SCIENTIFIC ABSTRACT

Tissue-resident memory $CD8^+$ T cells (T_{RM}) are implicated in the pathophysiology of psoriatic lesions, and have been found in inflamed skin, joints, eye, gut and peripheral blood of patients with psoriatic arthritis (PsA). T_{RM} are implicated in recurrent psoriasis flares, and are present in actively inflamed synovium, intestines, and eyes of PsA patients. Eomesodermin (Eomes) is a transcription factor critical for the maintenance of circulating memory $CD8^+$ T cells, but its role in the formation and maintenance of T_{RM} is tissue-specific. Preliminary data in a murine model of $CD8^+$ T cell antigen-induced arthritis shows that loss of Eomes increases the number of synovial T_{RM} observed in affected joints. The proposed research will investigate the transcriptional changes following Eomes ablation in established synovial T_{RM} that may underpin observed changes in synovial T_{RM} survival and retention. This work may identify mediators regulating synovial T_{RM} that may serve as future therapeutic targets for our patients with PsA and other inflammatory arthritis.

LAY ABSTRACT

Psoriatic arthritis (PsA) is an autoimmune disease characterized by chronic joint pain, swelling, and damage and recurring flares of synovial inflammation. While new treatments are highly effective in treating PsA, most patients do not reach remission or are refractory to multiple lines of treatment. Recurrent flares are thought to be due to tissue-resident memory $CD8^+$ T cells (T_{RM}) that remain within the affected joints and skin. Eomesodermin (Eomes) is a transcription factor, a gene that controls cellular programming, that is important for circulating memory T cells. Earlly experiments that look at what happens when Eomes is removed from CD8 T cells suggest that Eomes regulates the accumulation of T_{RM} within joints, and may play a significant role in PsA. Understanding how Eomes controls synovial T_{RM} in inflammatory arthritis may provide new strategies to treat PsA.

BACKGROUND

Psoriatic arthritis (PsA) is a chronic inflammatory disease arising from aberrant innate and adaptive immunity characterized by peripheral and axial inflammatory arthritis, gut inflammation, and uveitis, and psoriasis affecting around 25-40% of patients with psoriasis(*1*). While biologic therapies are highly effective, more than half of patients do not reach remission or are refractory to treatment, and maintaining treatment durability is a persistent challenge(*2-4*). Greater understanding of the pathophysiology of PsA is needed to broaden the therapeutic armamentarium. Tissue-resident memory $CD8^+$ T cells (T_{RM}) are implicated in the pathophysiology of recurrent synovitis flares and are enriched in synovium of PsA patients(*5-7*). Although clonally expanded populations of tissue-resident memory-like $CD8^+$ T cells are present in the circulation and tissue of PsA patients, the *mechanisms by which synovial T_{RM} persist in synovium and contribute to joint destruction is not well understood*.

<u>T_{RM} provide long-lived immunological memory within tissues:</u> $CD8^+$ T cells are being recognized to be increasingly central in the pathophysiology of PsA(*6*). T_{RM} are a subset of antigen-specific cytotoxic $CD8^+$ T cells and remain in tissue following initial activation to provide rapid immunologic defense to re-invading pathogens. In autoimmune disease like PsA, T_{RM} persist in chronic inflammatory lesions and are associated with disease recurrence and flares(*5, 7, 8*). Understanding the regulatory factors that promote T_{RM} formation and maintain persistence in health and disease is important for developing targeted therapies.

<u>Transcriptional programming controlling T_{RM} persistence and behavior is unique to tissue and disease context.</u> Work done by others and our lab has shown that transcription factors (TF) that regulate T cells are tissue specific(*9, 10*). Within the heterogeneous populations of skin T_{RM} for instance, T_{RM} that produce interferon gamma (IFNɣ) or interleukin-17 (IL-17) have differential cytokine requirements for long-term maintenance within tissues(*9, 11*). IFNɣ-producing T_{RM} require IL-15 while IL-17-producing T_{RM} require IL-7 to survive. Clinical trials of IL-15 blockade has been promising for the IFNɣ-driven skin disease vitiligo(*12*), but less promising for an IL-17-driven disease such as psoriasis. These translational insights follow robust investigation of transcriptional programming in skin T_{RM}. However, our lack of understanding of the TFs that regulate synovial T_{RM} remains a major barrier for PsA management.

