Mapping the Influence of Autoimmunity Associated, Non-Coding Genetic Diversity on T Cell Function

Stephanie DeVoe[1], Laura Shaw[2], Angela Prete[2], James Scott-Browne[1,2]

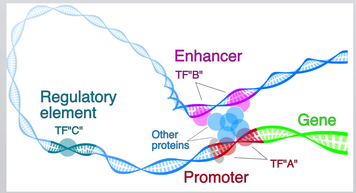
1. Department of Immunology and Microbiology University of Colorado Anschutz, 2. Department of Immunology and Genomic Medicine National Jewish Health

Introduction

Genetic variability between inbred strains of mice permits the study of susceptibility to disease or response to treatment. For example, the non-obese diabetic mouse (NOD) is prone to spontaneous development of autoimmune diabetes; whereas other inbred strains of mice do not carry this risk, such as the C57BL6J (B6) strain. The natural susceptibility of the NOD mouse to disease has been broadly mapped to certain regions of the genome referred to as **i**nsulin **d**ependent **d**iabetes (Idd) loci. Yet, there are over 5.5 million SNPs between the two strains that can influence immune responses. Even in the absence of disease, peripheral NOD T cells have been known to be hyperresponsive to α-CD3/CD28 stimulus. The epigenetic regulatory mechanisms governing the diverging behavior of NOD and B6 T cells has been explored in double positive thymocytes, a precursor population to all T cells, finding strain-specific active regulatory elements in addition to hyperconnectivity within the Idd loci of NOD mice. To understand how strain-specific chromatin regulation impacts responses to persistent antigen stimulation – found in chronic infection, cancer, and autoimmunity – the differences in regulatory elements needs to be assessed in several T cell contexts within the periphery.

Objectives

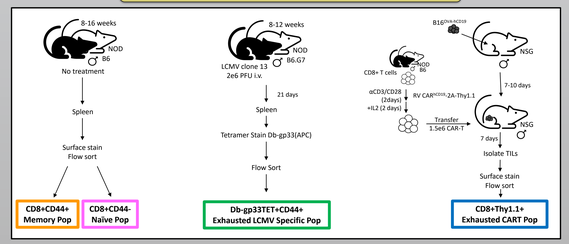
- Identify differentially accessible regulatory elements in peripheral CD8 T cells between NOD and B6 mice
- Understand the influence of genetic polymorphisms on CD8 T cell responses undergoing persistent stimulation
- Identify candidate regulatory elements that may contribute to aberrant T cell responses in NOD mice



Hypothesis

Fine mapping accessible regions of chromatin will reveal disease relevant differences in regulatory elements located near loci canonically associated with CD8 T cell exhaustion profiles in the B6 mouse with the NFAT and NR4A family motifs being most affected by sequence variation.

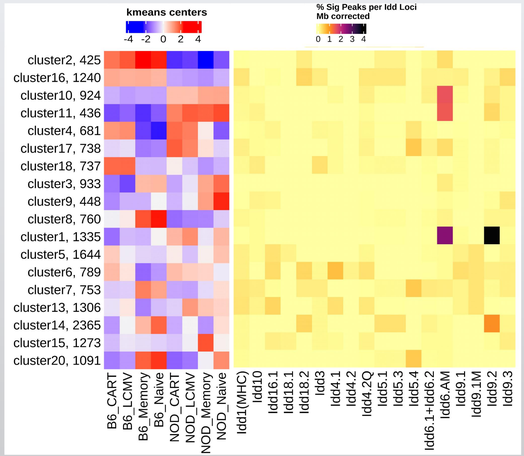
Methods



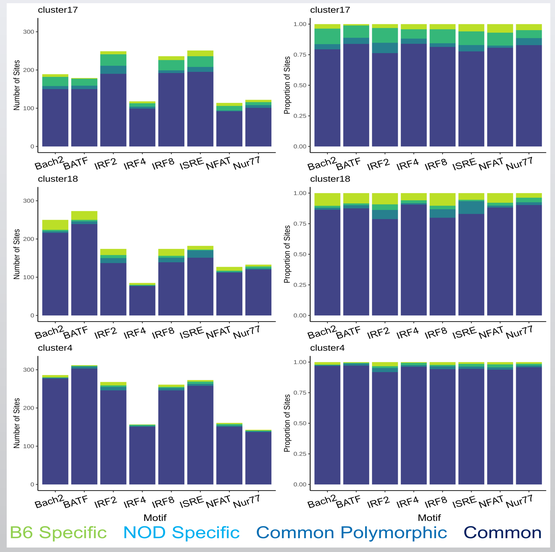
Low input-omni ATACseq was used to determine open chromatin regions in 4 CD8 T cell contexts: naïve, memory, exhausted LCMV-specific, and exhausted CART cells. Reads were processed to strain specific genomes using the ENCODE pipeline, and NOD reads were shifted back to B6 coordinates using MMARGE. Differential accessibility was determined through pairwise, inter-strain comparisons using limma-voom (ie B6 CART vs NOD CART). A fold change cutoff of 3 and an FDR of 0.05 was used to determine significant peaks. FIMO was used to locate transcription factor binding motifs within the NOD and B6 sequences of the differentially accessible peaks. Motif enrichment was calculated based on the number of motif sites per kb of each cluster per sequence normalized to a background set of sequences randomly selected by Homer to match the base content of 10,000 randomly sampled nonsignificant peaks.

