Sialyltransferases and influenza virus infection

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Introduction

Influenza type A has been established as an all-time leading killer of humans in the modern world. The "Spanish flu" of 1918 alone has killed 20-50 million people, and each year it is suspected that seasonal influenza is responsible for 250,000-500,000 deaths worldwide (World Health Organization, 2005). It is known that influenza type A recognizes receptor molecules composed of glycoproteins, glycolipids, or proteoglycans on cell surfaces, and that terminal sialic acid moieties mediate virus binding and infection. In vivo, enzymes collectively referred to as sialyltransferases (STs) mediate the addition of sialic acid residues to glycoconjugates in a highly regulated, tissue specific manner. ST3s, of which there are six (ST3Gal1-6), are responsible for adding sialic acid to oligosaccharide substrates through an α2,3 linkage, the configuration of sialylated molecules most common to the mucosal surfaces of most animals. Sialylated molecules are also thought to play important roles in cell-cell communication during immune responses. Therefore, sialylated glycoconjugates may have a confounding effect in influenza virus infection. The focus of this project will be to try to determine the importance of ST3s during influenza virus infection.

Hypotheses: (1) Assuming that the action of one (or more) ST3s is required for the formation of influenza virus receptors, a difference (decrease) in influenza virus infection will be observed in one (or more) ST3 knockout (KO) strains of mice when compared to wild type (WT) controls. (2) This trend will be mimicked at the cellular level by reduced infection of the corresponding KO mouse embryo fibroblasts (MEFs). These 'perfect-world' results would conclusively indicate a binding specificity pattern specific to the actions of particular ST3s.

Objectives: To determine if influenza A virus infection differs in ST3 KO mice and wild type controls.

To carry out, in parallel, infection of MEFs isolated from KO mouse strains (ST3Gal 1-5) to determine if the immune response to influenza virus infection is different in ST3 KO mice and wild type mice.

Experimental

Mice: KO mice for ST3 β-Galactoside α2,3-sialyltransferases (ST3 Gal) 1 and 2 and WT C57BL/6 controls were obtained from Jackson Labs, and then bred to increase the experimental population.

Virus: Influenza A/PR/8/34 (PR8; H1N1) and influenza A/rgHK/213/03xPR/8, containing the H5 and N1 genes of Influenza A/HK/213/2003 on the A/PR/8/34 background. Virus titres for infection experiments were calculated from plaque assays performed on Madin-Darby canine kidney (MDCK) cells.

Isolation and infection of mouse embryo fibroblasts: Mouse embryos were isolated from female mice at 14 days in the gestation period, trypsinized, and cells were cultured in growth medium (DMEM) containing 10% fetal bovine serum until confluency. MEFs were subsequently frozen in liquid nitrogen. Passage 2 MEFs were infected with H5N1 or PR8 at a multiplicity of infection (m.o.i.) of 3 and incubated for 24 hr.

Flow cytometry: MEFs were trypsinized, washed, fixed and permeabilized, and incubated in the presence or absence of mouse monoclonal antibody specific for the influenza A nucleoprotein (NP)(MAB8257; Millipore) and/or phycoerythrin-conjugated goat anti-mouse immunoglobulin.

Results

Table 1: Flow cytometric analysis of MEF infection by influenza A virus

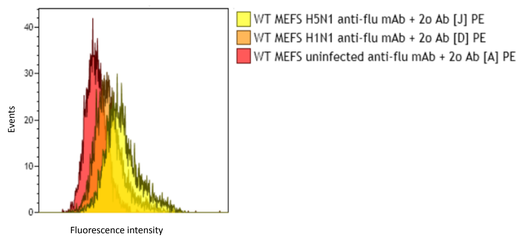
Experiment Description	M FI	MFI Ratio
WT MEF uninfected αflu mAb +2°Ab	1.77	
WT MEF H1N1 24hr αflu mAb +2°Ab	4.47	2.53
WT MEF H5N1 24hr αflu mAb +2°Ab	8.78	4.96
Gal 1 KO MEF uninfected αflu mAb +2°Ab	1.80	
Gal 1 KO MEF H1N1 24 hr αflu mAb +2°Ab	4.00	2.22
Gal 1 KO MEF H5N1 24 hr αflu mAb +2°Ab	9.69	5.38
Gal 2 KO MEF uninfected αflu mAb +2°Ab	2.27	
Gal 2 KO MEF H1N1 24 hr αflu mAb +2°Ab	3.29	1.45
Gal 2 KO MEF H5N1 24 hr αflu mAb +2°Ab	11.19	4.93

Note: Mean fluorescence intensity (MFI) ratios were calculated by dividing the mean FI values of the infected MEFs by the mean FI values of the corresponding uninfected control MEFs.

