Bidirectional transfer of human cytomegalovirus strains in donor and recipient seropositive lung transplant patients

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Abstract

Donor and recipient HCMV-seropositive (D+R+) lung transplant recipients (LTRs) often harbour multiple human cytomegalovirus (HCMV) strains, likely due to transmitted donor (D) strains and reactivated recipient (R) strains. To date, the extent and timely occurrence of each likely source in shaping the post-transplantation (post-Tx) strain population is unknown. Here, we deciphered the D and R origin of the post-Tx HCMV strain composition in blood, bronchoalveolar lavage (BAL), and CD45+ BAL cell subsets. We investigated either D and/or R formalin-fixed paraffin-embedded blocks or fresh D lung tissue from four D+R+ LTRs obtained prior to transplantation. HCMV strains were characterised by short amplicon deep sequencing. In two LTRs, we show that the transplanted lung is reseeded by R strains within the first six months after transplantation, likely by infiltrating CD14+ CD163+-/ alveolar macrophages. In three LTRs, we demonstrate both rapid D-strain dissemination and persistence in the transplanted lung for \textgreater 1 year post-Tx. Broad inter-host diversity contrasts with intra-host genotype sequence stability upon transmission, during follow-up and across compartments. In D+R+ LTRs, HCMV strains of both, D and R origin can emerge first and dominate long-term in subsequent episodes of infection, suggesting no replication advantage of one source over the other despite pre-existing R strain-specific immunity.

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Figure 1. HCMV genotypes present in pre-Tx donor (D) and recipient (R) lung tissue and post-Tx emergence in D+R+ LTR. (A) HCMV DNA loads in pre-Tx D and R lung FFPE sections, in post-Tx follow-up plasma and BAL samples, and in the cellular subsets of the BAL. Red circles indicate samples that underwent HCMV genotyping. Grey bars show the period under antiviral therapy. (B) CD4+ BAL cells were further sorted for CD163 and CD14 expression, distinguishing between CD14+CD163+ (left upper gate), CD14+CD163+ (right upper gate) and CD14+ cells (left lower gate). Red squares indicate HCMV-DNA positive cell subsets. (C) Schematic illustration of the D and R FFPE blocks with the number of sections investigated. HCMV DNA positive slides are dark green and red circles indicate the slides positive for HCMV genotyping. (D) Relative frequency of the genotype-specific reads in the indicated post-Tx samples. BAL, bronchoalveolar lavage, LTR, lung transplant recipient, FFPE, formalin-fixed paraffin-embedded, Tx, transplantation; Ul, unique long; gO, glycoprotein O; d, day.
Figure 2. HCMV genotypes present in pre-Tx recipient lung tissue and post-Tx emergence in D+R+ LTR. (A) HCMV DNA loads in R pre-Tx lung FFPE sections, in post-Tx follow-up plasma and BAL samples, and with the cellular subsets of the BAL. Red circles indicate samples that underwent HCMV genotyping. Grey bars show the period under antiviral therapy. (B) CD4+ BAL cells were further sorted for CD163 and CD14 expression, distinguishing between CD14+/CD163- (left upper gate), CD14+/CD163+ (right upper gate) and CD14- cells (left lower gate). Red squares indicate HCMV-DNA positive cell subsets. (C) Schematic illustration of the D and R FFPE blocks with number of sections investigated. HCMV-DNA positive slides are dark green and red circles indicate the slides positive for HCMV genotyping. (D) Relative frequency of the genotype-specific reads in the indicated post-Tx samples.

BAL, bronchoalveolar lavage; LTR, lung transplant recipient; FFPE, formalin-fixed paraffin-embedded; Tx, transplantation; UL, unique long; gh, gD, glycoprotein H; d, day; recipient, R, donor, D.
Figure 3. Temporal emergence and abundance of donor-derived genotypes in an HCMV-seropositive recipient post-Tx. (A) HCMV DNA loads in pre-Tx donor lung tissue, and in post-Tx plasma and BAL samples. Red circles indicate samples that underwent HCMV genotyping. Grey bars show the period under antiviral therapy. (B) Schematic illustration of the HCMV DNA positive lung tissue sections taken from the middle lobe of the donor lung before transplantation. (C) Relative frequency of the genotype-specific reads in the indicated post-Tx samples. The HCMV genotypes detected in the donor lung tissues pre-Tx are the sum of those detected in the cells and of those found in the 1x PBS storage buffer (supernatant, SN) and are therefore shown without relative frequencies. BAL, bronchoalveolar lavage; LTR, lung transplant recipient; PBS, phosphate buffered saline; Tx, transplantation; UL, unique long; g16, glycoprotein N d, day.
Figure 4. Temporal emergence and abundance of donor-derived genotypes in an HCMV-seropositive recipient post-Tx. (A) HCMV DNA loads in pre-Tx donor lung tissue, and in post-Tx plasma and BAL samples. Red circles indicate samples that underwent HCMV genotyping. Grey bars show the period under antiviral therapy. (B) Schematic illustration of the HCMV DNA-positive lung tissue sections taken from the middle lobe of the donor lung before transplantation. (C) Relative frequency of the genotype-specific reads in the indicated post-Tx samples. The HCMV genotypes detected in the donor lung tissues pre-Tx are the sum of those detected in the cells and of those found in the 1x PBS storage buffer (supernatant, SN) and are therefore shown without relative frequencies. BAL, bronchoalveolar lavage; LTR, lung transplant recipient; PBS, phosphate buffered saline; Tx, transplantation; UL, unique long; gH, gL, glycoprotein H, glycoprotein L.
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