Cell

Temporal Response of the Human Virome to Immunosuppression and Antiviral Therapy

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SUMMARY

There are few substantive methods to measure the health of the immune system, and the connection between immune strength and the viral component of the microbiome is poorly understood. Organ transplant recipients are treated with posttransplant therapies that combine immunosuppressive and antiviral drugs, offering a window into the effects of immune modulation on the virome. We used sequencing of cell-free DNA in plasma to investigate drug-virome interactions in a cohort of organ transplant recipients (656 samples, 96 patients) and find that antivirals and immunosuppressants strongly affect the structure of the virome in plasma. We observe marked virome compositional dynamics at the onset of the therapy and find that the total viral load increases with immunosuppression, whereas the bacterial component of the microbiome remains largely unaffected. The data provide insight into the relationship between the human virome, the state of the immune system, and the effects of pharmacological treatment and offer a potential application of the virome state to predict immunocompetence.

INTRODUCTION

The human microbiome is now recognized as an important component of human health (Turnbaugh et al., 2007). Community-level analyses have shed light on factors that shape the structure of the bacterial component of the microbiome, such as age (Yatsunenko et al., 2012), diet (De Filippo et al., 2010; Muegge et al., 2011), geographical location (Yatsunenko et al., 2012), antibiotic treatment (Jakobsson et al., 2010), and disease (Clemente et al., 2012). The viral component of the microbiome, the human virome, remains relatively understudied (Wylie et al., 2012) and little is known about the effects of immune modulation and antiviral therapies on virome composition. It was previously shown that the healthy gut virome remains remarkably stable over time (Reyes et al., 2010), and that the predominant source of variation is due to differences between subjects, although an association between diet and the virome composition was found (Minot et al., 2011). Here, we study the dynamic response of the human virome in plasma to antiviral drugs and strong perturbations of the immune system as experienced by organ transplant recipients.

Immunosuppressive therapies significantly reduce the risk of graft rejection in organ transplantation but increase the susceptibility of recipients to infections (Fishman, 2007). Infections with viral pathogens, in particular the herpesvirus cytomegalovirus (CMV), occur frequently and increase the recipient's risk of graft failure (Fishman et al., 2007). Organ transplant recipients are therefore frequently subjected to antiviral prophylactic or preemptive therapies directed against CMV (Slifkin et al., 2004). The inverse relationship between the level of immunosuppression and the risks of infection and rejection leaves only a narrow therapeutic window available for patient treatment (see Figure 1A). Posttransplant care is further complicated by numerous limitations of the currently available methods for the diagnosis of infection and rejection. Diagnosis of rejection mostly relies on invasive biopsies that suffer from interobserver variability, high cost, and patient discomfort (Marboe et al., 2005; Saraiva et al., 2011; Snyder et al., 2011). Diagnosis of infections is challenging given the fact that the symptoms of infection are diminished following immunosuppression (Fishman, 2007) and commonly used diagnostic methods, such as antigen-detection and PCR-based molecular tests, rely on a specific target and therefore an a priori hypothesis for the source of the infection. As a final complication, patient-to-patient variability in the sensitivity to immunosuppressive drugs can give rise to over- and underimmunosuppression, increasing the risk of infection or rejection, respectively (Budde et al., 2011; Wieland et al., 2010).

In this work, we sequenced cell-free DNA circulating in plasma to investigate drug-microbiome interactions following organ transplantation. We studied the patterns of infection in heart and lung transplant recipients subjected to a combination of

