

viral load ( $<16,000$  copies/ $10^5$ ); Group 3 ( $n=4$ ) stable, high viral load ( $>16,000$  copies/ $10^5$ ); Group 4 ( $n=4$ ) history of PTLD, no detectable/low viral load. Flow cytometric analysis with HLA-A2 or -B8 tetramer (TMR) probes was performed on peripheral blood. The polarization of EBV-specific CD8+T cells (IFN- $\gamma$ /IL-5/IL-10) was assessed by ELISPOT or ELISA.

**Results:** Overall, the "lytic" specific CD8+T cells were more frequent than the "latent" ones and displayed distinct memory phenotypes (Effector Memory vs Central Memory). In addition, higher EBV loads triggered higher frequencies of TMR+ cells ( $G2 = G3 \gg G1$ ), while patients with history of PTLD (G4) maintained high TMR+ frequencies. Interestingly, although patients in groups G2, G3 and G4 had high frequencies of "lytic" TMR+ cells ( $1.3 \pm 2\%$  vs  $2.3 \pm 3.4\%$  vs  $1.1 \pm 0.9\%$ ), G2 and G4 exhibited higher frequencies of IFN- $\gamma$  producing cells ( $30 \pm 30$  and  $32 \pm 23$  spots/ $10^5$ ), suggesting functional EBV-memory CD8+T cells, while G3 displayed impaired IFN- $\gamma$  ( $19 \pm 31$  spots/ $10^5$ ), indicative of functional exhaustion. Although EBV stimulation triggered preponderantly IFN- $\gamma$ , the IFN- $\gamma$ /IL-5 ratio was lower in all patients (2.5:1) as compared to adult controls (5:1) yet another unique feature of immune responses in pediatric patients. CD8+T cells in G3 produced higher levels of IL-10 as compared to other groups.

**Conclusion:** These results demonstrate significant differences in EBV-specific memory CD8+T cells from pediatric HTx patients based on their EBV clinical/viral load status. The functional impairment of CD8+T cells from G3 patients might be either a direct result of chronic EBV challenge, or might be due to biased polarization (intermediate IL-5) or to Treg development (high IL-10).

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### Chronic Rhinoviral Infection in Lung Transplant Recipients

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**Background:** *Rhinovirus* is the most frequent respiratory virus circulating in the community. Lung transplant recipients with viral respiratory tract infections are at risk of complications and protracted diseases.

**Objective:** To describe lung transplant recipients chronically infected by *rhinovirus*.

**Methods:** We first identified an index case and confirmed by sequencing viral isolates that he was chronically infected by *rhinovirus*. Then we conducted a prospective study to assess the incidence and the potential clinical impact of chronic rhinoviral infections in a cohort of 68 lung transplant recipients. Sequence analysis of viral isolates (all cases) as well immunochemistry on lung biopsies (in one case) have been performed.

**Results:** We describe 3 lung transplant recipients chronically infected by *rhinovirus* over a period of one year. *Rhinovirus* was mainly identified by RT-PCR but full virions were also isolated repeatedly in one case. The persistence of a unique strain was confirmed by the analysis of the 5' NCR and VP1 genes sequences and ruled-out re-infections. All cases presented lower respiratory symptoms as well as graft dysfunctions, 2 had repeated acute rejections episodes, and 2 died. In one case that failed to produce neutralizing antibodies we also showed the presence of *rhinovirus* within the lower respiratory tract parenchyma. Over a period of 19 months rhinoviral infections, screened in broncho-laveolar lavages, were documented in approximately 15% of cases; one fifth of them presented a persistent infection.

**Conclusions:** In lung transplant recipients with graft dysfunction we have documented that rhinoviral infection can be persistent. *Rhinovirus* was detected in the lung parenchyma in one case. Our investigation suggests that *rhinovirus* contributed to the graft dysfunctions.

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### Withdrawn

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### Clinical Features Associated with *Coronavirus* Infections: A Prospective and Hospital-based Study

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**Background:** Human-coronaviruses (HCoV) are the most frequent cause of upper respiratory infections after rhinoviruses. HCoV are also associated with lower respiratory tract symptoms and protracted disease in subjects at risk. Until recently only HCoV-OC43 and 229E were known in humans. The impact of the recently discovered HCoV-NL63 and HKU1 in hospitalized adults needs to be established.

