Methods: LLC-PK1 cells were exposed to FK506 with necrox-7, and cell viability was measured. Western blotting and RT-PCR analyses evaluated protein or gene expression of MDA, HO-1, Bcl-2, Bax, caspase-3, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), kidney injury molecule-1 (KIM-1), toll-like receptor-4 (TLR-4), and high mobility group box 1 protein (HMGB1) expression were assessed. The number of apoptotic cells was measured using an annexin V/PI staining with flow cytometry.

Results: Reduction in cell viability by 50mM FK506 was ameliorated significantly by cotreatment with necrox-7. MDA, KIM-1, IL-6, TNF-α, BAX, caspase-3, TLR-4 and HMGB1, increased markedly in LLC-PK1 cells treated with FK506 and significantly decreased after cotreatment with necrox-7. HO-1 and Bcl-2 significantly increased in LLC-PK1 cells treated with FK506 after cotreatment with necrox-7. Moreover, flow cytometry assay showed that apoptotic cell death was increased by FK506 treatment, whereas it was significantly decreased after cotreatment with necrox-7.

Conclusions: These results collectively provide therapeutic evidence that necrox-7 ameliorates the FK506-induced renal damage via antioxidant effect and inhibiting apoptosis and inflammation.

CITATION INFORMATION: Jang H. NecroX-7 Ameliorate FK506-Induced Nephrotoxicity in LLC-PK1 Cells (Pig Kidney Epithelial Cells) AJT, Volume 22, Supplement 3

DISCLOSURES: H.J. Jang: None.

Abstract# C83

Immunomodulatory Effects of Probiotic Bifidobacterium Bifidum with Tacrolimus and Sirolimus in Mouse Skin Graft Model.

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Purpose: Accumulating evidence suggests that gut microbiota actively crosstalks with the host immune system, and alterations of gut microbiota may exert immune regulatory effects. We aimed to identify probiotic strains capable of modulating the immune response in a mouse allogeneic skin graft model to evaluate its potential as a therapeutic adjunct in the transplantation field

Methods: Tail skin of BALB/c mice was grafted to the back of C57BL6 mice. Recipient mice were treated with one of five probiotic strains (Lactobacillus lactis, Lactobacillus fermentum, Lactobacillus rhamnosus, Bifidobacterium bifidum, and Lactobacillus reuteri) alone or in conjunction with tacrolimus or sirolimus, and survival of the skin grafts was compared to the control groups with either no medication, tacrolimus without probiotics, or sirolimus without probiotics. The experiment was repeated for selected probiotic strains that showed immunomodulatory effects for quantitative assessment of cytokines using qRT-PCR.

Results: Co-administration of B. bifidum with tacrolimus significantly improved skin allograft survival when compared to either the no medication group (mean 17 days vs. 11.7 days, $p<0.001$ by log-rank test) or to the tacrolimus only group (mean 17 days vs. 12.7 days, $p<0.001$ by log-rank test). B. bifidum also demonstrated synergistic survival improvement when administered with sirolimus (mean 15.6 days; $p=0.019$ and $p=0.036$ when compared to the control group and the sirolimus only group, respectively). Skin grafts from recipients treated with B. bifidum and tacrolimus or B. bifidum and sirolimus showed significantly increased expression of anti-inflammatory cytokine, IL-10. Expression of proinflammatory cytokine, IL-6 was also markedly inhibited in B. bifidum + sirolimus group.

Conclusions: B. bifidum promoted allogeneic skin graft survival in mice synergistically with tacrolimus or sirolimus, possibly through induction of IL-10 production. Our study suggests that such a synergistic effect of B. bifidum may be applicable as an adjunct to conventional immunosuppressive therapy.

CITATION INFORMATION: Han A., Chung C., Kim H., Ko H., Jo E., Min S., Kim S., Park H., Ha J. Immunomodulatory Effects of Probiotic Bifidobacterium Bifidum with Tacrolimus and Sirolimus in Mouse Skin Graft Model AJT, Volume 22, Supplement 3

DISCLOSURES: A. Han: None. **C. Chung:** None. **H. Kim:** None. **H. Ko:** None. **E. Jo:** None. **S. Min:** None. **S. Kim:** None. **H. Park:** None. **J. Ha:** None.

Abstract# C84

Immunoglobulin M (IgM) Inhibits Replication of SARS-CoV-2 In Vero E6 Cells In Vitro and Delays Disease in K18-hACE2 Mice Infected with SARSCoV-2 In Vivo.

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Purpose: To determine if IgM has a direct effect in preventing SARS-CoV-2 replication in Vero E6 cells, and delaying or preventing disease in infected K18-hACE2 mice.