Overall, the H5N1 infected MEFs expressed more influenza NP than H1N1-infected MEFs. More importantly, the mean FI ratios for Gal 1 KO and WT MEFs infected with H1N1 were similar (approximately 2.5). By contrast, the Gal 2 KO MEFs infected with H1N1 reported a slightly lower MFI ratio (1.45). Both KO strains and the WT control displayed an MFI ratio of approximately 5 when infected with H5N1.

Wild Type mice

Flow cytometry analysis of wild type MEFs infected with influenza virus

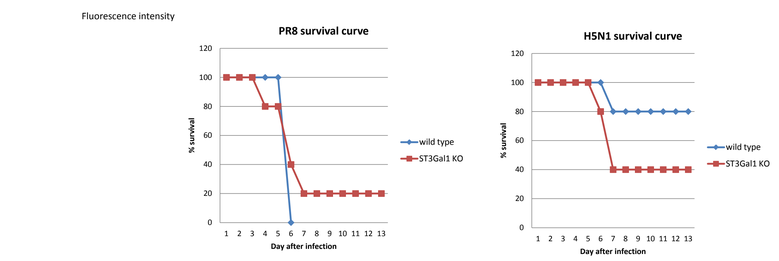


Observations:

1. H5N1 infected MEFs expressed more influenza nucleoprotein than H1N1-infected MEFs

ST3Gal 1 knockout mice

Flow cytometry analysis of ST3Gal1 KO MEFs infected with influenza virus

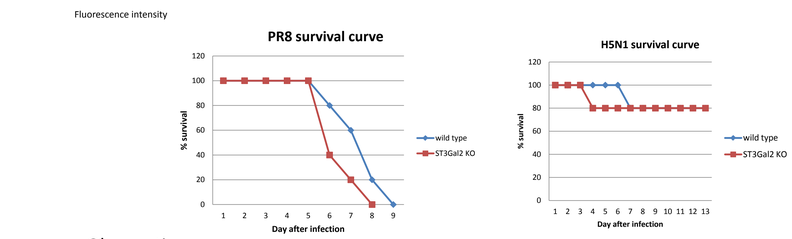


Observations:

1. H5N1 infected MEFs expressed more influenza nucleoprotein than H1N1-infected MEFs
2. Equivalent survival of ST3Gal1 KO mice compared to WT mice following PR8 influenza virus infection.
3. Decreased survival of ST3Gal1 KO mice compared to WT mice following H5N1 virus infection.

ST3Gal 2 knockout mice

Flow cytometry analysis of ST3Gal2 KO MEFs infected with influenza virus



Observations:

1. H5N1 infected MEFs expressed more influenza nucleoprotein than H1N1-infected MEFs.
2. H1N1 nucleoprotein expression was almost negligible.
3. Approximately equivalent survival of ST3Gal2 KO mice compared to WT mice following PR8 influenza virus infection.
4. Approximately equivalent survival of ST3Gal2 KO mice compared to WT mice following H5N1 virus infection.

Discussion

Contrary to the original hypothesis, H1N1 and H5N1 infectivity does not seem to vary between the MEFs of WT, Gal 1 KO and Gal 2 KO strains. H1N1 may have lower infectivity in Gal 2 KO MEFs; however more experimentation will be required to validate this observation. H5N1 virus appears to have an attenuated phenotype in mice compared to H1N1 virus. These studies also suggest that some strains of KO animals are more susceptible to influenza virus infection, and in particular, H5N1 infection, than WT animals. The decreased survival of the KO animals could be the result of a compromised immune response due to problems in sialic acid-dependent cell-cell communication. Recent studies by others have shown that ST3Gal 4 may affect the function of CXCR2, a protein that is crucial in initiating immune response and that may be implicated in the immune response against influenza A virus.

Supplementary Work

The experiments summarized in table 1 will need to be repeated (in part or in full) to confirm the data presented here. Additional flow cytometry experiments will be performed on Gal 3, Gal 4 and eventually Gal 5 KO strains in order to establish any differences in susceptibility to influenza A virus infection between mouse strains. Infectivity studies will continue to be performed in order to examine virus titres in the lungs of infected animals and to generate survival curves for both H5N1 and H1N1 on all mice strains.

Acknowledgements

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