Safety and Immunogenicity of Adjuvanted Recombinant Subunit Herpes Zoster Vaccine in Lung Transplant Recipients

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Supporting Information

METHODS

Inclusion and exclusion criteria

We enrolled the first 50 patients who were willing to participate, had received a single- or double lung transplant and were age ≥50 years. Detailed inclusion and exclusion criteria are available in the Supporting information. Patients were eligible if they were ≥ 90 days post-transplant, had positive varicella zoster IgG antibodies before transplantation, and were able to provide informed consent. We excluded patients who had treatment for allograft rejection during the past 30 days, had treatment with rituximab in the preceding six months or were anticipated to receive rituximab, had treatment with intravenous immunoglobulins (IVIG) in the preceding 30 days or were anticipated to receive IVIG, had plasmapheresis in the preceding 30 days or were anticipated to have plasmapheresis, had a history of a severe allergic reaction (anaphylactic reaction) after any vaccine, had a HZ episode during the past 12 months, had ongoing CMV viremia (≥ 200 IU/mL), were HIV positive, had a congenital immunodeficiency, had a febrile illness during the past 7 days or were unable to comply with the study protocol. The study protocol did not interdict the administration of other non-VZV vaccines during the study period. The institutional research ethics board approved the study (#17-6247).

Assessment of anti-gE antibody avidity

The antibody avidity describes the strength with which an antibody binds to an antigen. Diethylamine (DEA) is a mild protein-denaturing agent. If DEA is added to antibody-antigen mixture antibodies of low avidity (antibodies with week binding to antigens) are more likely to dissociate from the antibody-antigen complexes than those of higher avidity; this effect can be used for determining the antibody avidity which is expressed as avidity index (AI). Avidity index (AI), expressed in percentage, was calculated as the result of OD of wells washed with

PBS- diethylamine (DEA+), divided by the OD of wells washed with PBS (DEA-), and multiplied by 100, based on the formula; AI = OD (DEA+) / OD (DEA-) × 100 (high values indicate highly avid antibodies).

Assessment of cell mediated immune response

In order to assess the cell mediated immune response we modified previously described methods [1,2]. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll gradient centrifugation (GE Healthcare Life Science, Issaquah, WA, USA) and cryopreserved in fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) with 10% dimethyl sulfoxide (DMSO) (Fisher BioReagents, Thermo Fisher Scientific). After thawing and washing twice, a target count of $1x10^6$ (and a minimum of $5x10^5$) viable PBMCs were added to RPMI media plus L-glutamine (Wisent Bioproducts, Quebec, Canada) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin sulfate, 1% MEM nonessential amino acids, 100 mM sodium pyruvate, 50 mM 2-mercaptoethanol (all Gibco, Thermo Fisher Scientific, Waltham, MA, USA) in 96-well plates. The PBMC were stimulated with a pool of 153 15-mer peptides (1.25 ug/mL each, overlapping by 11 amino acids) which span the entire VZV gE protein (JPT Peptide Technologies, Berlin, Germany) in combination with the BD FastImmuneTM anti-human CD28/CD49d co-stimulatory reagent (BD Biosciences, Mississauga, ON, Canada) for 2 hours, before addition Brefeldin A (1 µg/mL; BioLegend, San Diego, CA) and incubation for an additional 18 hours at 37°C. Positive (PMA/Ionomycin, eBioscience, Thermo Fisher Scientific, Waltham, MA, USA) and negative controls (unstimulated) were included in each assay. Following incubation, cells were washed and stained with the Zombie Aqua™ viability dye (BioLegend, San Diego, CA). After Fc receptor blocking using Human BD Fc Block (BD Biosciences, Mississauga, ON, Canada), cells were incubated with a cell-surface staining cocktail consisting of mouse anti-human CD45 (clone HI30)-PerCP/Cy5.5 (Biolegend, San

