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Introduction. Human cytomegalovirus (HCMV) is the leading cause of congenital infection resulting in severe morbidity and mortality among infected newborns worldwide; however, mechanisms and virulence factors contributing to HCMV pathogenesis and particular clinical outcomes remain unclear. To successfully establish a persistent infection, the virus must have evolved multiple mechanisms to avoid host immune recognition. For example, HCMV infected cells exhibit remarkable resistance to natural killer (NK) cell-mediated cytolysis via encoding a large set of immunomodulatory proteins. Furthermore, HCMV demonstrates an exceptionally high degree of variability. Against this background, our aim was to determine whether and to what extent the differences in genetic composition affects viral fitness, its ability to modulate NK response, and clinical outcome.

Materials and Methods. For this purpose, we enrolled a cohort of 21 pediatric patients with confirmed HCMV congenital infection. We evaluated the degree of genetic polymorphism of HCMV clinical strains by next generation sequences (NGS), primarily focusing on viral genes known to encode proteins with potent NK immunomodulatory functions. In parallel, we ran an extensive *in vitro* analysis of all clinical isolates to characterize viral growth properties in different cellular models.

Results. Here we report extraordinary genetic and phenotypic diversity of the clinical isolates, reflected in both viral growth properties and ability to modulate NK cells. Growth analysis of HCMV clinical isolates revealed different patterns of replication and dissemination. Although we observed no difference in cell tropism, the fast-replicative isolates form a unique morphological pattern. The analysis of NK cell activating ligands at both RNA and protein level, demonstrated that HCMV clinical strains affect NK ligands to different degrees. For instance, all viruses downmodulate ULBP2/5/6 ligand, with the strongest effect observed with the phenotypically more aggressive isolates. Moreover, there was a strong up-regulation of PVR observed with fast-replicative isolates. Conversely, other NK ligands, such as MICA, MICB, ULBP3 and B7-H6, were not modulated by any of the HCMV isolates. Finally, to assess whether HCMV isolates affect NK recognition, we analyzed IFN-gamma production. Likewise, we observed a great variation between clinical strains, indicating that the genetic variability actually reflects variability in functionality.

Discussion and Conclusions. Overall, our study contributes to understanding the impact of viral genetic variability on viral fitness and immune system modulation, with the ultimate goal to identify valuable markers for the management of congenitally infected newborns.

Keywords: Human cytomegalovirus (HCMV), congenital infection, clinical isolates, genetic variability, viral phenotypes, immunomodulation, NK ligands.