opioids can cross from maternal to fetal circulation, the mechanisms underlying these adverse outcomes remain poorly defined. This study aims to uncover OUD-associated immunological changes in maternal and fetal circulation. METHODS/STUDY POPULATION: To study the effect of maternal OUD on the maternal immune system at delivery, we collected maternal blood samples at delivery from healthy pregnant women (controls) and pregnant women with a diagnosis of severe OUD. To study the impact of maternal OUD on newborn immunity, we also collected umbilical cord blood (UCB) at delivery. Flow cytometry was used to determine the frequency and phenotype of circulating immune cell subsets and responses to stimulation. Isolated monocytes were stimulated with bacterial/viral agonist cocktails, while T and NK cells were stimulated with PMA/ionomycin. The impact of maternal OUD on circulating immune mediators was determined by Luminex and on monocyte activation markers sCD14 and CRP by ELISA. RESULTS/ANTICIPATED RESULTS: In maternal circulation, OUD was associated with a significant decrease in markers of inflammation, cell proliferation, and activation. Frequencies of immune cell subsets were impacted by OUD, shown by an expansion of CD8+ EMRA T cells, marginal-zone B cells, mDCs, non-classical monocytes, and CD16low NK cells. While no differences were seen in T and NK cell responses to stimulation, monocytes and pDCs had significantly lower responses to bacterial and viral agonist stimulation. Analysis of UCB revealed increased levels of pro-apoptotic/T cell exhaustion mediators and pro-inflammatory cytokines, albeit decreased levels of several chemokines and growth factors. The UCB immune landscape is altered with maternal OUD, as demonstrated by a shift from naive to memory CD8+ T cells and a decrease in pDC frequency. DISCUSSION/SIGNIFICANCE: OUD dampens maternal peripheral immunity, possibly contributing to poor placental function or premature/delayed labor. Monocytes and pDCs lack antimicrobial functionality, suggesting increased infection susceptibility with OUD. Finally, these implications extend to the fetal compartment, shown by heightened immune activation in UCB.

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Dual TGFβR1/MAP4K4 inhibitor reduces kidney injury in a mouse model of renal fibrosis.

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OBJECTIVES/GOALS: Renal fibrosis is a critical pathophysiological event in chronic kidney diseases. Our goal is to determine the ability of dual-inhibitor of transforming growth factor beta receptor 1 (TGFα²R1) and mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4), TK850, on reducing kidney fibrosis. METHODS/STUDY POPULATION: To test the renal anti-fibrotic action ofdual TK850,8-10-week-old male and female C57BL/6mice with unilateral ureteral obstruction (UUO) induced kidney fibrosis were used. Mice were separated into 3 groups: group 1 contained mice that had UUO surgery (UUO control), group 2 contained mice prophylactically treated with TK850 thatstarted 7 days prior to UUO(UUO-P,20 mpk/d/ip), and group 3 contained mice interventionally treated with TK850 that started3 days after UUO(UUO-I,20 mpk/d/ip). Ten days following UUO the kidneys and blood were collected for analysis. Renal fibrosis was assessed from hydroxyproline content (measure of collagen)and histological collagen analysis using Picrosirius red stain. RESULTS/ANTICIPATED RESULTS: Renal hydroxyproline was increased equally in the UUO kidney of male (5.4 ± 0.41 μg/10mg, n=5) and female mice (5.5 ± 0.50 μg/10mg, n=5) compared to the contralateral control kidney (2.9 ± 0.14 μg/10mg, n=10). TK850 treatment in UUO-P mice (n=10, 3.4 ± 0.24 μg/10mg) and UUO-I mice (4.30 ± 0.20 μg/10mg, n=10) had significantly reduced hydroxyproline levels. Histopathological evaluation revealed that kidney injury increased collagen deposition in the UUO kidney (17.1 ± 0.43% collagen positive area, n=10) compared to the control kidney (2.0 ± 0.23%, n=10). TK850 treatment in UUO-P mice significantly attenuated collagen deposition (10.5 ± 0.38%, n=10), while UUO-I had significantly reduced collagen deposition as well (13.1 ± 0.25%, n=10). DISCUSSION/SIGNIFICANCE: Taken together, these results validate the dual TGFβR1/MAP4K4 inhibitor, TK850 as a potential therapeutic to mitigate renal fibrosis and supports the emergence of a combinational pharmacotherapeutic approach for multi-factorial kidney diseases.

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Investigation of the Epidemiological Differences associated with Post Acute Sequelae of Sars-CoV-2 infection

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OBJECTIVES/GOALS: In this work investigating the epidemiological differences associated with Post Acute Sequelae of SARS-CoV-2(PASC) patho genesis we willassess the sex differences inocular viral persistence and the immunologic profile in tear film obtained from COVID-19 patients. METHODS/STUDY POPULATION: Participants will be enrolled from the NIH funded RECOVER Consortium at the 15 adult hubs in the US and will include those with acute COVID-19 (n=250), followed up at baselineto 48 weeks post infection. RT-PCR will be used to detect viral RNA and electro chemiluminescence assays will be used to detect IgG, IgA1 and IgA2 antibodies.Tear film antibody titers will be measured longitudinally in all participants to assess the kinetics of the immune responses in those who developed PASC and those who did not. Tear film antibody titers will be correlated with antibody titers in the blood and compared between those individuals with or without measurable viral RNA in tear film. RESULTS/ANTICIPATED RESULTS: Logistic regression models will be used assess the association of viral persistence with PASC status controlling for relevant covariates. Linear mixed regression models will be used to assess the association of IgA1/IgA2 with PASC status. We expect to observe delayed clearance of viral RNA and elevation in SARS-CoV-2 specific IgA2/IgA1 in the tear film of patients with PASC compared with those without PASC. Given evidence of increased PASC risk in women we expect to observe higher rates of SARS-CoV-2 ocular viral persistence and higher SARS-CoV-2 specific IgA2/IgA1 ratios in women with COVID-19 when compared to men with COVID-19. DISCUSSION/SIGNIFICANCE: There is concern that PASC will pose a major global health challenge given the scale of the Covid-19 pandemicand the patho genesis remains unclear. This work is highly likely to improve our understanding of the mechanisms of PASC and the reasons why women are more vulnerable to this condition.

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