Diego, CA), mouse anti-human CD3 (clone OKT3)-BV786 mouse anti-human CD4 (clone RPA-T4)-Pacific Blue and mouse anti-human CD8 (clone SK1)-APC-Cy7 (all BD Biosciences, Mississauga, ON, Canada). Following fixation (Fixation Buffer, Biolegend, San Diego, CA) and permeabilization (Intracellular Staining Permeabilization Wash Buffer, Biolegend, San Diego, CA), intracellular staining with mouse anti-human interferon (IFN)-γ (clone B27)-FITC, mouse anti-human tumor-necrosis factor (TNF)-α (clone MAb11)-PE-Cy7, rat anti-human interleukin (IL)-2 (clone MQ1-17H12)-APC and mouse anti-human CD154 (CD40L) (clone 24-31)-PE (all BD Biosciences, Mississauga, ON, Canada) was performed. Flow cytometry was done on a BD LSR II (BD Biosciences, Mississauga, ON, Canada) cytometer at the SickKids-UHN Flow and Mass Cytometry Facility (Toronto, Ontario, Canada) with a target event count of 100,000 live, CD45+ cells. Data was analyzed using FlowJo software v10 (FlowJo LLC, Ashland, OR, USA). To account for background cytokine production, the frequency of cytokine-producing T-cells obtained in the unstimulated specimen was subtracted from the frequency obtained in the stimulated specimen. Cell mediated immune responses were expressed as the frequency of viable CD4 T-cells expressing two or more activation markers ($CD4^{2+}$ T-cells) among tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin-2 (IL-2), and CD40 ligand (CD40L) per 106 CD4+ T-cells as previously established by others [3-10].

Grading solicited adverse events

Redness at injection site: *Grade 1* if diameter < 3cm

Grade 2 if diameter 3-6 cm

Grade 3 if diameter >6 cm

Swelling at injection site: *Grade 1* if diameter < 3cm

Grade 2 if diameter 3-6 cm

Grade 3 if diameter >6 cm

Symptoms (Pain at injection site, myalgia, fatigue, headache):

Grade 1 if classified as mild by the patient

Grade 2 if classified as moderate by the patient

Grade 3 if classified as severe by the patient

RESULTS

Table E1 Unsolicited adverse events

Adverse event	Days after last RZV dose	Treatment	Vaccine related
Lower respiratory tract infection (*E. coli*)	116	Antibiotic treatment (outpatient setting)	Not related
Aspiration pneumonia (pathogen unknown)	77	Antibiotic treatment (outpatient setting)	Not related
Invasive pulmonary aspergillosis (*Aspergillus ustus*)	30	Antifungal treatment (outpatient setting)	Not related
CMV infection (asymptomatic replication)	15	Antiviral treatment (outpatient setting)	Not related
Gastroenteritis (pathogen unknown)	88	No specific treatment	Not related
Lower respiratory tract infection (*E. coli*)	4	Antibiotic treatment (outpatient setting)	Not related
Traveler's diarrhea (southeast Asia, pathogen unknown)	75	No specific treatment	Not related
Agranulocytosis	29	G-CSF treatment	Not related
Clostridium difficile infection	6	Antibiotic treatment (outpatient setting)	Not related
Impetigo contagiosa (pathogen unknown)	19	Antibiotic treatment (outpatient setting)	Not related
CMV infection (asymptomatic replication)	14	Antiviral treatment (outpatient setting)	Not related
Shingles (localized)	2	Antiviral treatment (outpatient setting)	Not related
Severe adverse event	Days after last RZV dose	Treatment	Vaccine related
Lower respiratory tract infection (pathogen unknown)	15	Antibiotic treatment (hospital admission)	Not related
Skin cancer (melanoma in situ)	12	Surgical resection	Not related
Shingles (disseminated)	32	Antiviral treatment (hospital admission)	Not related
Allograft rejection (clinically diagnosed)	89	Methylprednisone (hospital admission)	Not related
Cellular rejection grade A1 (histologically proven)	17	Methylprednisone (hospital admission)	Possibly related
Cellular rejection grade A1 (histologically proven)	90	Methylprednisone (hospital admission)	Not related
CMV infection (viral syndrome)	71	Antiviral treatment (hospital admission)	Not related
Abdominal aortic aneurysm	16	Endovascular repair (hospital admission)	Not related
Lower respiratory tract infection (pathogen unknown)	45	Antibiotic treatment (hospital admission)	Not related
Allograft rejection (clinically diagnosed)	130	Anti-thymocyte globulin treatment (hospital admission)	Not related
Congestive heart failure	25	Optimizing cardiac medication (hospital admission)	Not related
Allograft dysfunction (unknown etiology, histologically not compatible with rejection)	92	Methylprednisone (stopped after histological result was available; hospital admission)	Not related
Bacteremia (*Pseudomonas aeruginosa)*	125	Antibiotic treatment (hospital admission)	Not related
Influenza B infection (resulting in death)	139	Antiviral treatment (hospital admission)	Not related

Supplementary References

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