P2-22 | Imaging changes and immune-checkpoint expression on T cells in bronchoalveolar lavage fluid from patients with pulmonary sarcoidosis

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Background and Aims: Sarcoidosis is a systemic granulomatous disease caused by unknown immunological abnormalities. The organs most vulnerable to sarcoidosis are the lungs. Patients often resolve spontaneously, but the lungs can also be severely affected. Although details regarding prognostic factors in sarcoidosis patients with lung involvement remain unclear, several reports have suggested that immune checkpoint molecules are involved in the pathogenesis of sarcoidosis.

Methods: We enrolled 23 patients who were newly diagnosed with pulmonary sarcoidosis between March 2017 and August 2018 at Kyushu University Hospital. We analyzed 11 patients who were subjected to bronchoalveolar lavage fluid (BALF) collection and who were subsequently followed up by chest computed tomography (CT) imaging 3 to 9 months after the diagnosis. All patients analyzed were clinically asymptomatic or mildly ill and did not receive systemic steroids during the follow-up. We divided patients into two groups (improved or unimproved) based on chest CT findings. We then compared immune checkpoint molecules (PD-1, PD-L1, TIM-3, TIGIT, and LAG-3) expressed on T cells in BALF between the two groups, using flow cytometry.

Results: We found elevated PD-1 or TIM-3 expression on T cells in BALF in patients with spontaneous improvement on CT findings compared with those in patients without improvement on CT findings. Expression levels of PD-L1, TIGIT, LAG-3 expression on T cells in BALF were not different between the groups.

Conclusion: Our study implies that PD-1 or TIM-3 expression on T cells in BALF may be a prognostic factor for pulmonary lesions in sarcoidosis.

P2-23 | Hypercapnia accelerates adipogenesis: A novel role of high CO2 in exacerbating obesity

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Obesity is a major risk factor for the development of obstructive sleep apnea (OSA) and obesity hypoventilation syndrome (OHS), which manifest as intermittent hypercapnia and sustained intermitted hypercapnia, respectively. In this study, we investigated whether CO2 affects adipocyte differentiation (adipogenesis) and maturation (hypertrophy). Human visceral or subcutaneous preadipocytes were grown to confluence and then induced to differentiate to adipocytes under hypocapnia, normocapnia, and hypercapnia with or without hypoxia. Adipogenesis was also induced under intermittent or sustained hypercapnia. Differentiated adipocytes were maintained to maturity under normocapnia or hypercapnia. Our main findings are as follows: (1) hypercapnia accelerated adipogenesis in visceral and subcutaneous preadipocytes whereas hypocapnia inhibited adipogenesis; (2) hypercapnia did not affect adipocyte hypertrophy; (3) hypercapnia-accelerated adipogenesis was independent of extracellular acidosis, oxygen concentration, or either intermittent or sustained exposure to high CO2; (4) the mechanisms underlying hypercapnia-accelerated adipogenesis involved increased production of cyclic AMP via soluble adenyl cyclase, leading to the activation of protein kinase A and exchanger protein directly activated by cyclic AMP, which in turn activated pro-adipogenic transcription factors, such as cyclic AMP response element binding protein, CCAA T/enhancer binding protein b and peroxisome proliferator-activated receptor g. This study reveals a novel role of high CO2 in promoting adipogenesis, which provides mechanistic clues to a pathoetiological interaction between OSA/OHS and obesity. Our data suggest a vicious cycle of disease progression via the following mechanism: OSA/OHS® hyperventilation® hypercapnia® increased adipogenesis® increased fat mass® exacerbated OSA/OHS.

