TITLE: Investigating the Genomic Basis of Severe Life-Threatening Viral Infections in Immunocompetent Children.

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BACKGROUND

Over the last decade, a number of immunocompetent children have presented to our center with multi-organ failure due to severe cytokine storm triggered by viral infections. These diseases are associated with poor outcomes, in part due to a lack of understanding regarding the genetic basis of the disease. The causative viruses responsible for this severe presentation are typically the Epstein-Barr Virus (EBV) and, less commonly, cytomegalovirus (CMV).

EBV is a human herpesvirus that causes a spectrum of disease ranging from a mild viral syndrome to life-threatening malignant disease. By adulthood, 90-95% of individuals are infected with EBV, with primary EBV infection frequently occurring in childhood (Cohen et al. 2011). While the majority of EBV infections are asymptomatic or cause a transient, self-limited viral illness, rare individuals develop malignancies with significant associated morbidity and mortality. While the vast majority of these cancers affect B-cells, a spectrum of life-threatening EBV-associated T-cell lymphoproliferative disorders (LPD's) has recently been identified, causing a severe clinical presentation secondary to cytokine storm. With few exceptions (e.g. as with X-linked Lymphoproliferative Disorder), it is unclear what underlies this rare susceptibility to uncontrolled EBV-mediated lymphoid proliferation.

EBV-associated T-cell LPD differs from other EBV-associated malignancies in that it occurs during or immediately following primary EBV infection, whereas others typically occur in latently affected cells long after primary infection (Sevilla et al. 2011). It has been shown that EBV-infected T-cells demonstrate clonal integration of the EBV viral genome and rearrangement of the T-cell receptor genes (Campo et al. 2011). These cells develop the ability to

evade the host immune system by down-regulation of viral proteins and, subsequently, reduced antigenicity (Kimura 2006). Even so, the vast majority of hosts are able to overcome the infection and it is unclear why a small fraction of individuals cannot. There is a significantly higher prevalence of this disease among Asian and Native American individuals (Kimura et al. 2003), raising suspicion for the presence of an underlying genetic immunodeficiency in these patients that is not yet recognized.

CMV is another human herpesvirus that typically affects immunosuppressed hosts, but has been noted in rare cases to cause similarly severe clinical presentations characterized by cytokine storm in immunocompetent or only mildly immunosuppressed patients. CMV disease has a variety of manifestations, including retinitis, pneumonitis, hepatosplenic disease, and, in rare cases, hemophagocytic lymphohistiocytosis (HLH). CMV viremia occurs in 13.6% of patients with lymphoid malignancies who do not undergo transplantation (Han 2007), but severe, life-threatening disease has been reported only twice in the literature (Rahbarimanesh et al. 2015). A similar patient presented recently to our center with severe multi-organ damage due to this same process. We aim to establish the genetic basis for the rare presentation of this patient (and any future patients who may present) with virus-associated cytokine storm using genetic sequencing.

Through the use of whole-exome sequencing (WES), we now have the ability to sequence a patient's genetic information to help establish a diagnosis. The goal of this study is to use WES to identify previously unrecognized immunodeficiency-inducing germline mutations in patients who develop severe viral disease out of proportion to the perceived degree of their immune function. The discovery of such genes and further examination of their function and activity will

enable deeper understanding into the pathogenesis of virus-associated cytokine syndrome, ultimately allowing for improved screening, counseling, treatment, and clinical decision-making.

STUDY OBJECTIVE

The objective of our study is to identify new candidate genes that confer a predisposition to severe cytokine storm in the setting of viral infection. The aim of this project is to determine why, in a small minority of patients, common viruses such as EBV and CMV can lead to a spectrum of life-threatening disease including T-cell lymphoproliferation, lymphoma, and hemophagocytic lymphohystiocytosis, in many cases progressing rapidly to multi-organ failure and, ultimately, death.

STUDY METHODS

General Design

We will prospectively perform genetic sequencing techniques on patients diagnosed with viral infection-associated cytokine storm in order to identify potential constitutional genetic alterations that cause underlying immunodeficiency in this population. For those patients who are newly diagnosed, consent and diagnostic samples will be obtained prospectively.

