iDfellows project: Parvovirus B19 evolution and pathogenesis

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Background: The clinical presentation of a parvovirus B19 (B19V) infection can range from an asymptomatic condition to life-threatening diseases such as myocarditis, aplastic crisis, and hydrops fetalis in pregnant women. Degree and severity of the disease correlate strongly with the immune status and hematopoietic maturation. Interestingly, B19V infection has also been associated with different diseases, including rheumatoid arthritis in adolescents and adults as well as kidney disease (PMIDs: **9529776**; **17040883**; **31202393**). Primary B19V infection is common in childhood and small epidemics have been reported every few years (PMIDs: **14762186; 34391051)**. In young adolescents, B19V seroprevalence is approximately 50%, increasing to 80-90% with increasing age. Interestingly, Israel recently described a marked increase in symptomatic B19V infections. Children aged 6-11 years and pregnant women showed the most significant increase in B19V infection, when comparing the incidence rate ratio from 2015 – 2023 (PMID: **38005937**).
B19V is a small, non-enveloped, single-stranded DNA virus with a genome of approximately 5.5 kb that encodes six viral proteins. In contrast to the previously thought strict tropism of B19V for erythroid progenitor cells in the bone marrow, additional receptors and co-receptors of B19V and infection of non-erythroid cells and vascular endothelial cells mediated by antibody enhanced uptake (**PMID: 24807719**) have been reported . This may explain the wide range of clinical manifestations of B19V infection (PMID: **29923512**).
B19V is divided into three genotypes which form one serotype. The worldwide distribution of the genotypes varies, with genotype 1 accounting for 60% of infections in Europe, and genotype 1 accounting for nearly 95% of all described infections in Africa and Asia. Different genotypes are not only prevalent in different age groups, but have also been associated with different clinical presentations. Although there is insufficient data on the disease association of the different genotypes, genotype 2 has been described more frequently with cardiac manifestations. Regardless of genotype, viral genomic DNA was detected in multiple tissue types after primary infection in both symptomatic and asymptomatic individuals. However, a high viral load of genotype 1 was only detected in erythroid precursor cells of the human bone marrow (PMID: **29923512**).
Limitations in cell culture systems, infection models as well as technical difficulties in genome recovery and sequencing have led to the fact that knowledge about the biology of B19V is still very limited. Currently, genetic diversity and many crucial aspects of B19V pathogenesis such as tissue tropism, persistence, and tissue damage remain very poorly understood.

Consistent with the observation of the largest so far described epidemic of B19V infections in Israel (PMID: **38005937**), we have observed a significant increase in acute symptomatic B19V infections at the UKE with **> 30 cases from Oct 2023 – Mar 2024, compared < 5 cases/year during the last 5 years**. Of note, due to no reporting obligation for B19V infection in Germany benchmark data of B19V infections across Germany are missing.

We propose that the current surge in B19V infections provides a unique opportunity to apply advanced molecular analytical and bioinformatical methods, many of them established during the SARS-CoV-2 pandemic (1-5), to improve our understanding of the disease.

We propose to study the evolution of B19V in the current epidemic in the German population.

Our aims are:

1. To study the current epidemic of the virus in Hamburg:

In the 30 cases observed in the UKE, viral load, viral diversity and viral infectivity will be characterized using retained saliva and serum samples. This will allow first conclusions on genotypes, intra-host diversity and viral load of this current outbreak.

2. To study genotypes, intra-host diversity and tissue tropism:

We will complement the data obtained in 1. with data obtained from the UKE autopsy sample collection, allowing us to study primarily the tissue tropism of B19V.

3. To study possible disease association in immunocompromised hosts:

As a long-term perspective, the data collected in this project will be analyzed and deepened in collaboration with the DZIF (TTU infections of immunocompromised hosts) on an existing large patient cohort.

WP1 (Virus kinetics and evolution): Analysis will be conducted on B19V positive samples from patients with acute disease (defined as anti-B19V-IgM positive, PCR > 10^6 IU/ml). Samples will be taken from the current epidemic (n=30 samples already retained) and acute samples from the past 10 years (n=10 samples). Whole genome sequences will be obtained using Parvovirus capture panel next generation sequencing. We will determine intra-host and inter-host diversity and follow the evolution of the virus within the current epidemic. Further, we will address prolonged, persistent infections in patients who fail to clear B19V DNA or experience a slow decline in levels (n=5) at 6 and 12 months after primary infection. This will give us first ideas on intra-host genome variability and possible viral factors accounting for persistence.

WP2 (infectivity and humoral immunity): Saliva and serum samples from at least 10 patients will be analyzed by digital PCR to quantify and correlate B19V DNA levels with anti-B19V IgG and IgA levels. To test for infectivity, selected samples will be employed for virus isolation using in vitro cell culture models (e.g., commercially available UT7/Epo-ST1 cells and/or IPSC-derived erythroid progenitor cells). Successfully isolated viruses will be further used in virus neutralization tests (VNTs) to assess the specificity and neutralizing capacity of the detected humoral immune responses.

WP3 (Tissue Tropism and Host Immune Response): To analyze long-term tissue tropism, archived autopsy samples from patients will be screened by qPCR of DNA pools covering multiple organs (intestine, heart, liver, kidney and spleen, and serum). B19V-positive pools will be deconstructed by digital PCR to define individual B19V load. Samples with high B19V DNA loads will be further analyzed by immunofluorescence staining and DNA in situ hybridization (DNA-Scope) to visualize infected cells in the organs and by capture panel sequencing as described in WP2 to obtain whole genome sequences. Finally, we will perform spatial transcriptomics (10x Xenium technology in collaboration with E. Whyler and M. Landthaler, MDC Berlin) on selected samples to identify B19V infected cell types and to analyze the host response to B19V infection/persistence at the single cell level.

Long-term perspective: To study B19V infection or reactivation of the virus in immunocompromised patients and **the possible association with disease** after kidney transplantation, we will apply to the DZIF Transplant Cohort for access to the biosamples

https://www.dzif.de/de/arbeitsgruppe/transplantationskohorte. The transplant cohort includes more than 800 kidney transplant patients, more than half of whom had an infectious event in the first year after infection. While this cohort has been well studied for BK polyomavirus reactivation and herpesvirus reactivation (PMID: **35855001**), no data are available yet on B19V.

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