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Lung Transplant Recipients with SARS-CoV-2 Infection Induce Circulating Exosomes with SARS-CoV-2 Spike Protein S2 Which Are Immunogenic in Mice

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Purpose: Exosomes are nanosized vesicles released by cells into body fluids. We have demonstrated the presence of circulating exosomes containing viral antigens in lung transplant recipients (LTxR) undergoing rejection. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an important risk factor for LTxR undergoing immunosuppression. Our goal is to determine whether exosomes with SARS-CoV-2 spike protein are induced in LTxR with SARS-CoV-2 infection and exosomes are immunogenic in mice, inducing immune responses to the spike protein

Methods: We analyzed 67 patients with SARS-CoV-2 infection for the induction of circulating exosomes with SARS-CoV-2 spike protein. Exosomes were isolated from plasma by an exosome precipitation protocol followed by 0.2 micron filtration and size determination by NanoSight300. Exosomes were first analyzed by western blot with specific antibodies to SARS-CoV-2 spike and its nucleoprotein. Eluted proteins from the gel were analyzed by mass spectrometry. Exosomes were subjected to transmission electron microscopy (TEM) to detect spike and nucleocapsid antigens. To determine the immunogenicity of isolated exosomes, C57BL/6 mice were immunized with exosomes carrying SARS-CoV-2 spike protein.

Results: Exosomes from SARS-CoV-2 infected LTxR expressed SARS-CoV-2 spike protein S2 and increased levels of RNA related to SARS-CoV-2. Peptides specific for SARS-CoV-2 spike protein in exosomes were confirmed by mass spectroscopy. TEM also revealed the expression of spike protein and nucleocapsid antigens on the exosome surface. Mice immunized with exosomes carrying the spike protein, developed antibodies to SARS-CoV-2 spike antigens. Severe inflammation and lesions were also demonstrated in the lungs of mice immunized with exosomes carrying SARS-CoV-2 spike protein. Splenic lymphocytes from mice immunized with exosomes carrying SARS-CoV-2 spike antigen also demonstrated increased frequency of T-cells which are spike protein antigen specific and secreting IFN-γ and TNF-α .

Conclusion: SARS-CoV-2 infected LTxR induce circulating exosomes with spike protein and nucleic acids related to SARS-CoV-2. Since the induced exosomes are highly immunogenic, we propose that the exosomes induced by SARS-CoV-2 will have immunological consequences relevant to the COVID19 disease process.

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Analysis of Humoral and Cellular Immunity of Lung Transplant Recipients Following SARS-CoV-2 Infection and BNT162b2 mRNA Vaccination

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Purpose: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in lung transplant recipients (LTxR) under immunosuppression carries higher risk with 14-39% mortality. Immune responses of LTxR under immunosuppression following SARS-CoV-2 infection or vaccination remains unknown. Our goal is to determine the humoral and cellular immunity to SARS-CoV-2 in LTxR with infection and following vaccination.

Methods: We performed a single center analysis to determine immune responses of LTxR with infection and following BNT162b2 mRNA vaccination. The results were compared with controls (non-transplant individuals). ELISA was developed to determine the antibody (Ab) concentration (IgG) to SARS-CoV-2 spike (CSP) and nucleocapsid (CNP) antigens. PBMCs from LTxR were isolated by ficoll-hypaque centrifugation to determining the frequency of cells secreting IFNγ and TNFα to CSP and CNP by ELISpot.

Results: Concentration of Abs developed and T-cell frequencies secreting TNFα and IFNγ against CSP and CNP in LTxR and controls are given in **Table 1**. Infected LTxR and controls developed Abs to both CSP and CNP. In contrast, vaccinated LTxR induced 10 fold less Abs to CSP in comparison to control. Frequencies of cells secreting TNFα for both CSP and CNP were significantly reduced in LTxR with infection. However, vaccination of both LTxR and control induced similar levels of TNFα secreting cells upon stimulation with both CSP and CNP. It is of interest that frequency of IFNγ producing cells against both CSP and CNP were significantly higher in LTxR in comparison to control.

Conclusion: Infection with SARS-CoV-2 in LTxR and controls produced comparable levels of Abs both against CSP and CNP. However, vaccinated LTxR didn't induce significant levels of Abs against CSP. Frequency of T-cells, secreting IFNγ were significantly increased by vaccination in LTxR and in controls suggesting that T cell responses against SARS-CoV-2 has been induced in LTxR by mRNA vaccine**.**

Antibody and T-cell responses in SARS-CoV-2 infected/vaccinated healthy controls and LTxR

Antibody Response	SARS-CoV-2 Infected (ng/ml)		Pfizer-BioNTech BNT162b2 mRNA vaccine (ng/ml)	
	LTxR (n=50)	Controls (n=7)	LTxR (n=100)	Controls (n=20)
SARS-CoV-2 Spike	4982.93±1784.97	3073.65±104.64	284.07±772.99	3613.27±896.17
SARS-CoV-2 Nucleocapsid	21.44±20.02	25.82±12.39	3.79+3.02	5.66±3.00
T-cell Response: SARS-CoV-2 Spike Antigen	**Spots per Million**		**Spots per Million**	
	LTxR (n=13)	**Controls (n=7)**	**LTxR (n=50)**	**Controls (n=20)**
TNF-alpha	903.51±227.37	5394.8±801.32	2782.90±561.78	4160.60±910.81
IFN-gamma	617.47±220.87	31.81±63.12	269.12±189.96	120.80±163.91
SARS-CoV-2 Nucleocapsid Antigen				
TNF-alpha	342.56±53.06	8925.98±788.81	5981.59±64.03	5374.61±407.32
IFN-gamma	422.21±96.60	30.14±4.98	180.70±71.33	41.19±8.04

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Immunogenicity of Two Doses of ChAdOx1 nCoV-19 Vaccine in Lung Transplant Recipients

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Purpose: Lung transplant recipients (LTR) are at higher risk to develop severe SARS-CoV2 pneumonia, due to the immunosuppressive regimen, which further hampers their immune response to vaccination. Indeed, is has been shown that LTR mount weak antibody response after SARS-CoV2 mRNA vaccination. Nevertheless, the immunogenicity of ChAdOx1 nCoV-19 vaccine has not yet been studied in LTR.