P2-24 | Ways to tackle the highly transmissible delta virus

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Delta virus is a newly widespread mutant that shows increase resistance to most vaccine due primarily to the mutation of spike protein. Epidemiological study, wearing mask, limitation of gathering, social distancing and city closure may help. Herd immunity can be achieved by more than 70% population being vaccinated. However, the virus mutates again during the vaccine production, reducing the effectiveness of vaccine. So preventing the receptor binding by neutralizing antibody is fruitful to reduce the mortality of critically ill patients. However, convalescent serum is limited in supply and is not in a concentrated form. To manufacture SARS-CoV-2 neutralizing mAbs, we first isolate antibodies from convalescent patients, immunized animals or phage-displayed human antibody libraries. Then they are amplified using germline genes. Mass industrial production through stable cell lines can be employed in the treatment. Hollow fiber bioreactor allows the production of high concentration of antibodies. The hybridoma cells are grown in fresh medium, which is changed regularly to reduce the accumulation of toxic by-products. By centrifugation, ion exchange chromatography column, and electrophoresis, purified
antibodies can be obtained. Humanized mAbs have the advantages of being less immunogenic, more effective and have a longer half-life than chimeric antibodies (11-24 days). As COVID attacks not only the lung but also the neurological system, this makes antibody-conjugated nanoparticles particularly useful as it can penetrate into various tissue better, e.g. photothermal nanoparticles conjugated with neutralizing antibodies can inactivate SARS-CoV-2 on shining light of wavelength 500-850nm. The traditional hybridoma techniques in mouse also work.

P2-25 | Iron mediates cigarette smoke-induced ACE2 expression in bronchial epithelial cells

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Background: Compared to non-smokers, current smokers were found to be at an increased risk of developing symptomatic coronavirus disease 2019 (COVID-19) owing to their increased airway expression of angiotensin-converting enzyme 2 (ACE2), which is the entry receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Moreover, accumulating evidence suggests that cigarette smoke (CS) induces accumulation of iron in bronchial epithelial cells. Iron metabolism has also been recently implicated in viral infection. However, despite this evidence, the precise relationship between SARS-CoV-2 infection and iron metabolism has yet to be completely understood.

Aim: In this study, we aimed to determine whether iron metabolism was involved in CS-induced ACE2 expression in bronchial epithelial cells.

Methods: We used deferoxamine (DFO) as an iron chelator to assess the role of iron metabolism in CS-induced ACE2 expression.

Results: We demonstrated that CS did induce mRNA expressions of ACE2 and type II transmembrane serine protease (TMPRSS2), another entry receptor for SARS-CoV-2, in bronchial epithelial cells. Furthermore, the administration of DFO reduced CS-induced ACE2 mRNA and protein expressions in these cells.

Conclusions: Our findings indicated that iron metabolism mediated CS-induced ACE2 expression, suggesting a novel mechanism for increased susceptibility to SARS-CoV-2 infection in smokers.

P2-26 | Effect of lycopene on lung aging and oxidative stress in senescence-accelerated mouse P1 strain

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Background and Aims: Senescence-accelerated mouse P1 strain (SAMP1) is an established animal model for investigating lung oxidative stress, inflammation, and aging, since SAMP1 shows an accelerated senescence after normal development and maturation. Lycopene is a potent antioxidant and has been shown to reduce the risk of chronic diseases associated with oxidative stress. However, the effect of lycopene on lung oxidative stress and aging remains elusive. We hypothesized that treatment with lycopene improves oxidative stress and accelerated aging in SAMP1.

Methods: SAMP1 mice (n=8) were fed with a commercial chow and received either lycopene solution (0.05 mg/mL) or plain water for a year. Body weights and aging scores of mice were measured every month. After the bronchoalveolar lavage fluid (BALF) was collected, all mice were sacrificed at 12-months of age. Structural changes and oxidative stress of the lungs were assessed by measuring mean linear intercept (MLI) and reactive oxygen species (ROS) in the lungs homogenate, respectively.

Results and Conclusions: Treatment with lycopene significantly attenuated increase of total cell counts of BALF and ROS in the lungs, suggesting improvement of lung inflammation and oxidative stress. Lycopene supplementation resulted in attenuation of MLI increases and improved aging score. Our results indicate that oxidative stress has some role in the accelerated aging process in SAMP1 and that lycopene might have a protective effect against lung oxidative stress and aging.

P2-27 | Identification of inhibitors targeting heat shock protein 47 for the development of anti-fibrotic therapeutics

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Background and Aims: Idiopathic pulmonary fibrosis is a chronic progressive fibrotic disease and has an extremely poor prognosis. Although the anti-fibrotic drugs including nintedanib and pirfenidone inhibit the progress of disease, effective treatment is limited and the development of curative therapeutics is required. Heat shock protein 47 (HSP47) is a collagen specific molecular chaperone, which is essential for collagen synthesis and secretion and causally related to fibrotic disease. The identification of compounds that inhibit the HSP47-collagen interaction is major first step for the development of anti-fibrotic therapeutics. We aim to identify HSP47 inhibitors as novel therapeutics for pulmonary fibrosis.