<u>Eomesodermin (Eomes) may be a critical TF in chronic psoriatic disease.</u> Eomes, a TF critical for the long-term maintenance of circulating memory $CD8^+$ T cells and had previously been shown to inhibit skin T_{RM} formation(*9*).

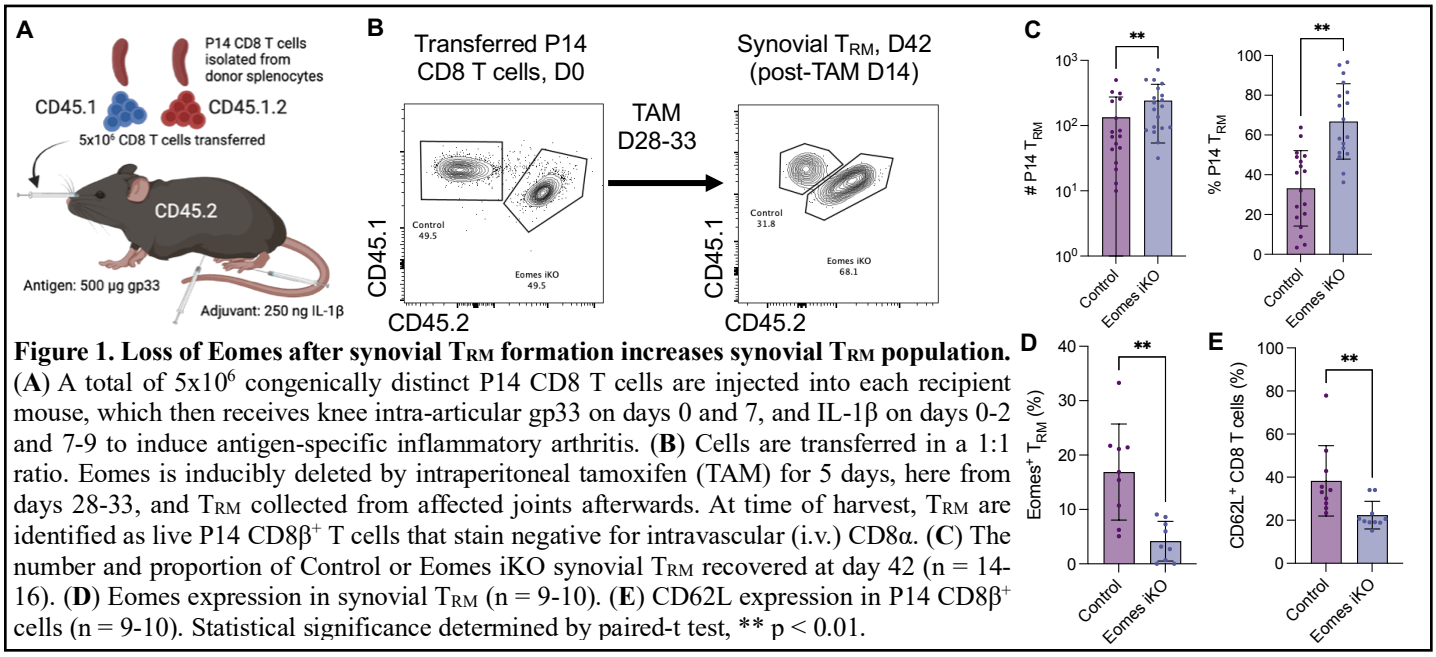

Figure 1. Loss of Eomes after synovial T_{RM} formation increases synovial T_{RM} population. (**A**) A total of $5x10^6$ congenically distinct P14 CD8 T cells are injected into each recipient mouse, which then receives knee intra-articular gp33 on days 0 and 7, and IL-1β on days 0-2 and 7-9 to induce antigen-specific inflammatory arthritis. (**B**) Cells are transferred in a 1:1 ratio. Eomes is inducibly deleted by intraperitoneal tamoxifen (TAM) for 5 days, here from days 28-33, and T_{RM} collected from affected joints afterwards. At time of harvest, T_{RM} are identified as live P14 CD8β$^+$ T cells that stain negative for intravascular (i.v.) CD8α. (**C**) The number and proportion of Control or Eomes iKO synovial T_{RM} recovered at day 42 (n = 14-16). (**D**) Eomes expression in synovial T_{RM} (n = 9-10). (**E**) CD62L expression in P14 CD8β$^+$ cells (n = 9-10). Statistical significance determined by paired-t test, ** $p < 0.01$.

We have previously reported this differential requirement for Eomes in small intestinal T_{RM} where loss of Eomes had no effect on small intestinal T_{RM} formation but was required for long-term maintenance(*10*). However, in the context of chronic psoriasis, Eomes expression in T cells is significantly increased in lesional and non-lesional skin compared to healthy controls(*13*). This raises the question of whether Eomes may be important for the maintenance and function of established synovial T_{RM} in inflammatory arthritis.

