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THE ROLE OF DIFFERENTIAL VIRAL PROFILES IN THE PATHOGENESIS OF SJÖGREN'S SYNDROME

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Background

Primary Sjogren's syndrome (pSS) is a chronic autoimmune syndrome that predominantly impacts females and is characterized by decrease in saliva and tear production, salivary gland inflammation and development of autoantibodies[1]. The underlying triggers of pSS are attributed to chronic viral exposures in conjunction with genetic susceptibility factors that limit patients' abilities to effectively clear viral infections. The Weller lab has identified the presence of multiple unique virus profiles in pSS patients and has shown the capacity of viral antigens to trigger the complete pSS phenotype in vivo[2]. Preliminary studies were conducted on 3 murine strains (NOD-ShiltJ, C57Bl/6 and BALB/c) to identify viral signatures present in the salivary gland tissue. Here we demonstrated that NOD-ShiltJ murine model of pSS has a significant increase in Mouse Mammary Tumor Virus (MMTV), a Retrovirus, in disease affected salivary gland tissue. Presence of MMTV in salivary gland tissue in NOD-ShiltJ has not been previously characterized. This study details the detection of MMTV and other viral signatures in the NOD-ShiltJ pSS model.

Methods

CLC Genomics Workbench (Qiagen bioinformatics, USA) was utilized to analyze viral gene expression in salivary glands of 12 week old female C57BL-6, BALB/c and Nod-ShiltJ mice and from 4, 8 and 12 week old female NOD-ShiltJ mice. Microarray data was normalized using quantile normalization protocols in CLC workbench. Background threshold was set for normalized expression intensity above 50 and fold change above 1.5 or below -1.5. T-test and ANOVA statistical analysis was performed and false discovery rate (FDR) algorithm was applied to identify statistically significant ($p<0.05$) viral probes in each analysis group. *GeneVenn* (genevenn.sourceforge.net/) was used to perform Venn diagram analysis and identify shared viral probes between NOD-ShiltJ 4, 8, and 12-weeks of age datasets.

Results

In Aim 1, we characterized baseline viral profiles of 12-week NOD murine strain against BALB/c and C57BL-6 (C57) control strains. Analyses showed there were minimal differences between the two control strains, BALB/c and C57. Only 6 viral probes were significantly different between the two control groups (Figure 1). In addition, there were a total of 18 probes shared between the NOD-C57 and the NOD-BALB/c comparison (Figure 1). Further analysis yielded 4 significant probes, which were upregulated and shared between the aforementioned comparisons (Table 1). This analysis identified a significant upregulation of retroviral transcript probes in the

salivary glands of NOD-ShiltJ relative to both C57 and BALB/c controls. These results warranted further investigation of Retroviridae profiles as potential contributors to the pSS phenotype.

In Aim 2, we characterized the natural history of viral profiles present in the NOD-ShiltJ salivary gland tissue over 4, 8, and 12 weeks of age. Based on our finding in Aim 1, we further narrowed our focus to further investigate significant Retroviridae probes observed over 4-12 weeks. Retroviridae 2342 and Retroviridae 297 showed statistically significant upregulation in expression over the course of our study. Retroviral expression did not change in any single direction over the 4-8 week course, however it increased between 8-12 weeks consistent with the natural progression of pSS phenotype in vivo (Figure 2).

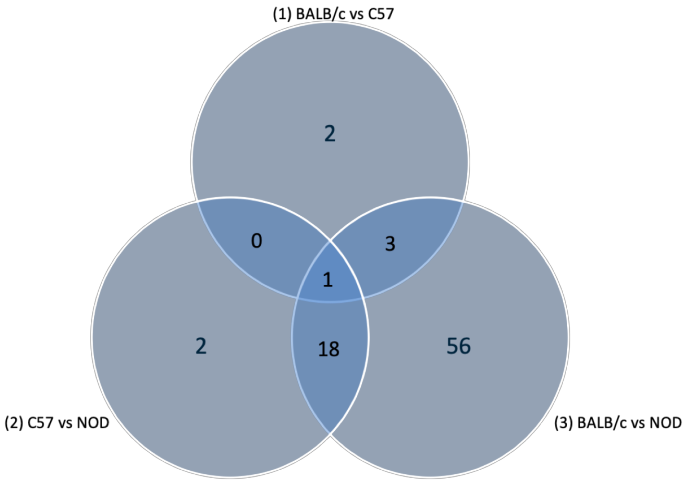

Figure 1. Comparative viral transcript profiles between NOD-ShiltJ and control strains, BALB/c and C57/BL6, identified viral signatures upregulated in the NOD-ShiltJ salivary gland. Eighteen viral probes were differentially expressed in the NOD-ShiltJ relative to control strains and 4 of these probes were significantly upregulated in the NOD-ShiltJ.

