**036**

Functional interrogation of immune cell types identified by single-cell RNA sequencing in alopecia areata

**EY Lee1, Z Dai1, E Chang1 and AM Christiano1,2**

Alopecia areata (AA) is an inflammatory disease in which autoreactive CD8+ T cells attack the hair follicle (HF) and result in non-scarring alopecia. Previous work from our lab, using both human samples and the graft-induced C3H/HeJ mouse model, established that CD8+ NKGD2+ T cells are the major pathogenic drivers of AA. However, the role of other immune cell types in mediating AA was unclear. In this study, we performed single-cell RNA-sequencing (scRNAseq) of CD8++ immune cells in skin harvested from affected and unaffected mice. We also performed antibody-based depletion of major immune cell populations to functionally interrogate their role in disease onset, in which 7-week-old C3H/HeJ mice were treated with antibodies one week prior to disease induction via engraftment, followed by continued antibody treatment for two weeks. Consistent with our previous work, depletion of a single population of CD8+ T cells in AA, and depletion of CD8+ T cells resulted in complete disease prevention. Non-Treg CD4+ T cells comprised a minor population in our dataset, and accordingly, their depletion resulted in only a slight delay in disease onset with the graft model. Depletion of other minor cell types in our dataset, such as γδ-T cells, NK cells, and B cells, had no effect on the kinetics of AA disease onset. Interestingly, depletion of CFSE++ melanoid cells delayed disease onset in similar extent as non-Treg CD4+ T cell depletion. Although CFSE+ cells comprised a smaller proportion of the immune landscape in AA compared to control skin, they showed upregulation of inflammatory cytokines such as CCL5 and CXCL10, which are known to activate T cells. Functional dissection of immune cell populations in AA supported that CD8+ T cells are the major pathogenic cell type in disease, and uncovered novel roles for myeloid lineages in AA onset.

**037**

IL-7 regulates the PD-1 signaling pathway via degradation by E3 ubiquitin ligase F-box Protein 38

**Z Dai1, Y Chang1 and AM Christiano1,2**

Alopecia areata (AA) is a T cell-mediated autoimmune disease attacking the hair follicle (HF). The IL-7 signaling pathway plays an important role in T cell survival and it has been therapeutically targeted in several T cell-dependent autoimmune disease models. Here, we showed that C3H/HeJ mice with AA exhibited hair regrowth after anti-IL7Ra treatment. Mechanistically, we observed that IL-7Ra blockade significantly reduced the number of alopecic effector CD8+ T cells. We also found that C3H/HeJ mice treated with anti-IL7Ra showed a significant increase in the frequency of PD-1+CD44+ T cells within SLOs compared to controls. Our previous results indicated that IL-7 may antagonize the function of PD-1 by downregulating the expression of PD-1 in T cells, however, the mechanism of this downregulation is not understood. Recently, the F-box protein FBXO38, a member of the SKP1–CUL1–F-box protein family of E3 ubiquitin ligases, has been shown to interact with PD-1 and decrease PD-1 cell-surface expression via degradation. Based on this findings, we postulated that IL-7 might decrease PD-1 expression through upregulation of FBXO38. We found that IL-7 significantly increased the transcription levels of FBXO38 and concurrently decreased the expression of PD-1 on the surface of T cells in vitro. We further observed that FBXO38 knockdown using siRNA increased the PD-1 expression on the surface of T cells compared to control, and moreover, this effect could not be rescued by IL-7 treatment. Our results indicate FBXO38 as a critical mediator of PD-1 degradation and suggests that targeting IL-7-mediated regulation of FBXO38 expression may represent a potential strategy to enhance PD-1 signaling in T cell-mediated autoimmune diseases including AA.

**038**

High-throughput single-cell β2 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, W Zeng1, P Perez-Lorenzo1 and AM Christiano1,2**

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, remain 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 β2 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.

**039**

CXCR3 blockade reduces skin germinal center B cells and autoantibody titers in murine cutaneous lupus erythematosus

**HS Raef1,2, L Wong1, C Garelli1, E Kim1, M Ahmed1, K Pike1, S Moses1, J Harris1, A Marshak-Rothstein1, M Rashighi1 and J Richmond1**

Cutaneous Lupus Erythematosus (CLE) describes a broad range of autoimmune dermatologic diseases that are characterized histopathologically by interface dermatitis and autoantibody deposition. Racial and ethnic disparities have been reported in disease activity and outcomes in CLE, with African Americans presenting with greater disease severity. Treatment options available to patients with CLE are unfortunately limited. It is important to understand the pathogenesis of CLE to develop effective treatments for patients. One of the most highly upregulated chemokine receptors in CLE is the CXCR3 chemokine family. The interaction between CXCR3-expressing T cells and its ligands have been associated with tissue damage in CLE subtypes. To further understand the role of CXCR3 in CLE immunopathogenesis, we performed functional studies using a mouse model of CLE and human tissue. Here, we characterize CXCR3-bearing immune cells in the skin of this mouse model and in blister biopsies obtained from CLE patients. We observed higher expression of CXCR3 on T cells and B cells, supporting the role of CXCR3 in the pathogenesis of CLE. We then wanted to determine whether CXCR3 blockade with a monoclonal antibody could prevent CLE disease development. We show that CXCR3 blockade in CLE mouse models stabilized skin lesions and helped reduce autoantibody titers and germinal centers. This suggests that blockade of CXCR3 may have preventative effects, resulting in reduced autoimmune body titers. These results provide further rationale for targeting CXCR3 in CLE.

**040**

Single-cell RNA sequencing identifies a disease-promoting dominant CD8+ T cell population co-expressing both activating and inhibitory receptors of the NK2 family

**Z Dai1, EH Wang1, EY Lee1, I Moriga1, M Zhang1 and AM Christiano1,2**

Alopecia areata (AA) is a T cell-mediated, autoimmune form of hair loss characterized by lymphocytic infiltration of the bulge region of the hair follicles (HF), inflammation, and destruction of the HF. We previously showed that CD8+ NKGD2+ 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 CD69 and CD40LG, 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 NKGD2, an activating receptor, but also NKGA2, a known inhibitory receptor of the NK2 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 NKGA2 receptor pharmacologically, we found that treatment of mouse T cells in vitro with an NKGA2 agonist antibody reduced increased IFNG production, and treatment of C3H/HeJ mice with a blocking antibody against Qa-1 (an NKGA2 ligand) prevented disease induction via engraftment resulted in earlier AA onset and accelerated disease progression in vivo. Therapeutic manipulation of the NK2 family of activating and inhibitory receptors represents a novel treatment approach in AA.