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Identification of Alopecia Areata Autoantigens in C3H/HeJ Mice using Whole-Skin Homogenates

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Alopecia Areata (AA) is a non-scarring cell mediated autoimmune inflammatory disease of the hair follicle (HF). In most AA patients, histopathological examination reveals Dystrophic Anagen stage hair follicles that are surrounded by a peri- and intra-follicular inflammatory cell infiltrates, consisting primarily of CD4 and CD8 T cells. We hypothesize that the hair follicle immune privilege is lost and normally sequestered antigens are exposed to CD8 T cells, which preferentially target Anagen HFs, leading to their destruction. Here, we present an unbiased screening approach to assess whether Anagen HFs are being specifically targeted, and to identify the autoantigen epitopes in C3H/HeJ mice. We isolated skin-draining lymph node cells (LNCs) and extracted protein homogenate from Anagen and Telogen skin of AA-affected and AA-unaffected C3H/HeJ mice. LNCs were cultured with protein homogenates and assessed for T cell activation via IFN γ ELI spot assays. We found that Anagen skin protein homogenates induced a higher frequency of T cell activation in both AA-affected and AA-unaffected mice, showing that T cells are more activated by anagen HFs than telogen HFs, consistent with preferential expression of AA autoantigens in the Anagen phase of the hair cycle. To further narrow down candidate antigen targets, protein homogenates were separated by column chromatography into individual fractions of proteins. Using this approach, we found that one out of four Anagen fractions, which contains molecules with larger molecular weight, preferentially induced an immune response. We are currently performing a bias screening approach and testing AA associated proteins against melanogenesis related antigens and keratinocyte derived antigens. Characterizing the protein content and autoantigen epitopes will facilitate the identification of specific antigens and aid in the rational development of new therapies for AA.