Results

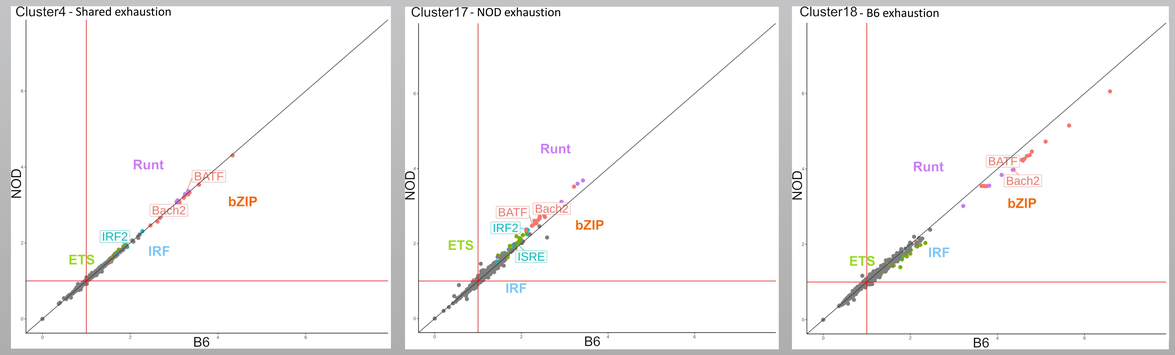
Integration of differentially accessible chromatin regions through k-means clustering reveals profiles of strain-specific, context-specific, as well as strain and context-specific regulatory elements. NOD-specific clusters tended to contain a higher proportion of peaks falling within the Idd6.AM and Idd9.2 loci. Cluster 4 demonstrates a shared exhaustion profile, while clusters 17 and 18 demonstrate and NOD or B6 specific exhaustion profile, respectively.



Shared regions of regulatory elements in exhausted CD8 T cells contain conserved TF sites known to be associated with development of an exhausted state. Fewer than 10% of exhaustion-related TF sites are mutated within cluster 4. Whereas NOD-specific exhaustion elements display the highest proportion of polymorphic sites.



Motif enrichment (below) reveals exhausted CD8 T cells share complementary TF bias in strain-specific profiles. All 3 exhaustion profiles were found to be enriched for bZIP, IRF, ETS, and Runt family motifs compared to background in either strain. Little variability is seen strain-wise within Cluster 4 pertaining to conserved exhaustion-related regulatory elements. However, in Clusters 17 and 18 we see a bias away from equivalency towards the given strain for more enrichment in IRF, RUNX, and bZIP family motifs.

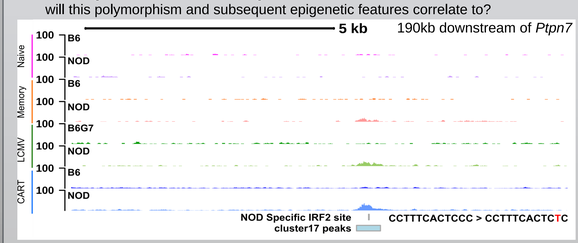


Conclusions

CD8 T cells in NOD and B6 mice will display regions of open chromatin in response to the genome/strain regardless of the T cell context, to the T cell context regardless of the strain, and unique strain and context specific contexts. Exhausted CD8 T cells in NOD and B6 mice have regulatory elements enriched for bZIP, IRF, RUNX and ETS family members. NFAT and NR4A family sites, known to play a prominent role in driving exhaustion states, displayed decreased polymorphisms compared to certain bZIP and IRF family sites. This reinforces the importance of NR4A and NFAT as core mediators of acquiring an exhausted phenotype. Furthermore, genetic polymorphisms are not impacting which mediators are being used to regulate persistent antigen stimulation, as seen in the complementary enrichment of IRF2 and Bach2 within strain-specific clusters. However, the polymorphisms are influencing downstream consequences by dictating *where* binding sites are located. Due to the sequence variation, different sets of genes will have differentially active regulatory elements nearby.

Implications

The number of polymorphisms between NOD and B6 mice are approximately the same as the amount between any two humans. Understanding how ablation or acquisition of a new TF binding site from the standard reference genome will aid in understanding polymorphisms that influence phenotypic nuances of T cells. With additional research we hope to translate findings to address public health issues for prevention and treatment of disease. As shown below, there is a SNP introducing an IRF2 site downstream of Ptpn7 in NOD mice. Ptpn7 is a negative regulator of TCR signaling via dephosphorylation of ERK and p38. Would CART therapy be more or less successful in an individual with an orthologous SNP? Is destruction of pancreatic islets slowed in a Type 1 Diabetic with this SNP? In other words, are effector functions more likely to be retained when the T cell is receiving continuous activation signals and what disease or treatment outcome will this polymorphism and subsequent epigenetic features correlate to?



Future Research

ATACseq reveals where active regulatory elements are, but it reveals little about the resulting functional activity and consequences or long-range looping interactions. Further experiments are needed to obtain a more comprehensive picture of where polymorphisms are influencing diverging mechanisms of chromatin regulation and functionality. For example, a promoter site may be differentially accessible with no change in gene expression, or a site may be equally open with a change in bound TF. In vitro experiments of NOD and B6 splenic CD8 T cells stimulated with α-CD3/CD28 for 24 hours with downstream evaluations of chromatin accessibility, histone modifications, TF occupancy, and gene expression will be done to address the current limitations of our analysis.

References

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