The Role of ST6 GAL-I sialyltransferase on immune responses to LCMV Infection

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The role of ST6 Gal-I sialyltransferase on immune responses to LCMV infection

Senior Honors Project
University Honors 499
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By

Natalie Wilson
Senior Honors Project

During the course of the senior honors project, the student:

1. Worked in Dr. Onami's research lab for seven months
2. Conducted personal research and experiments pertaining to her project
3. Gave a 30 min presentation of her research to Dr. Onami's lab (powerpoint slides given)
4. Wrote a paper detailing the methods and results of her research
The role of ST6 Gal-I sialyltransferase on immune responses to LCMV infection

Abstract:

ST6Gal-I is a sialyltransferase enzyme that catalyzes the addition of alpha-2,6 sialic acids to galactose on N-linked glycoproteins post-translation. Previous research in this lab has shown that this gene plays a role in both B cell and T cell responses to LCMV infection. Specifically, ST6Gal-I deficient mice show reduced B cell, CD4 T cell, and CD8 T cell responses on day 8 post-infection. Because of these reduced responses, we examined the role of ST6Gal-I in viral clearance. After performing plaque assays to detect viral titers, we determined that viral clearance in ST6Gal-I deficient mice was no different from viral clearance in wild type mice on day 8. This leads us to conclude that although ST6Gal-I plays a role in B cell and T cell responses to LCMV infection, it does not affect viral clearance by day 8 post infection.

Introduction:

Previous research in this lab has shown that ST6Gal-I plays a role on immune responses to infection. In figure 1 we show that ST6Gal-I knockout mice have reduced LCMV specific antibody titers for both IgG and IgM at all time points shown, compared to the wild type B6 mice.
Research also shows that T cell responses are affected by ST6Gal-I. ST6Gal-I knockout mice have reduced viral specific CD4 T cell responses on day 8 post infection, as seen in Figure 2.

Figure 2. Viral specific IFNγ producing CD4 T cells, showing reduced response in ST6Gal-I knockout mice. (Rajini Bheemreddy)
The CD8 T cell response is also affected by ST6Gal-I. In figure 3 we show that ST6Gal-I knockout mice have a reduced viral specific CD8 T cell response by day 8 post infection. This is significant because the CD8 T cell response is the key population of cells involved in LCMV to viral clearance. This data was highly influential in this research project.

Because ST6Gal-I plays a role in both B cell and T cell responses, we decided to look at its role in viral clearance. Previous research showed that by day 8, the virus was cleared from the serum in both wild type and ST6Gal-I knockout mice. My project involved looking at viral clearance in the tissues.

Objective:

ST6Gal-I deficient mice show reduced B cell, CD4 T cell, and CD8 T cell responses. Considering this, we looked at the effects of ST6Gal-I on viral clearance. Specifically, we studied how the loss of ST6Gal-I affects viral clearance in the tissues.
Materials and Methods:

To examine viral clearance in tissues, we used a plaque assay method. This is a technique used to determine viral titers. First, I maintained a VERO cell line for several days. These cells are optimal for infection by LCMV. This is done prior to the plaque assay. On the day before the assay begins, we count the cells and dilute them using specific calculations. Next, we add cells to each well of several six-well plates. This step is important because it allows an equal number of cells to be added per well, ensuring that every well has the same chance of being infected by the virus, barring any cell contamination.

On day one of the plaque assay, I homogenize a tissue suspected of containing virus. The tissues we examined include the spleen, kidney, liver, and lung from both wild type and knockout mice. All were from day 8 post-infection. I then add the tissue to a 96-well plate and make serial dilutions. This step requires precision in pipetting technique and maintaining sterilization. Then I transfer the diluted virus into the six-well plates. The plates are incubated at 37°C for one hour and rocked every fifteen minutes. This allows the virus to begin mixing with and infecting the cells. Finally, I add a 1:1 mixture of hot agarose and 2x199 media to the wells. When it solidifies, the plates are incubated at 37°C for three days. During this time, the cells are infected with virus and plaques begin to form. A plaque is a clearing on the plate indicating cell death due to viral infection.