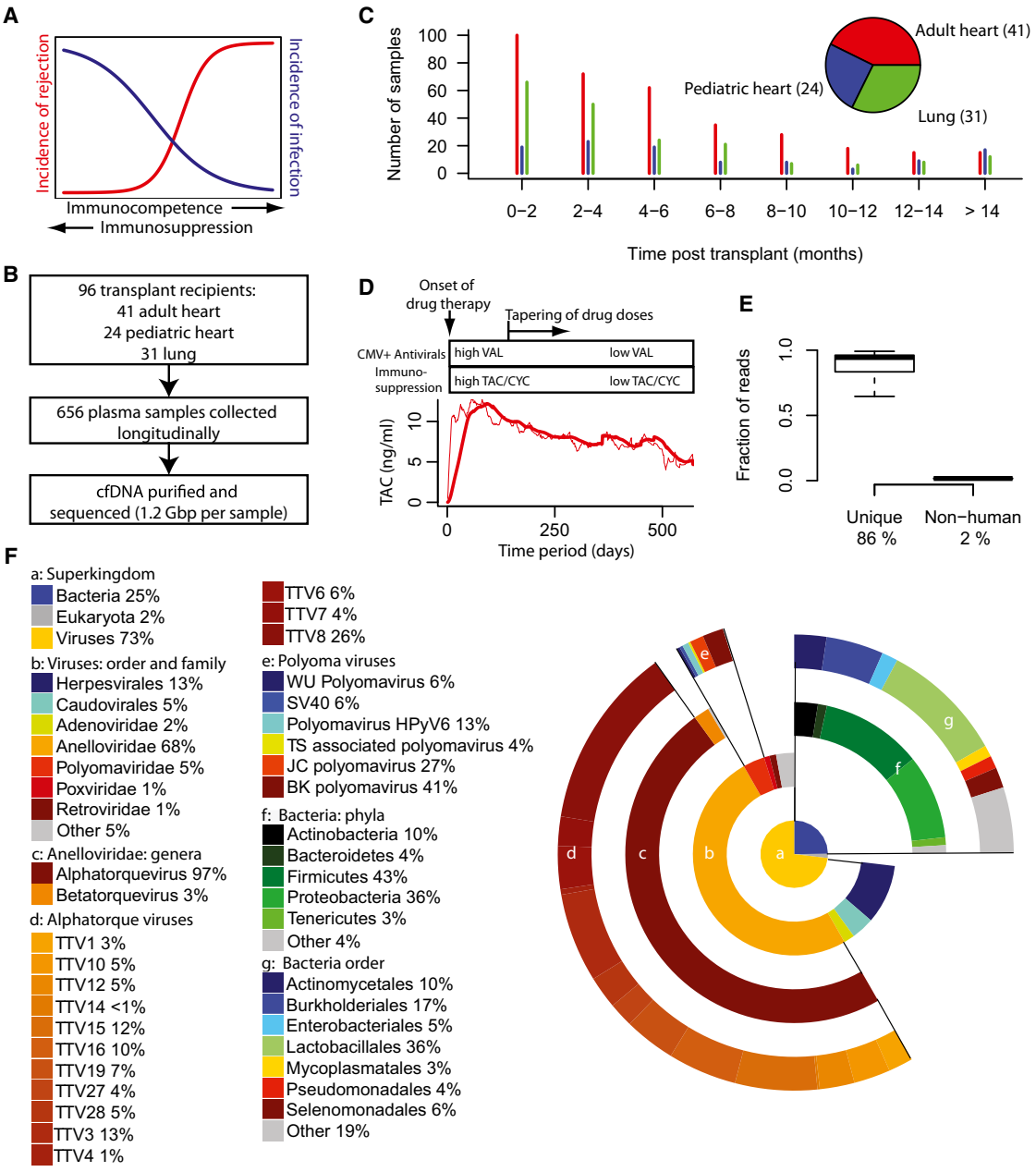

Figure 1. Study Design, Read Statistics, and Phylogenetic Distribution

(A) Immunosuppression reduces the risk of rejection in transplantation but increases the risk of infection.

(B) Design of study: 656 plasma samples were collected, cell-free DNA was purified and sequenced to an average depth of 1.25 Gbp per sample.

(C) Number of samples collected as function of time for the different patient groups part of the study.

(D) Treatment protocol for patients in the study cohort, all patients are treated with maintenance immunosuppression (tacrolimus-based [TAC] for adult heart and lung transplant recipients and cyclosporine [CYC] for pediatric patients). CMV positive (donor or recipient, CMV+) transplant cases are treated with anti-CMV prophylaxis, valganciclovir (VAL). Mean level of tacrolimus measured in blood of transplant recipients treated with a TAC-based protocol (dashed line actual, solid line window average filter).

(E) Fraction of reads that remain after filtering of lower quality and duplicate reads (mean 86%, left) and after removal of human and low-complexity reads (mean 2%, right).

(F) Relative genomic abundance at different levels of taxonomic classification after removal of human reads (average over all samples from all organ transplant recipients [n = 656]). The central pie chart shows the composition at the superkingdom level of classification. Lower levels of classification are shown in donut charts with progressively larger radius.

See also Figure S1.