<u>Eomes-deficient synovial T_{RM} increases synovial T_{RM} populations.</u> To study transcriptional regulation of synovial T_{RM} and the effects of Eomes deletion, I used the antigen-specific P14 mouse/gp33-peptide system as an antigen-induced arthritis model. My preliminary data shows that Eomes ablation after the establishment of synovial T_{RM} increases the number and proportion of synovial T_{RM} (**Fig. 1A-D**). Eomes-deficient CD8$^+$ T cells had decreased expression of Eomes target gene CD62L (**Fig. 1E**), a lymph node homing marker on circulating lymphocytes, suggesting that Eomes alters tissue retention phenotype.

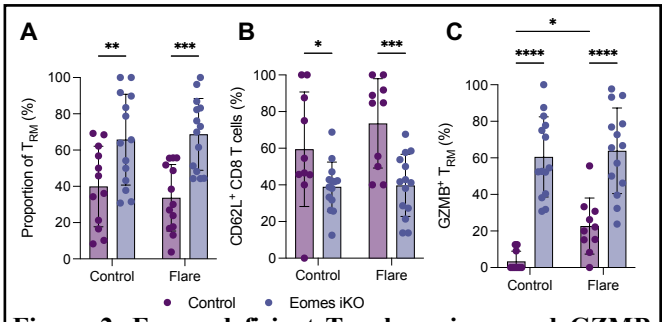

Figure 2. Eomes-deficient T_{RM} have increased GZMB. Eomes was inducibly deleted from synovial T_{RM} on D28-33, and 100 µg gp33 was injected i.p. to induce a flare. T_{RM} are defined as CD8 T cells that were negative for i.v. CD8α. (**A**) Proportion of synovial T_{RM}. (**B**) CD62L expression in CD8 T cells. (**C**) Expression of GZMB in synovial T_{RM}. Statistical significance determined by two-way ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; n = 10-14.

<u>Eomes-deficient synovial T_{RM} have increased cytotoxicity.</u> I next evaluated the effect of a flare on Eomes-deficient T_{RM}. Using the model depicted in **Fig. 1**, I induced a flare by intraperitoneal (i.p.) gp33 injection on day 37 post-induction, and evaluated synovial T_{RM} on day 42 post-induction. My preliminary data shows that in an acute flare, Eomes iKO T_{RM} populations maintain their survival advantage over Control T_{RM}, and that Eomes iKO T_{RM} have decreased CD62L expression (**Fig. 2A** and **B**). Eomes iKO have = increased expression of cytotoxic granzyme B compared to Control synovial T_{RM} (**Fig. 2C**).