On day four, another mixture of agarose and 2x199 is added to the wells. However, during this step, 1% neutral-red dye is also added. Again, the solution solidifies and the plates are incubated overnight. During this time, the viable cells take up the dye.
and stain red. The cells infected by the virus are killed and do not take up the dye. They remain clear, and the plaques can be seen.

On the fifth day of the assay, the plaques on the plates are ready to be counted. I count the plaques in each well, and then calculate the plaque forming units per ml for each tissue used. This gives the viral titer. For each assay, a control virus with a known viral titer was also used to ensure that the technique was performed correctly. For these assays, LCMV-Armstrong was used as the control.
Results:

Our results show that no virus was found in any tissue the wild type or the knockout mice, as seen in Figure 4. Any virus in the tissues was below our limit of detection, which was about 50pfu/ml (plaque forming unit per milliliter). These data suggest that there is no difference in viral clearance between the wild type and the ST6Gal-I knockout mice at day 8 post-infection.

Figure 4. Viral titers in various tissues for wild type mice, ST6Gal-I knockout mice, control virus, showing that viral clearance in the tissues is the same in the wild type and knockout mice.
Conclusions:

The loss of ST6Gal-I does not affect viral clearance at day 8 post-infection. Virus (above the level of detection) was cleared from both the wild type and the ST6Gal-I knockout mice.

There were some possible sources of error during these experiments. First, my viral titers of the control plates were often lower than what they should have been. Because the control plates still produced plaques, just at a lower rate than normal, it is probable that there was error in my pipetting technique. For this reason, I can confidently say that the control plates contained virus while the tissues did not, as far as my limit of detection conveyed. (It is possible that because my technique might have been flawed, my limit of detection was not as good as it should have been. It is possible that there were traces of virus that I could not detect.) However, I could not confidently say the exact viral titer for each tissue. Another important factor when considering this data is the unit of measurement of the viral titer. In the future, should there be a need to compare viral titers of these tissues, it would be better to measure in pfu/gram of tissue. This way, tissues of different sizes can still be compared. It is also important to note that for the control plates, straight LCMV-Armstrong was used instead of a tissue that we knew to be infected. It would be beneficial to use infected tissue viral titers as a comparison, versus the straight virus.

Future research concerning the role of ST6Gal-I on viral clearance should be conducted. Although ST6Gal-I does not seem to affect viral clearance at day 8, it could still play a role in clearing the virus at earlier time points. By comparing tissues from day 3 or day 4 post-infection from wild type and ST6Gal-I knockout mice, a difference in
viral clearance might be seen. This could lead to further knowledge of the role of ST6Gal-I on immune responses to LCMV infection.
Works Cited:


The role of ST6Gal-1 on immune response to LCMV infection

November 24, 2008

**ST6Gal-1**
- Sialyltransferase
- Catalyzes the addition of α2,6 sialic acids linked to Galβ1-4GlcNAc
- ST6Gal-1 knockout mice show reduced B cell and T cell responses

**LCMV**
- Enveloped RNA virus
- Arenavirus
- Used armstrong strain as control

Antibody titers at various time points

- IgM
- IgG
**Question:**
How does the loss of ST6Gal-I affect viral clearance?

**Plaque Assay**
- Technique used to determine viral titers
- Infect VERO cells with LCMV
- Cells take up virus → Virus replicates → causes cell death
- Stain cells (only viable cells take up dye)
- Count plaques
Determining Viral Titers:

Plaque Count $\times 5 \times 10^x = \text{pfu/mL}$

Problems:

- Maintaining VERO cells
- Control titers too low

Conclusions:

- In ST6Gal-I knockout mice, although there is a reduced B cell and T cell response, the virus was cleared at day 8.
- Need to look at earlier time points to observe differences