, and the potent modification of the cell response in the presence of QS-inhibitors: furanone and N-pyrimidyl butanamide (C11), an original compound designed in our laboratory.

Methods: Among eight blood donors tested, both P aeruginosa HSLs were found to induce IL-6 production, and seem to have cumulative pro-inflammatory effect when added together. These data suggest that 3-oxo-C12-HSL, but also C4-HSL, may possess immuno-modulatory activity, playing a role in P aeruginosa infection pathophysiology. Furanone and C11 do not show similar effects and their association with HSL induce various modifications in the IL-6 production in human monocyte derived macrophages.

Conclusion: To improve these results about QS-inhibitors immuno-modulatory properties, we need to pursue our investigations using LPS-stimulated human macrophages, various timing of reagent associations and tests in normal and cystic fibrosis bronchial epithelial cells.

Reference(s)

171 Anti-Pseudomonas aeruginosa IgY antibodies promote specific bacterial aggregation and internalization in polymorphonuclear neutrophils

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Objectives: Oral treatment with pathogen-specific IgY antibodies poses a promising complement to antibiotics in averting the devastating chronic lung infections with Pseudomonas aeruginosa (PA) in Cystic Fibrosis (CF). Anti-PA IgY antibodies augment the PMN-mediated bacterial killing in vitro, indicating a faster bacterial clearance of pathogens in the airways. The present study aimed at exploring whether IgY-mediated bacterial aggregation improves the internalization of bacteria in PMNs.

Methods: Anti-PA IgY solution was obtained from ImmunSystem AB (Sweden). Pseudomonas aeruginosa PA01 strain was grown in LB broth. Stationary phase organisms were obtained from overnight cultures (ON).

Neutrophil isolation: PMNs were isolated from whole human blood.

Bacterial assay: Measuring the loss of bacterial viability over time by mixing (non)opsonized bacteria and PMNs and plating diluted samples overnight followed by colony counting.

Immunofluorescence microscopy: PA01 was mixed with S-IgY and secondary TexasRed-conjugated rabbit anti-chicken IgG followed by smearing the solution on microscope slide. Immunofluorescent micrographs of specimens were obtained using a confocal laser scanning microscope (CLSM) after 24h. PMN mediated phagocytosis of PA01 was visualized and captured by CSLM imaging. ON of fluorescent PA01 (GFP) was mixed with S-IgY or PBS and allowed to aggregate prior to addition of PMNs.

Conclusion: Anti-Pseudomonas aeruginosa IgY antibodies improve the bacterial internalization in PMNs and subsequent bacterial killing in vitro by conferring bacterial aggregation, suggesting a mode of action to the observed clinical impact of oral IgY treatment.

172 Basophil activation test: A novel approach to the diagnosis of allergic bronchopulmonary aspergillosis in cystic fibrosis patients

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Objectives: Basophil Activation Test (BAT) is a novel flow cytometric method that enables the assessment of IgE mediated hypersensitivity reactions to specific antigens. Being such a reaction, Allergic Bronchopulmonary Aspergillosis (ABPA), may represent a disease for which BAT could be applied. The purpose of this study was to investigate the role of BAT using Aspergillus fumigatus extract antigens, in the diagnosis of ABPA in the Cystic Fibrosis setting.

Methods: Starting as of 09/2005, BAT has been applied to 5 normal controls and to 59 samples of 49 CF patients (3–23 years old), of which 10 without signs of ABPA, 13 with definite ABPA under corticosteroid treatment, 7 with definite ABPA prior to corticosteroid administration, 20 with probable ABPA diagnosis and 9 patients in whom ABPA was resolved. BAT results (percentage of basophils with CD203c and CD63 up regulation) were correlated to the patients’ clinical course within 1.5 to 7 years and with ABPA diagnosis using consensus 2003 criteria.

Conclusion: BAT results were significantly correlated with ABPA diagnosis and SPT results. CD203c up regulation results were also significantly correlated with IgE serum levels. BAT was normal in all controls and CF patients without ABPA. Among CF patients with probable ABPA, there was a statistically significant temporal correlation of BAT levels with final development of the disease, by Cox regression. As a conclusion, BAT was found to be predictive of ABPA development and could be a candidate additional criterion to ABPA diagnosis.