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Case Study: Observing the Effects of Length of Exposure to MNV on Mice Colonies

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Case Study: Observing The Effects of Different Lengths of Exposure of MNV on Mice Colonies

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Abstract

Four groups of experimental mice are studied in this experiment following intentional exposure to murine norovirus (MNV). Two groups are exposed to the virus at different points in their lives, and the other two are not infected and studied alongside the other groups as controls.

Past research has indicated that B and T immune cells are not essential to MNV resistance[2]. Additionally, antibody production against MNV has shown to be slow as infection progresses[1]. Thus, the expected results from the experiment are that the mice with longer infections of MNV will have larger quantities of IgG than those more recently infected with the virus. Immunological data gathered from these groups is compared to IgG counts from control mice, which are comprised of pathologically clean mice of the same strain as the sentinel mice infected with MNV and the same strain as the colony with innate exposure to MNV.

Introduction

Murine norovirus (MNV) is the most common pathogenic species found in laboratory mice. An RNA, non-enveloped virus, MNV is of the family Caliciviridae and is transmitted from host to host via the fecal-oral route. Though the virus is typically asymptomatic in mice with competent immune systems, it has caused more detrimental pathology in mice with immunodeficiencies, particularly mice with damaged or lost STAT1 genes, which encode proteins that respond to interferon stimulation, particularly interferon-gamma (IFN-γ), a critical component to development of innate and adaptive immunity[6]. Mice with deficiencies in STAT1 genes that are infected with the virus have symptoms including wasting and death. This lead to the assumption that STAT1 genes are crucial in MNV defense in mice[1,2]. Less severe infections have shown inflammation in the liver, lungs and peritoneal cavities[3].

Interest in the virus has been limited due to its noninvasive nature with research, and thus little literature is found on MNV. However, investigators with an interest in studying immunology using murine models may have their results questioned if their mice are infected with MNV. Those studying mice with knocked-out or knocked-down STAT1 genes may also have difficulty in their experiment if the colonies used are infected with the virus. It is for these reasons and the fact that the virus is so prevalent that the experiment chose to focus on mice colonies infected with MNV.

The experiment detailed in this report is a case study of two different groups of mice housed in The University of Akron Research Vivarium (UARV). The key focus was the relationship between length of infection of MNV and the immunological results, showcased in this experiment as Immunoglobulin G (IgG) counts of infected mice. IgG counts were chosen as the dependent variable because of the nature of antibody count, or IgG count, increasing during viral infection.

One group of mice, which represent a colony with historically positive serology test results for MNV, are assumed to have been exposed to the virus since birth, yet innate immunities have prevented any symptoms from evolving. The other group are sentinel mice that are exposed to the virus after being introduced into the colony from an external, MNV-negative source, and thus are infected well after birth. Two other groups act as controls, in that neither are exposed to MNV and have never received positive serology test results for MNV.

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Methods

The experiment focused on 4 experimental groups. Group 1 (n=3) was composed of sentinel mice intentionally exposed to soiled bedding and feces from Group 2. Group 2 (n=2) included mice representing the colony with historically positive serology tests for MNV. Group 3 (n=3) included sentinel mice not exposed to MNV, yet exposed to feces from Group 4. Group 4 (n=2) included control mice with historically MNV-negative serology test results.

Group 2 (1 female and 1 male, strain C57BL6) mice were bred in-house. Group 4 (female, strain C57BL6) were imported from The Jackson Laboratory in Sacramento, California. Sentinel mice, which were used in experimental Groups 1 and 3 are female, strain CF-1 and originate from Charles River Laboratories in Raleigh, North Carolina.

The experiment performed was completed in tandem with the sentinel testing program at the UARV, which was approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Akron under protocol 13-3C.

Mice in Group 1 were housed with a colony of mice with a history of MNV infection, showcased by positive results following serology tests. During weekly cage changes, dirty bedding containing fecal material from each cage in the colony was placed in Group 1's cage, allowing for transmission of any pathogens present in the colony. This procedure was performed for Group 3, with the exception that these mice were housed with a colony with historically clean serology test results. Groups 2 and 4 were donated from the colony with MNV and the clean colony, respectively.

Sentinel mice were housed with the colonies of experimental mice for a period of eight weeks, allowing enough time for transmission of any present pathogens. At the end of the eight weeks, the sentinel mice and the groups representing the colonies the sentinels were housed with were euthanized and serology samples were collected.

Prior to euthanasia, mice in each group were anesthetized intraperitoneally with a combination of ketamine (75-125 mL/kg) and acepromazine maleate (2.5-4 mL/kg) in a volume of approximately 0.1-0.25 mL per 100 g of body weight. The ketamine and acepromazine maleate were distributed from Vedco, Inc. and Boehringer Ingelheim Vetmedica, Inc., respectively, from St. Joseph, Missouri.

