Impact of Traf3 Copy Number on IL-6 Signaling

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Introduction High specificity and diversity are key features of adaptive immunity and are critical for protection against reinfection. Plasma cells (PCs) are fully differentiated B cells that are specifialized to constitutively produce a singular product: one specific, high-affinity antibody (note: PCs are different from memory B cells which are dormant). As such, PCs are major contributors to the afforementioned featues of adaptive immunity. After immature B cells with high-affinity B cell receptors differentiate into PCs, they are sustained by IL-6R signaling. IL-6 is also a growth factor for malignant PCs and therefore must be tightly regulated. Previous work from our laboratory has shown that the adaptor protein Tumor Necrosis Factor Receptor (TNFR)-Associated Factorr 3 (TRAF3) is an important negative regulator of IL-6R signaling in B cells (Figure 1). Upon IL-6 stimulation of the receptor, TRAF3 recruits phosphatase PTPN22 to the IL-6R signaling complex (Figure 1). PTPN22 reverses IL-6 dependent activation of JAK1 and STAT3 by removing the activating phosphorylation, which suppresses downstream effects such as PC development (Figure 1). B cells deficient in TRAF3 (knockouts) have increased IL-6 dependent phosphorylation of JAK1 and STAT3 as a reesult. Most of our work to date has been performed on B cells with a homozygous loss of *Traf3*, but further investigation of how *Traf3* copy number affects TRAF3-dependent phenotypes is justified based on: 1) an increasing number of patients with heterozygous loss-of-function *Traf3* mutations 2) preliminary work indicating B cells TRAF3 levels may decline with age The goal of this project is to reproduce Figure 2 which shows that following IL-6 stimulation of B-*Traf3* heterozygous primary mouse cells, there is enhanced early STAT3 phosphorylation compared to wildtype mice (Figure 2).

[Figure 1. IL-6R signaling pathway.]{E:Documents/My_Project/reference_figure/IL6R_signalpath.png}

[Figure 2. Wildtype, *Traf3* heterozygous, and *Traf3* homozygous primary mouse cells stimulated with IL-6 for given time, then blotted for pSTAT3, STAT3, and actin. * p<0.05, ** p<0.1, and * * * p< 0.001 between WT and KO. X p<0.05 between WT and het.]{E:Documents/My_Project/reference_figure/referencefig.png}

Materials and Methods Western blotting was used to determine the differences in the amount of protein produced by WT, hets, and KO. Figure 2 shows the pooled data from western blots asseessing the differences in the amount of STAT3 phosphorylation in response to IL-6. The data I have is the quantification of the sizes of the bands in the western blots that I have ran and imaged over the last year. This data for 4-5 mice of each genotype has been added to my git repository as an excel spreadsheet. Data for additional mice will be added in the coming weeks. I also have a representative Western blot which will be included

for reference. To create my target graph, I will be using the language R on RStudio. I will use R to organize the data in the optimal way and create means and standard deviations for each time point and genotype prior to creating the graph. The mean and standard deviation will be useful in determining potential outliers and in demonstrating the spread of the data on the graph. I also plan to use R to perform an ANOVA test on these data. This statistical analysis will be used to mark significant differences between wildtype and heterozygous mice, wildtype and knockout mice, and heterozygous and knockout mice. Following this processing of the data, I will be able to construct my graph in the same fashion as the model figure (Figure 2). Up to now (April 08) the data of the female mice has been plotted onto a rudimentary graph using R. The data has been put on the correct axes and is labeled according to mouse genotype. Moving forward, the graph will show averages and standard error bars. There will also be a line connecting the genotypes. Lastly, the ANOVA test will be performed and the p-score will be reflected on the graph with asteriks (added based on feedback from HW2).

Results Thus far the results have been separated by males and females, then the female datapoints have been plotted according to time point and colored according to genotype (Figure 3).

[Figure 3. Preliminary results of a graph made using R. Wildtype, *Traf3* heterozygous, and *Traf3* homozygous female primary mouse B cells stimulated with IL-6 for 0, 15, 30, or 60 minutes, then blotted for pSTAT3Y705 and total STAT3]{E:Documents/My_Project/output/Rplot.png}