**Objectives:** To assess the clinical impact of the 4 human HCoVs in hospitalized patients.

**Methods:** All patients of our university hospitals who needed a bronchoalveolar-lavage (BAL) for an acute respiratory event were enrolled. BAL specimens were screened by RT-PCR for the presence of HCoV-OC43, 229E, NL63 and HKU1 and ten other respiratory viruses (*influenza A-B*, *respiratory syncytial virus A-B*, *parainfluenza 1-2-3*, *human-rhinovirus*, *enterovirus* and *human-metapneumovirus*) as well as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Legionella pneumophila*.

**Results:** There were 539 cases enrolled in the study and 29 (5.4%) had an HCoV identified in the BAL specimen. HCoV-OC43 was the most frequent (n = 12) followed by HCoV-229E (n = 7), HCoV-NL63 (n = 6) and HCoV-HKU1 (n = 4). The median age was 47 years and 69% of the cases were male. Transplantation was the most frequent underlying diseases and only 28% patients were not considered as immunosuppressed. The microbiological cultures of the BAL were negative for bacteria in 69% cases and no other respiratory viruses or herpes were identified. Nine patients (31%) were in the intensive care unit at the time of the BAL procedure (eight of them on mechanical ventilation). Twenty-one (72%) patients presented acute respiratory symptoms, sixteen (55%) had cough and sputum, and 13 (45%) dyspnea. A new chest X-ray infiltrate was documented in 18 patients (62%) at the time of the BAL procedure. Fifteen (52%) patients were already treated for a respiratory infection at the time of the BAL. The most frequent final diagnosis was a lower respiratory tract infection that remained of unspecified etiology in one third of cases.

**Conclusion:** All four HCoVs are recovered in bronchoalveolar lavages of hospitalized patients. Our analysis suggests that they contributed to respiratory symptoms in most cases.

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### Analysis of the Cytomegalovirus (CMV) gB Vaccine with MF 59 Adjuvant (CMVgB) in CMV Seronegative Patients with Renal Failure on a Transplant List

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**Background:** Previous studies have shown that the live attenuated Towne CMV vaccine improved graft survival in CMV seronegative renal transplant recipients of CMV seropositive donor organs. However,

the Towne vaccine is not available and recombinant vaccines are being developed. Trials have demonstrated that recombinant glycoprotein B CMV vaccine (CMVgB) when combined with the adjuvant MF 59 is immunogenic and safe in normal individuals.

**Objective:** To assess the safety and immunogenicity of the CMVgB vaccine in immunocompromised individuals with renal failure on dialysis, who are awaiting renal transplantation.

**Methods:** A multicenter, randomized, placebo-controlled trial in CMV seronegative patients with renal failure on transplant lists. Randomization was 2:2:1 with doses of CMVgB vaccine at either 20 mcg, 100 mcg or placebo. Injections were administered by intramuscular injection at 0, 1 and 6 months post enrollment. Sera were obtained at enrollment, and 1, 1.5, 3, 6, 6.5, 8, and 12 months. Patients transplanted post enrollment were followed but did not continue immunization. Sera were tested for antibody to CMVgB by ELISA, and for neutralizing antibody to CMV. Only a limited set of sera were tested before the trial was halted by the sponsor due to slow enrollment.

**Results:** 46 patients were enrolled: 18 received 20 mcg per dose, 19 received 100 mcg per dose and 9 received placebo. Patients were well matched for degree of renal failure and age. Renal transplantation occurred in 14 patients. Neutralizing antibody was transiently present in 1 of 5 patients who received 3 doses of the 20 mcg dose. In 5 patients who received 3 doses at 100 mcg, neutralizing antibody was detected in 3 of 5 at titers that ranged from 35 to 57. Two patients in the 100 mcg dose group developed CMV syndromes post transplantation both received only 2 doses of vaccine prior to transplantation. A neutralizing antibody titer of 248 was detected 4 months after infection in one subject, the other was not tested. No significant adverse events were noted with injections in any subject.

**Conclusions:** The CMVgB vaccine was safe. Some evidence of neutralizing antibody could be detected in patients at the 100 mcg dose. Two patients who did not complete the 3 dose series developed CMV infection and syndrome. Future studies may need to incorporate larger doses of vaccine or an improved adjuvant or both.