Molecular Mechanisms of Autoimmune Disease

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Seminal work by Yaron Tomer, M.D., has revealed various single nucleotide polymorphisms (SNP) that influence the likelihood of acquiring Autoimmune Thyroid Disease (AITD). Through whole genome screens and associated studies, these SNPs were found to reside in the *thyroglobulin (Tg)*, *MHC II*, *CD 40*, and *CTLA-4* genes. Furthermore, deeper analysis has unearthed striking genetic interactions between disease causing SNPs in the *Tg* and *MHC II* gene, which may mirror a *bona fide* biochemical interaction between the protein products of these genes. My work in the summer focused heavily on developing assays to better elucidate these molecular mechanisms and demonstrate their correlation to AITD.

The first part of my project focused entirely on a SNP in the promoter element of the *Tg* gene. A promoter luciferase system was created and utilized as a way of testing an individual SNP's contribution to the activity of the *Tg* promoter. Interestingly, a disease-causing variant at position -1690 displayed an enhanced activity relative to its protective counterpart. Moreover, the disease causing SNP was shown to harbor an Ets-responsive element. Additional transcription factors that may bind to this region in both the disease causing SNP as well as the protective allele are feverishly being explored using a Yeast-One-Hybrid system. Results from this screen could potentially yield definitive molecular culprits for this SNP's propensity for causing AITD.

In the second part of my study, a reverse immunological approach was employed to identify disease-causing variants of the *Tg* protein. From a list of putative peptides generated *in vivo* by endoprotease digestion, two were selected for further experimentation; one spanning a known disease causing SNP, while the other encompassed a potential iodination site. Both variants of the peptide were synthesized, representing changes in amino acid sequence and iodination state. Using a bacterial recombinant system, which I showed to bind at least as effectively as cell-cultured molecule, empty *MHC II* molecules were constructed and tested by ELISA to check their ability to bind the different peptides. Various other peptides were also assayed using this system to check their capacity as *MHC II* binders as well. Based on our assays, we report that these polymorphic distinctions can have dramatic consequences in terms of peptide binding and capture. Combined, our results shed new light on the subtleties in the relationship between DNA composition, amino acid variations, and post-translational modifications and the pathoetiology of autoimmune disease.