Blood draws, anesthesia, and euthanasia were completed for each group using a 1-mL syringe and 25-gauge, 5/8-inch long needles vended from Becton-Dickinson. Blood was collected via cardiac puncture and placed into a Becton-Dickinson vacutainer serum tube, each group having its own tube. The serum tube contained an clot-activator additive and was coated with silicon on the interior. Blood from each mice belonging to the group was placed in the tube to properly represent all in the experimental group. All mice were euthanized via an overdose of pentobarbital-containing euthanasia solution Beuthanasia-D Special (390 mg/mL), distributed by Schering-Plough Animal Health in Union, New Jersey. Euthanasia was given at a minimum dose of 100-125 mg/kg. Death was

confirmed by lack of respiration and heartbeat. Following death, a gross necropsy was performed on each mouse to observe for any anomalies. Anal tapes, fecal floats, and cage swabs were performed to test for any presence of ecto- and/or endoparasites. Anal tapes and fecal floats were performed in-house. Cage swabs were sent to IDEXX BioResearch and analyzed via PCR.

The blood in each vacutainer tube was separated into serum and plasma components via a Speedfuge Centrifuge HSC10K made by Savant Instruments, Inc. from Farmingdale, New York. Serum from each tube was collected using a microliter pipette and placed inside four individual Eppendorf microcentrifuge tubes for each group. The serum samples were sent to IDEXX BioResearch in Columbia, Missouri, where serology tests were performed to test for antibodies against common pathogens in laboratory mice colonies. In addition to the Basic Serology examination, IDEXX BioResearch also provided immunoglobulin counts (µg/mL) for each serum sample.

Results

Detection of MNV by IDEXX BioResearch was completed via Multiplex Fluorescent Immunoassay (MFI). A MNV antigen created by IDEXX was tagged with fluorescent beads to identify presence of IgG antibodies against the virus. Intensity of fluorescence was calculated for each treatment group in the experiment if MNV was present in the serum.

According to the Basic Serology results from IDEXX, Group 1 tested positive for MNV infection and Groups 2-4 tested negative (**Table 1)**. Group 1 showed MFI (median fluorescent intensity) values of 14,500 – 15,499 for MNV, high above the baseline for detection (MFI > 2.000). No MFI values were reported for any other group.

Group 2 showed the highest number of IgG in the serum collected from the members (1,907 µg/mL). Group 1 had the second highest number at 1,114 µg/mL. Groups 3 and 4 had the lowest immunoglobulin counts, at 757 µg/mL and 812 µg/mL respectively. Results from the t-test showed that the differences in values are statistically significant (p-value = 0.011). A graph exhibiting the IgG counts can be found in **Figure 1**.

No significant findings of abnormal physiological detail, clinical health concerns, or ectoparasites were found upon gross necropsy. Anal tapes, fecal floats and cage swabs showed no evidence of parasitic infection. Basic Serology results from IDEXX displayed no pathogenic species, other than MNV, had infected the mice from Groups 1-4.

MNV	Baseline	Group 1	Group 2	Group 3	Group 4
MNV Purified Virus	MFI > 2.000	**+15**	--	--	--
MNV VP1 Recombinant	MFI > 3.750	**+18**	--	--	--

Table 1. Detection of MNV was completed by MFI. Fluorescence was detected in Group 1 mice with a value between 14,500 – 15,499 MFI. Recombinant viruses were detected as well at a value between 17,500 – 18,499 MFI. MNV was not detected in any other treatment group in the experiment. Table adapted from IDEXX BioResearch Case 2423-2016.
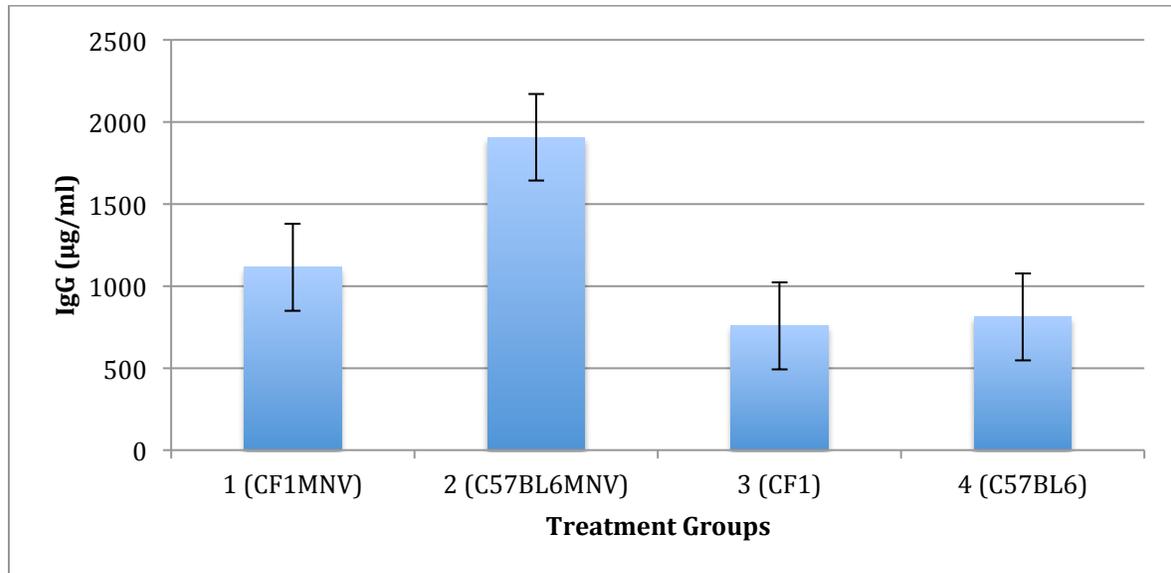