Inclusion criteria: Patients, ages 0-30, who were treated at our center from 2000 to the present time, who fit any of the following descriptions:

1. Immunocompetent patients presenting with severe organ dysfunction secondary to viral-associated hemophagocytic lymphohistiocytosis (HLH)
2. Immunocompetent patients with recurrent episodes of EBV or CMV infections

3. Patients with lymphoid malignancies, who have not undergone allogeneic hematopoietic cell transplantation, who develop severe cytokine storm in the setting of viral infection

Exclusion criteria:

1. Patients with HIV
2. Patients with history of allogeneic hematopoietic cell transplant
3. Patients with solid organ transplant-related lymphoproliferative disorder
4. Patients with known primary immunodeficiency

Description of study subjects and method of recruitment

Patients followed by CUMC physicians (age 0-30 years) who meet the inclusion criteria listed below will be eligible for this study. Participants will be recruited by Pediatrics Hematology-Oncology staff, Genetic Counselors, Medical Genetics staff and Genetic Counselors. The target accrual for this study is 15 subjects. Ten patients who meet criteria have already been identified by the pediatric oncology department. For these patients, and any others identified who meet inclusion criteria, families will be contacted by phone or at the time of a routine clinic visit and invited to participate.

Patients who meet the inclusion criteria for the study will be enrolled after consent is obtained. Blood samples for genetic testing will be obtained from both patients and parents when possible. In some cases, leftover surgical tissue may be available from prior biopsies; if sufficient in quantity, this tissue will be used to sequence the patient's constitutional DNA and will obviate the need to draw blood from these patients. We will aim to use leftover tissue samples stored in

the Department of Surgical Pathology whenever possible. Although parents' genomes will be sequenced as well, parents are not considered research subjects because their samples are simply being used as reference sequences in order to assess the subjects' genomes. Parental samples are essential in differentiating between heritable and de novo genetic mutations.

For participants > 5 years of age, 4 cc of blood will be obtained; for those < 5 years, only 2 cc of blood will required. Clinical data (such as age, gender, diagnosis, presence of hemophagocytic lymphohistiocytosis, relevant virus, treatment, outcomes) will be collected from institutional medical records. Following enrollment into this study, all study subjects will be assigned a unique identification number, which will allow linkage of clinically relevant information with the sequencing data generated from this study.

Molecular Pathology Procedures

Only surplus specimens or biopsies obtained at the time of routine, clinically necessary diagnostic or therapeutic procedures will be used. Specimens will be only be utilized after all assurances are made that usage for the purposes of this study will not deplete the tissue required for diagnostic studies or clinical care. DNA will be prepared from normal tissue specimens. Genome sequencing, analysis of all datasets and interpretation of results will be performed by the Columbia University Laboratory for Personalized Genomic Medicine.

RETENTION

Records relating to a specific research activity, including research records collected by investigators, must be maintained for at least three years after completion of the research (45

CFR 46.115(b); 21 CFR 56.115(b); 21 CFR 312.62). Clinical records, including consent forms that document clinical intervention or clinical diagnostic procedure research-related procedures, must be retained in medical records by the institution for at least seven years, per CUMC and NYP policy.

CONFIDENTIALITY OF STUDY DATA

Names, Medical Record Numbers, and any characteristics or codes that could identify an individual will be removed. Subject confidentiality will be ensured through de-identification procedures, wherein all subjects will be assigned a unique identifier. All analyses conducted for this project will be performed on data that has no subject identifiers. Information about patients who have been sequenced will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Only the principle investigator and study team will be allowed to access identified study data. To safeguard the PHI, any PHI collected, and/or subsequent data generated from the study results, will be stored on the CUMC IT approved MC Domain network drive P (Network Storage System ID 3959). If data is stored or transported on an end-point device, the end-point device will be encrypted and protected with a strong password.

Due to the unique nature of these subjects' diseases and the implications of the directed sequencing results, we would like to obtain permission to publish this as a case series. Any publication of information related to these studies will not include any identifying data.

PRIVACY PROTECTION

Collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no extraneous sensitive information is collected at any point. Any information collected during this study that can identify a subject by name will be kept as confidential as possible. However, complete confidentiality cannot be promised. Despite all of our efforts, unanticipated problems, such as a stolen computer may occur, although it is highly unlikely. Research findings will not be part of a medical record and will not be released to anyone without the consent of the participant. Access to names and personal identifying information will be strictly controlled by the principal investigator. Genetic results will only be released to the physician caring for the participant upon the participant's request. Participants are protected against genetic discrimination in employment and health insurance by the Genetic Information Non-Discrimination Act (GINA).

