S-RC-6

PSEUDOMONAS AERUGINOSA PYOCYANIN (PYO) DOWN-REGULATES INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) EXPRESSION IN HUMAN BRONCHUS

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Pseudomonas aeruginosa (PA) is a problematic but common respiratory pathogen in cystic fibrosis and bronchiectasis. Chronic infection with PA is responsible for considerable morbidity among these patients. PA produces PYO, which is a phenazine pigment that causes slowing of ciliary beating and disruption of respiratory epithelium. It has also been reported recently that iNOS down-regulation occurs in the airways of patients with cystic fibrosis although the cause of this remains obscured. We have therefore investigated the effect of PYO on iNOS expression in human bronchial organ cultures. Paired endobronchial biopsies were collected from 11 otherwise healthy subjects, who underwent bronchoscopy for suspected bronchial carcinoma, for incubation in FAD medium with either additional *PYO* or control medium for *16h* at *37°C* and *5% CO2*. *iNOS* immunostaining was performed on 3μm paraffin sections and intracellular expression of iNOS was quantified on the immunostained sections with computer image analysis. The mean ± SD intensity of iNOS expression in the control and test intraepithelial, endothelial, and submucosal cells were 84.62± 32.15 & 120.79± 25.33 (p=0.003); 193± 59 & 130± 63 vessesl/mm2 (p=0.04); and 169± 63 & 137± 150cells/mm2 (p=0.37) respectively. Our results indicate that PA pyocyanin down-regulates iNOS expression in bronchial epithelial and submucosal vessels. Further studies should follow to evaluate the clinical and pathological implications of our findings.

S-RI-1

B CELL APOPTOSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS Adrian Wu, Albert Chan, Stanley Chik, CS Lau. Department of Medicine, University of Hong Kong.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder characterized by the formation of autoantibodies against nuclear antigens. Apoptosis plays an important role in peripheral tolerance to self-antigens. Mice inheriting the *lpr* and *gld* mutations develop autoimmune syndromes and excessive production of many of the antibody specificities associated with human SLE. These animals also develop nephritis, and has uncontrolled proliferation of B cells. Recent studies revealed that the *lpr* and *gld* genes encode the apoptosis-inducing cell surface molecules Fas and Fas ligand (FasL), respectively. This study hopes to identify possible defects in apoptosis during B cell activation in patients with SLE.

We identified patients who fulfilled the 1982 ACR criteria for SLE. These patients were clinically inactive at the time of the study and on low-dose immunosuppression only. Age-matched healthy subjects were recruited as controls. PBMCs were separated from 40 ml of peripheral blood and B cells were isolated by negative selection using the MACS magnetic cell sorting system. B cell purity after isolation ranges from 35 to 55%. Apoptosis and Fas (CD95) expression of freshly isolated B cells was evaluated by double staining with anti-CD19 antibody and Annexin V-FITC conjugate or anti-CD95 antibody respectively, followed by FACS analysis. B cells were incubated overnight with an agonist anti-CD95 antibody. These cells were analyzed for apoptosis by FACS the following morning. Peripheral B cells were cultured in the presence of anti-CD40 antibody, with or without IL4. Cell surface Fas expression was evaluated after 48 hours. Anti-Fas antibody was added after 48 hours, and the cells were incubated overnight before FACS analysis for apoptosis. We have analysed 15 patients and 8 controls. The percentage of freshly isolated B cells staining positive with annexin V is low (<5%), and they express low level of Fas. There is no significant difference between patients and controls. After overnight incubation, the percentage of B cells undergoing spontaneous apoptosis ranged from 35 to 70%. The addition of anti-Fas antibody did not significantly change the percentage of cells undergoing apoptosis in patients or controls. B cell expression of Fas increased after 48-hour incubation with anti-CD40 antibody to the same extent in patients and controls. However, almost all B cells underwent spontaneous apoptosis in vitro after 64 hours of incubation without stimulation. The presence of anti-CD40 and IL4 reduced the percentage of apoptotic cells to the same extent in patients and controls. The addition of anti-Fas after 48 hours did not change the percentage of apoptotic cells. Data so far does not suggest the presence of defects in B cell apoptosis in patients with inactive SLE. B cells rapidly undergo apoptosis in vitro without stimulation, and anti-Fas antibody did not affect the rate of apoptosis in patients or controls. Preliminary experiments have shown that a combination of anti-IgM, anti-CD40 and IL-4 can stimulate B cell proliferation in vitro. We will further look into Fas-mediated apoptosis of these activated B cells from SLE patients.