Figure 1. Graph shows immunoglobulin counts between Groups 1-4. Serum used per group was a collection of blood donated by each member of the group, so counts are aggregates. Group 2 had the highest number of IgG, followed by Group 1, 4 and 3. T-test showed relationship between treatment group and IgG count is statistically significant (p-value = 0.011449). Raw data supplied by IDEXX BioResearch.

Discussion

The purpose of the experiment was to observe the effects of progression of MNV infection on the immunology of mice. Groups 1 and 2 were the key focus, as they differed in length of exposure to the virus, with Group 2 having the virus since birth and Group 1 being exposed to it around 6 weeks of age. Groups 3 and 4 acted as controls, as neither group was exposed to MNV or had any history of pathogenic infection. The expected result of the case study was that mice immediately exposed to the virus after birth (Group 2) were going to have higher IgG counts than mice exposed to it after being introduced into the colony (Group 1).

The results from the experiment showed that the hypothesis held true: Group 2 had a higher immunoglobulin count (1,907 µg/mL) than Group 1 (1,114 µg/mL). Exact measurements are shown in the results and a bar graph exhibiting the relationships between IgG counts between groups are shown in **Figure 1**. However, serology test results showed that Group 2 tested negative for MNV

infection (**Table 1**), a possibility not initially considered prior to experimentation. The colony that Group 2 was donated from had previously tested positive for MNV, yet this fact shows that even though the virus is present in the colony, it does not necessarily mean all mice are infected. This explains the results from the serology test.

However, this calls into question the results seen in the IgG counts. Group 1's serum contained antibodies against MNV, which would explain the higher IgG counts when compared to mice of the same strain in Group 3, which were not exposed to the virus. Group 2 had higher IgG counts than mice of the same strain not infected with MNV in Group 4. Group 2 had higher numbers of antibodies in their serum than Group 1, the only group infected with MNV.

Experimental error could have caused the results seen in the experiment. For example, MNV antibodies could have been present in Group 2's serum sample but at too low of concentrations to be detected by MFI. Excluding the possibility of experimental error, higher IgG counts in Group 2 mice could have been diet-induced.

C57BL6 mice are highly susceptible to diet-induced obesity, a trait known to cause higher levels of serum IgG[4]. In an experiment conducted by Mohammed N. et al. in 2012, strain C57BL6 mice fed a high-fat diet, when compared to mice with normal and low-fat diets, had more antibodies present in their serum. The IgG targeted specific pathogenic strains of common gut bacteria, including Escherichia coli[5].

The mice in Group 2 were fed high-fat diets prior to being donated to encourage breeding behavior. The diet caused the dam and sire to become obese, which, given the results of Mohammed N. et al.'s experiment, may have explained why their IgG counts were elevated, even more so than mice infected with MNV. Group 4, the same strain as Group 2, was fed a diet containing normal quantities of fat and results in **Figure 1** show that, relatively speaking, its IgG numbers were low. Thus, it is possible that prolonged consumption of high-fat food and subsequent obesity causes more of an immune response than MNV infection.

The fact that healthy B and T cells do not have as much as an effect on fighting MNV infection as STAT1 genes might also explain why Group 1 did not produce as much IgG. Additionally, if Group 2's obesity caused an increase in harmful species of gut bacteria, multiple IgG's targeted against multiple antigens created by these bacteria could easily explain heightened IgG levels in blood serum. Group 1, while having MNV, was not fed a high-fat diet and therefore could have not been hosts for associated pathogenic bacterial species, thus no antibodies were produced.

Acknowledgements

Resources

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