Evaluation of Differential Blood Stain for Detection of Enterovirus D68

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Introduction: Enterovirus D68 (EV-D68) is a small, non-enveloped RNA virus, and is suspected to be the cause of respiratory and neurological disease in young children.[1] At the Institute for Antiviral Research, and under the direction of Dr. Bart Tarbet, we have developed an animal model for studying EV-D68 infection. With this model, we will pursue a better understanding of the pathogenesis of EV-D68. We will evaluate a differential blood stain for detection of EV-D68 to gain a better understanding of cell association with viral infection.

Abstract

Enterovirus D68 (EV-D68) is a small, non-enveloped RNA virus, and is suspected to be the cause of respiratory and neurological disease in young children.[1] The most concerning clinical sign is an acute flaccid paralysis of the lower limbs.

At the Institute for Antiviral Research, and under the direction of Dr. Bart Tarbet, we have developed an animal model for studying EV-D68 infection. With this model, we will pursue gaining a better understanding of the neuropathogenesis of EV-D68. It has been shown, in in-vitro studies, that EV-68 is able to produce infectious progeny in leukocyte cell lines.[2] We will, therefore, compare the white blood cell counts of infected and non-infected mice from our animal model.

We will be taking blood samples from AG129 mice pre- and post-infection. With the blood collected, we will use a differential stain kit to stain for percentages of the different cell types in the blood. With the results from the stain, we will look at Complete blood count (CBC) from the collected blood. This will then be analyzed against a baseline CBC for AG129 mice. We hope that this information will provide an extra parameter when examining EV-D68 infection in our mouse model. If we identify a specific cell type associated with EV-D68, then further research may include examining the pathway of the infected cells throughout the body.

Objectives

- Collect blood via the submandibular vein from mice pre- and post-infection
- Analyze blood with Diff-Quik stain to receive a blood cell count percentage.
- Analyze the data for differences in cell percentages.

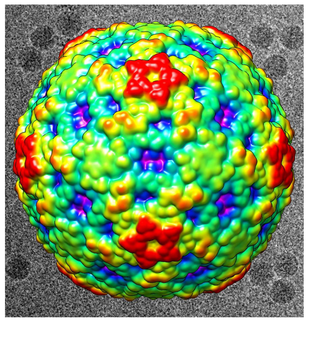

Figure 1: Surface view of Enterovirus D68. Areas in red represent the highest peaks on the viral capsid surface and areas in blue represent the lowest. (Purdue University image/Yue Liu and Michael G. Rossmann)[3]

Methods

Animals: AG129 mice will be obtained from mice colonies at the Institute for Antiviral Research. The animals will be fed and watered using Teklad Rodent Diet (Harlan Teklad) and tap water from the Laboratory Animal Research Center at Utah State University. Proper IACUC protocol will be used when handling the mice.

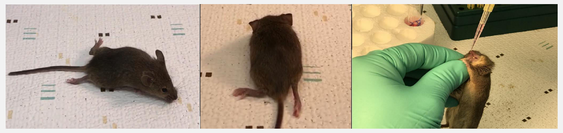

Figure 2: EV-D68 mouse model The first 2 pictures are examples of paralysis in our mouse model. The third is a picture of the intra-nasal infection route.

Viral Infection: Mice will be anesthetized by intraperitoneal injection with a ketamine/xylazine mixture prior to virus challenge. Once properly anesthetized, mice will be challenged by an intranasal route with 90 µl of EV-D68 (diluted 1:2). Following the infection, the mice will be observed for 7 days.

Blood collection through Submandibular vein: Collection of blood through the submandibular vein of the mouse is the preferred method, due to it being the most non-invasive. The mouse is restrained and a 4mm lancet is used to puncture the submandibular vein slightly behind the mandible but in front of the ear. A blood volume of 250 µl will be collected in EDTA tubes. EDTA tubes are used for analysis of cell count due to blood clot prevention, enabling a successful reading with the differential quick stain. After sufficient blood is collected the top of the tube will be gently pressed against the puncture wound allowing the hair follicles to interact with the blood. This will help clot the blood due to the clotting factors of the fur on the mice.

Fixation of blood samples: A single drop of blood is placed on the surface of the slide. The blood is then applied to the surface at an acute angle. The slide is then air-dried until it is apparent that the sample is free of a "wet" appearance. The samples are then placed in our clear solution from our differential staining kit, for 10 seconds. They are then dipped 5 times in our red staining solution and then dipped 5 times in our blue staining solution. The samples are then washed with distilled water and air dried. Once dried the samples will be dehydrated in 100% EtOH, then xylene and then mounted in a synthetic resin. We will then read the slides using an electron microscope to manually identify the white blood cells.

Figure 3: Differential Quick staining Kit The clear solution is the fixative. It will be used to adhere our cells to the slide. The blue and red solutions are our stains. These solutions will be used to help identify the white blood cells in the blood collection.

Reading the CBC: We will manually count the number of the different cells in four different small areas twice and then average the numbers. This will be done with post-infected mice samples as well as the pre-infected baseline samples for our mice.

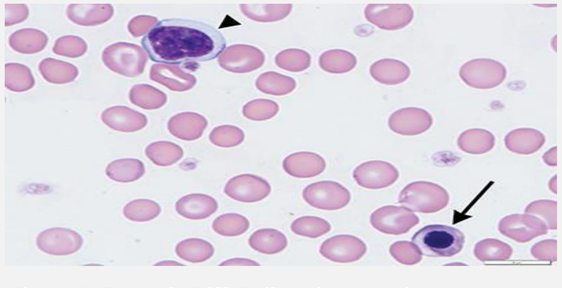

Figure 4: Example Diff-Quik stain. A single metarubricyte (arrow) and reactive lymphocyte (arrowhead). (In-Clinic Hemotology)[4]

Future studies

Future studies may include:

- Further determination of the pathogenesis of the virus through bronchoalveolar lavage
- Analysis of cells, by flow cytometry, in determining which specific cells are associated with the virus

Acknowledgments

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References:
1. Messacar, Kevin, et al. "A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado,
USA." The Lancet, vol. 385, no. 9978, 25 Apr. 2015, pp. 1662–1671., doi:10.1016/s0140-6736(14)62457- 0.
2. Smura, Teemu, et al. "Cellular tropism of human enterovirus D species serotypes EV-94, EV-70, and EV-68 in vitro: Implications for pathogenesis." Journal of Medical Virology, vol. 82, no. 11, 2010, pp. 1940–1949., doi:10.1002/jmv.21894.
Figure References:
3. Venere, Emil. "Purdue research suggests approach to treat virus causing respiratory illness, possible paralysis in children." Purdue University, 1 Jan. 2015, www.purdue.edu/newsroom/releases/2015/Q1/purdue-research-suggests-approach-to-treat-virus-causing-respiratory-illness,-possible-paralysis-in-children.html
4. Sharkey, Leslie, DVM, PhD, Diplomate ACVP (Clinical Pathology), and Daniel Heinrich, DVM University of Minnesota. "In-Clinic Hematology: The Blood Film Review." Todays Veterinary Practice, In-Clinic Hematology, 2 June 2016, todaysveterinarypractice.navc.com/in-clinic-hematology-the-blood-film-review/.

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