High-throughput single-cell β TCR sequencing identifies pathogenic CD8+ T cell clones that are sufficient to induce alopecia areata in a C3H/HeJ retrogenic model. Z Dai1, EH Wang1, EY Lee1, YJ Mong1, M Zhang1, and AM Christiano1,2 | Dermatology, Columbia University, New York, New York, United States and 2 Genetics & Development, Columbia University, New York, New York, United States

Alopecia areata (AA) is an autoimmune disease in which CD8+ T cells attack hair follicles and leads to nonscarring hair loss. AA is postulated to be an antigen-driven disease, however, whether pathogenesis requires T cell-mediated antigen recognition, and if so, what epitope(s) the T cells recognize, remains unclear. Our previous work showed that clonal expansion of CD8+ T cells coincides with AA disease onset, and we identified dominant T cell receptor (TCR) sequences shared among independent samples from the C3H/HeJ mouse model of AA, supporting the antigen-driven nature of AA. To understand whether specific subsets of CD8+ T cells undergo clonal expansion and drive AA pathogenesis, here we performed parallel single-cell RNA-sequencing and single-cell VDJ-sequencing on individual immune cells harvested from the skin and lymph nodes of affected vs. unaffected C3H/HeJ mice. In AA mice, the clonal repertoire of CD8+ T cells in the skin becomes significantly restricted, whereas cells in the lymph node retain clonal diversity, suggesting that pathogenic CD8+ T cells undergo clonal expansion in the skin after antigen recognition. This independent dataset validated our previously reported TCR sequences, and we found that the degree of clonal expansion correlated with gene signatures suggestive of T cell activation and pathogenicity. Using one of the highly expanded β TCR pairs, we generated TCR retrogenic mice, in which SCID C3H/HeJ mice devoid of endogenous T cells were reconstituted with bone marrow cells expressing only the TCR sequence of interest. TCR retrogenic mice displayed normal T cell development, including CD8+ T cells, and by 7 weeks post-transplantation over 80% of mice developed AA-like hair loss. Our results indicate that this pathogenic CD8+ T cell clone is sufficient to induce spontaneous AA in C3H/HeJ mice, and strongly supports the antigen-driven nature of AA.

Single-cell RNA sequencing identifies a disease-dominant CD8+ T cell population co-expressing both activating and inhibitory receptors of the NKG2 family. Z Dai1, EH Wang1, EY Lee1, YJ Mong1, M Zhang1, and AM Christiano1,2 | Dermatology, Columbia University, New York, New York, United States and 2 Genetics & Development, Columbia University, New York, New York, United States

Alopecia areata (AA) is a cell-mediated, autoimmune form of hair loss characterized by lymphocytic infiltration of the hair follicles (HFs), inflammation, and destruction of the HF. We previously showed that CD8+ NKG2D+ T cells are necessary and sufficient to induce AA in the graft-induced C3H/HeJ mouse model. Here, we used single-cell RNA sequencing to comprehensively profile the T cell component of the inflammatory infiltrate in AA. We first isolated CD8+ T cells from the skin of affected and unaffected control mice and focused our analysis on CD8+ T cells. We observed a marked expansion of all CD8+ T cells in AA mice (41% versus 4% of all CD4+ T cells in control mice). CD8+ T cells in both AA and control mice were clustered into 5 distinct populations, each with an associated set of marker genes. Shared CD8+ populations included antigen-experienced effector cells with high expression of IFNG, GZMA, and a memory T cell population with high CCR7 and CD44L, both of which were expanded in AA mice. Interestingly, we discovered a population of CD8+ T cells that is largely predominant in AA mice, marked by increased expression of not only NKG2D, an activating receptor, but also NKG2A, a known inhibitory receptor of the NKG2 family. These cells were also characterized by increased expression of T cell exhaustion markers (PD1, TIM3, CTLA4) as well as the co-stimulatory markers CD137 and ICOS. To probe the NKG2A receptor pharmacologically, we found that treatment of mouse T cells in vitro with an NKG2A agonist antibody resulted decreased IFNG production, and treatment of C3H/HeJ mice with a blocking antibody against Qa-1 (an NKG2A ligand) prevented disease induction via engraftment resulting in earlier AA onset and accelerated disease progression in vivo. Therapeutic manipulation of the NKG2 family of activating and inhibitory receptors represents a novel treatment approach in AA.