LOCATION OF STUDY

All samples will be obtained in the clinical care areas of the New York Presbyterian Hospital. Investigations using the materials will be conducted in the laboratories of the Department Pediatrics investigators or within CUMC or members of the HICCC.

COMPENSATION AND COSTS TO SUBJECTS

No compensation will be offered to subjects participating in this study, but they will incur no additional costs based on participation in this study.

RISKS AND BENEFITS

Loss of confidentiality is a potential risk of this study. Strict guidelines will be maintained to protect patient confidentiality. Furthermore, loss of confidentiality will be addressed by assigning each patient a unique ID code. This unique ID code and corresponding medical record numbers will be stored on a password-protected server (Network Storage System ID 3959). Computers used to access the protected server are stored in locked rooms at CUMC. No publication of information concerning patient-related material will include any identifying data.

There exists the risk of genetic discrimination. Participants will be advised that results will not be reported to any medical insurance, life insurance, or other insurance forms. There is small risk of pain during the blood draw and, rarely, a risk of bruising at the site of venipuncture.

This research is not designed to serve the subjects or their families directly, although it does have the potential to do so in the future. If a genetic source is identified in any of the patients, genetic testing could be beneficial to family members and offspring of the patient in order to better appreciate their susceptibility to similar virally-mediated lymphoproliferative disease.

Benefits to society may include identification of currently unidentified immunodeficiencies, enabling earlier identification and treatment of life-threatening disease in potentially affected individuals.

REFERENCES

Campo, E., S. H. Swerdlow, N. L. Harris, S. Pileri, H. Stein, and E. S. Jaffe. 2011. "The 2008 WHO Classification of Lymphoid Neoplasms and beyond: Evolving Concepts and Practical Applications." *Blood* 117 (19): 5019–32. doi:10.1182/blood-2011-01-293050.

Cohen, Jeffrey I., Elaine S. Jaffe, Janet K. Dale, Stefania Pittaluga, Helen E. Heslop, Cliona M. Rooney, Stephen Gottschalk, et al. 2011. "Characterization and Treatment of Chronic Active Epstein-Barr Virus Disease: A 28-Year Experience in the United States." *Blood* 117 (22): 5835–49. doi:10.1182/blood-2010-11-316745.

Han, Xiang Y. 2007. "Epidemiologic Analysis of Reactivated Cytomegalovirus Antigenemia in Patients with Cancer." *Journal of Clinical Microbiology* 45 (4): 1126–32. doi:10.1128/JCM.01670-06.

Kimura, Hiroshi. 2006. "Pathogenesis of Chronic Active Epstein-Barr Virus Infection: Is This an Infectious Disease, Lymphoproliferative Disorder, or Immunodeficiency?" *Reviews in Medical Virology* 16 (4): 251–61. doi:10.1002/rmv.505.

Kimura, Hiroshi, Tsuneo Morishima, Hirokazu Kanegane, Shouichi Ohga, Yo Hoshino, Akihiko Maeda, Shosuke Imai, et al. 2003. "Prognostic Factors for Chronic Active Epstein-Barr Virus Infection." *The Journal of Infectious Diseases* 187 (4): 527–33. doi:10.1086/367988.

Rahbarimanesh, Aliakbar, Mohammadali Ehsani, Maedeh Karahroudi, Armin Rashidi, Motahareh Aghajani, Alipasha Meysami, Elham Shahgholi, Azim Mehrvar, Maryam Tashvighi, and Hossein Keyvani. 2015. "Cytomegalovirus Disease in Children With Acute Lymphoblastic Leukemia in the Nontransplant Setting: Case Series and Review of the Literature." *Journal of Pediatric Hematology/Oncology* 37 (6): 429–32. doi:10.1097/MPH.0000000000000298.

Sevilla, Deborah W., Nader Kim El-Mallawany, Foxwell N. Emmons, Suzy Alexander, Govind Bhagat, and Bachir Alobeid. 2011. "Spectrum of Childhood Epstein-Barr Virus-Associated T-Cell Proliferations and Bone Marrow Findings." *Pediatric and Developmental Pathology: The Official Journal of the Society for Pediatric Pathology and the Paediatric Pathology Society* 14 (1): 28–37. doi:10.2350/09-12-0753-OA.1.