Pro-fibrotic M2 macrophage markers may increase the risk for COVID19 in type 2 diabetes with obesity

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To the Editor:

Diabetes and obesity are associated with severe COVID19-associated disease including acute respiratory distress syndrome (ARDS). Alveolar macrophage-derived cytokines contribute to the inflammation underlying ARDS, resulting in pulmonary fibrosis and edema, central facets of acute lung injury. Plasma lipopolysaccharide (LPS), elevated in obesity, is the key component for activation of M1 and a subtype of M2 macrophages (1). Chronic M2 macrophage activation leads to profibrotic mediator production, Transforming Growth Factor- β (TGF-β) and Platelet-derived Growth Factor (PDGF) for example, that enhance continuous fibroblast activation and myofibroblast proliferation (2). Lung alveolar M2 macrophages also produce matrix metalloproteinases, MMP7 and MMP9, and their overexpression promotes fibrogenesis.

Here, we hypothesize that alveolar M2 macrophages are activated in response to elevated plasma LPS in obese subjects with T2D (OT2D), resulting in excess pro-fibrotic inflammatory mediators, and thereby making OT2D patients more vulnerable to COVID19-related infection with severe disease. To test our hypothesis, we measured circulatory lung alveolar M2 macrophage markers in obese subjects with type 2 diabetes (OT2D) and controls.

A parallel study was performed in the Diabetes Research Centre at Hull Royal Infirmary in adults with type 2 diabetes (n=23) and nondiabetic controls (n=23). The male to female ratio of the subjects was similar between cohorts (12 males and 11 females in both control and OT2D cohorts). The OT2D subjects were older (62±7 vs 55±10 years, OT2D vs control, $p<0.0001$) with a higher BMI (32±4 vs 28±3 kg/m^2, OT2D vs control, $p<0.0001$). All

participants were Caucasian and in the fasting state for 10-hours before venipuncture. Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement (3) was used to determine LPS-binding protein (LPB), TGFβ-1, PDGF-β, MMP7 and MMP9 protein concentration expressed as relative fluorescent units (RFU). Statistical analysis was performed using the Student's t test (GraphPad Prism 8.0, San Diego, CA).

LPS-binding (LPB) protein was reduced in plasma (85311.3±1453.1 vs 91746.9±3047.9 RFU, OT2D vs control, $p<0.05$). Plasma LPB was also reduced in obese versus non-obese subjects regardless of diabetic status (84343.5±1455.7 vs 91398.5±2798.8 RFU, obese vs non-obese, $p<0.05$). Basal levels of TGFβ-1, PDGF-β, MMP7 and MMP9 were significantly higher in OT2D versus control: TGFβ-1 (1122.9±72 vs 932.8±27.1 RFU, $p<0.01$); PDGF-β (40610.0±5853.6 vs 25129.0±3271.6 RFU, $p<0.05$); MMP7 (1241.5±93.4 vs 1004.6±42.0 RFU, $p<0.05$) and MMP9 (30192.7±3745.9 vs 19532.3±1562.4 RFU, $p<0.05$).

We report here that LPS-related markers were associated with activated lung alveolar M2 macrophages in OT2D, with a reduction in plasma LPB as a surrogate marker of circulatory LPS elevation. Previously increased LBP levels were reported in obesity (4, 5), a discrepancy compared to our observations might be due to inclusion of obese cases who were smokers and alcoholic in those studies. LBP was increased in the bronchoalveolar lavage fluid (BALF) of smokers (6). Serum levels of LBP are also increased in heavy drinkers, probably reflecting high LPS exposure due to alcohol-induced damage of the gastrointestinal barrier (7, 8) However, in our study none of the OT2D subjects were smokers or consumed alcohol, as those were exclusion criteria. Moreover, LBP, the serum glycoprotein, plays a concentration-dependent dual role in determining LPS-induced macrophage activation; low concentrations of LBP enhance the LPS-induced activation of mononuclear cells (MNC), whereas the acute-phase rise in LBP concentration inhibits LPS-induced cellular stimulation (9). Furthermore, LBP is bound and internalized by host cells and colocalizes with LPS in the

cytoplasm (10). Therefore, the significantly lower LBP levels reflect the elevated LPS levels in OT2D subjects in our study.

The elevated TGF-β1 shown here may predispose to alveolar pre-fibrosis with their collapse following SARS-CoV-2 infection. TGF-β is detected in lung bronchoalveolar lavage fluid of 90% of patients with ARDS, major cellular sources of TGF-β in pulmonary fibrosis being alveolar macrophages and metaplastic type II alveolar epithelial cells. Activation of TGF-β1 is affected by MMP9 that was elevated here in OT2D, contributing to enhancement of the pool of active TGF-β1. MMP9 also weakens the airway epithelia barrier function by altering transepithelial electrical conductance and epithelial permeability to macromolecules (11). MMP7, also elevated here, is increased in ARDS and associated with idiopathic pulmonary fibrosis (IPF), whilst PDGF-β, again elevated here, contributes to fibrosis development with TGF-β in ARDS. Elevated plasma levels of TGF-β1, PDGF-β, MMP7 and MMP9 determined early in the course of COVID19 infection in a patient with OT2D may indicate potential risk for more severe disease. One limitation of this study is that all participants were Caucasian, and the results may not be generalizable to other ethnic groups. Moreover, it is also possible that, apart from alveolar macrophages, other cellular sources, for example lung epithelial cells (12), arterial smooth muscle cells (13), epithelial cells of glandular tissues like prostate (14) or bile duct epithelia (15), might contribute to LPS-induced elevated plasma levels of those pro-fibrotic markers.

In conclusion, in OT2D the lung epithelial barrier integrity is likely destabilized in response to fibroproliferative activity of elevated TGF-β1, PDGF-β, MMP7 or MMP9 derived from lung alveolar macrophages, increasing vulnerability to inhaled pathogens. This might lead to irreversible structural alterations and tissue stiffening in the lungs of OT2D patients even prior to SARS-COV-2 infection and thereby make these patients more vulnerable to COVID19-related infection with severe disease.

DECLARATIONS

Ethics approval and consent to participate: The Yorkshire and Humber Research Ethics Committee approved this study. All patients gave written informed consent.

Consent for publication: All authors gave their consent for publication.

Availability of data and materials: All the data for this study will be made available upon reasonable request to the corresponding author.

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Author contributions

ASMM and AEB analyzed the data and wrote the manuscript. TS supervised clinical studies and edited the manuscript. SLA contributed to study design, data interpretation and the writing of the manuscript. All authors reviewed and approved the final version of the manuscript. Alexandra E Butler is the guarantor of this work.

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