Methods: 1) Vero E6 cells, grown to confluence in 12 well plates, were used to test the effect of IgM in reducing the number of plaque-forming units (PFU).There were 4 groups: a) 25PFU WA-1 SARS-CoV-2 was combined with 20, 5 or 0.8µg IgM in growth medium, and incubated for 1hr in a final volume of 500ul. 100µl was added to Vero E6 cells in replicate wells and incubated for 1hr; b) 100µl of 20, 5 or 0.8µg IgM was added to Vero E6 cells and incubated. Media was aspirated and the cells were then inoculated with 25PFU WA-1 and incubated for 1hr; c) Virus control - as above, but with no IgM; d) No virus or IgM. FBS growth medium containing Avicel was overlain in the wells and incubated for 48 hours. Virus replication was stopped by incubating with 10% buffered formalin. Following removal of formalin, plates were stained with Giemsa violet, dried, and photographed. 2) A COVID -19 Spike-ACE2 binding assay kit was used to determine if IgM (2ug, 4.5ug, 20ug, 45ug IgM) inhibits the interaction between the Spike-receptor binding domain (S-RBD) and Angiotensin I ConvertingEnzyme 2 (ACE2) receptor. 3) K18-hACE2 mice were divided into 3 groups based on treatment regimen; Group 1: with IgM, No virus; 2: with Saline, with virus; 3: with IgM, with virus. 35ug IgM was injected intraperitoneal in a single dose, 2 days prior to infection. Mice were innoculated intranasally with 1250 pfu of HK SARS-CoV-2.

Results: 1) Exposure of 25PFU SARS-CoV-2 to IgM (at all concentrations) prior to incubation with Vero E6 cells, inhibited its replication in Vero E6 cells. When Vero E6 cells were incubated with IgM prior to infection, no plaques were seen in wells with 20ug and 5ug IgM but were observed in wells with 0.8ug IgM. Plaques were also observed in the Virus alone group, but none were seen in the 'No IgM-No virus' group. 2) 45ug IgM/100uls inhibited the binding of S-RBD to ACE2 by ~94-100%, 20ug IgM/100uls inhibited it by ~80%, and 2 or 4.5ug/100ul by ~70-75%. Control without IgM did not inhibit the S-RBD─ACE-2 binding. 3) Pretreatment with a single low dose IgM injection delayed weight loss and mortality.

Conclusions: IgM inhibits the replication of SARS-CoV-2 in Vero cells in vitro. It also inhibits the interaction between S-RBD that is present on the viral surface and the ACE2 receptor, by binding to S-RBD. A single low dose of IgM given prechallenge delayed disease in infected mice. The discovery that IgM interferes with the formation of the S-RBD─ACE2 complex, and that a single low dose can delay disease, indicates its translational potential as a vaccine/therapeutic to prevent or treat COVID-19.

CITATION INFORMATION: Chhabra P., Mann B., Ma M., Feldman S., Brayman K. Immunoglobulin M (IgM) Inhibits Replication of SARS-CoV-2 In Vero E6 Cells In Vitro and Delays Disease in K18-hACE2 Mice Infected with SARSCoV-2 In Vivo AJT, Volume 22, Supplement 3

DISCLOSURES: P. Chhabra: None. **B. Mann:** None. **M. Ma:** None. **S. Feldman:** None. **K.L. Brayman:** None.

Abstract# C85

Adenosine A2A Receptor (A2AR) Agonist Apadenoson Promotes Survival in K28-hACE2 Mice and Syrian Hamsters Infected with Sars-CoV-2.

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Purpose: To determine if Apadenoson or Regadenoson has a therapeutic effect in attenuating hyper-inflammation and improving survival rate in K18-hACE2mice or Syrian hamsters infected with SARS-CoV-2.

Methods: 6-8 weeks old male K18-hACE2mice were divided into Control group that received vehicle; Test group 1 that received the drug (Apadenoson or Regadenoson) 24hrs prior to challenge with SARS-CoV-2; and Test Group 2 (Drug-delay), that received the drug with a 5 hr delay post-viral infection (n=6/grp). Viral dose was 1250 PfuHong Kong/VM20001061/2020 delivered via intranasal route. Drug was delivered subcutaneously using 1007D ALZET pumps. 6 weeks old Syrian hamsters were divided into Control group that received Vehicle and Virus (n=4) and 2 test groups (n=5/group) that received Apadenoson+Virus and Regadenoson+Virus. Drugs were delivered by 2ML2 ALZET pumps (4ug/kg/hr). Hamsters were inoculated intratracheally with 750PFU SARS-CoV-2 WA1 strain prior to treatment. Mice