Table 1. Viral probes upregulated in NOD-ShiltJ salivary gland relative to C57BL/6 and BALB/c control murine strains. Four viral probes were positively upregulated and shared between the BALB/c vs NOD and C57 vs NOD comparisons. Note that all values included in this table are statistically significant with a p-value<0.05.

Positively Upregulated and Statistically Significant NOD- Viral Probes			
Probe ID	**Normalized Fold Change (NOD vs BALB/c)**	**Normalized Fold Change (NOD vs C57)**	**Viral Family**
Retroviridae 2217	103.0637834	8.905814401	Retroviridae
Retroviridae 2342	2.997221619	2.581586854	Retroviridae
Retroviridae 297	2.167464964	1.889341271	Retroviridae
Totiviridae 3050	10.68498606	16.78546753	Totiviridae

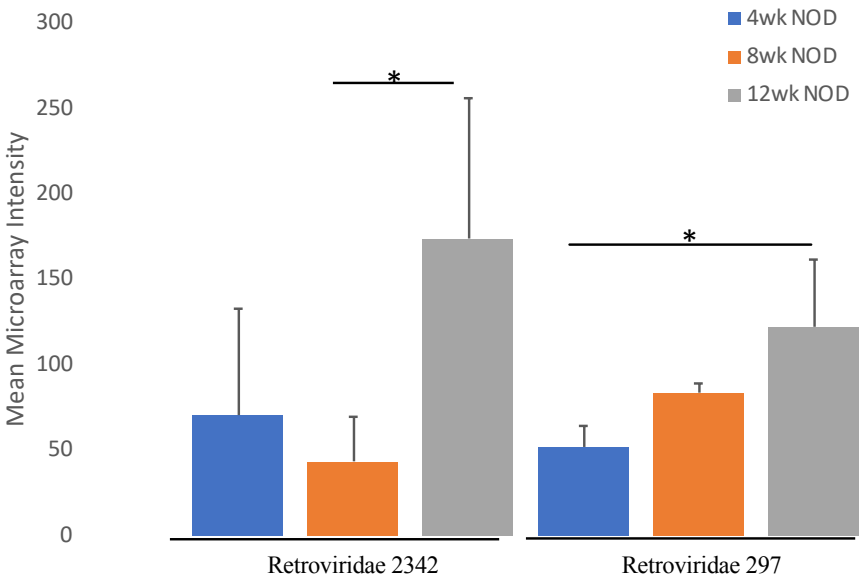

Figure 2. Increased expression of MMTV transcripts in female NOD-ShiltJ salivary gland tissue. A natural history study of NOD-ShiltJ at 4, 8 and 12 weeks of age further confirmed the increase of MMTV transcripts in NOD-ShiltJ salivary gland tissue. ANOVA analysis of all significant microarray viral probes over the course of pSS progression in the NOD mice were analyzed. The mean expression values are plotted at 4, 8 and 12-week for each relevant viral family. (*p-value < 0.05)

Conclusions

Our analysis identified a unique Retroviridae viral profile in the salivary glands of female NOD-ShiltJ mice. Retroviridae probes are significantly upregulated between 8-12 weeks during the natural history of pSS murine model. Prior studies have identified NOD-ShiltJ mice start to present disease phenotype after 8 weeks of age[3]. Thus, the increase in the Retroviral signature coincides with the development of the pSS phenotype in the NOD-ShiltJ model. Our finds suggest there may be a relationship between the pSS phenotype and retroviruses. Further studies are required to confirm the presence of MMTV in NOD-ShiltJ salivary gland and the association between MMTV replication and/or gene expression with the development of pSS phenotype in the NOD-ShiltJ murine model.

References

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