My preliminary findings raises the hypothesis that Eomes may play a complex role in inflammatory arthritis, and may suppress synovial T_{RM} populations and cytotoxicity. This pattern of increased cell numbers with deletion of Eomes has also been reported with tissue-resident tumor infiltrating CD8$^+$ T cells in B16-IL33 melanoma model, where the loss of Eomes promoted the accumulation of CD8$^+$ T cells within the tumor microenvironment, increased expression of IFNγ, and inhibited of tumor growth(*14*). This proposal aims *to investigate transcriptional changes following Eomes deletion in synovial TRM by leveraging single-cell RNA sequencing to identify potential mediators of observed phenotypic changes.*

METHODS

Antigen-induced arthritis model: P14 mice are C57Bl/6 mice with a transgenic T cell receptor to the gp33 peptide from LCMV on a TCRα-deficient background used to study of antigen-specific responses. P14 mice have been crossbred with B6.129-*Gt(ROSA)26Sor*$^{tm1(cre/ERT2)Tyj}$ ($Cre^{+/-}$) mice, containing tamoxifen (TAM)-inducible Cre recombinase, and B6.129S1(Cg)-*Eomestm1.1Bflu* ($Eomes^{f/f}$) mice, which have *loxP* sites flanking exon 2 of the *Eomes* gene (Eomes iKO); P14 $Eomes^{+/+}Cre^{+/-}$ mice are used as Controls. I adoptively transfer $5x10^6$ CD8 CD45.1 T cells into congenically distinct CD45.2 recipient mice on day 0 by retro-orbital injection. Recipient mice receive intra-articular gp33 (250 μg/10 μL) into each knee on day 0 and 7 and IL-1β (250 ng/20 μL) by subcutaneous footpad injection on days 0-2 and 7-9. I administer TAM at 75 mg/kg body weight by intraperitoneal injection on days 28-32 to delete Eomes, and harvest synovial tissue on day 42.

Isolation of synovial T_{RM}. Prior to harvest, I label intravascular (i.v.) cells with anti-mouse CD8α by retro-orbital injection to distinguish cells without access to circulation from those in residing tissue. I harvest synovium from knees by removing skin, fat and muscle tissue along the leg followed by amputation at the distal femur and the proximal tibia. Synovial tissue is digested enzymatically (RPMI1640, 10% FBS, 0.1 mg/mL DNase I, 1 mg/mL collagenase type IV) at 37°C for 30 minutes in a shaking incubator, followed by mechanical dissociation, and resuspension in media fluorescence-activated cell sorting. Synovial T_{RM} are defined and sorted as $CD45.1^+CD45.2^-CD8\beta^+$ lymphocytes from that stain negative for i.v. CD8α.

Single-cell gene expression of synovial T_{RM}. Sorted cells will be processed for single-cell gene expression via the Chromium Single Cell 3' Gene Expression workflow (10X Genomics) as I have done previously(*10, 15, 16*). Synovial T_{RM} will be pooled from 10 mice receiving Control cells or 10 mice receiving Eomes iKO cells, which would yield approximately 4,000 and 5,000 synovial T_{RM}, respectively. Samples will then be sequenced by the UCSD Institute of Genomic Medicine Core Facility. Sequencing data will undergo quality control, be processed through Cell Ranger (10X Genomics), and analyzed with Seurat in R (R Project). The primary endpoint for this experiment is the average gene expressions of Control and Eomes iKO synovial T_{RM}. Secondary endpoints include differentially expressed genes, Gene Ontology enrichment, gene set enrichment analysis, and comparison with publicly available PsA patient-derived gene expression data.

EXPECTED RESULTS

Based on my preliminary data, I expect that the average gene expression of Control vs Eomes iKO will corroborate existing flow cytometry data. Additionally, I expect differences in genes involved in cell survival or tissue retention, which may identify candidate genes for further study and validation. Regarding feasibility, I have experience performing, analyzing and publishing single-cell gene expression experiments from murine and patient-derived intestinal tissues(*10, 15, 16*). An alternative approach to evaluating single-cell gene expression of synovial T_{RM} would be to adoptively transfer CD8 T cells from female mice into male recipients, and running bulk cells through the Chromium Single Cell 3' Gene Expression workflow. In this approach, donors cells are identified by expression of *XIST*, but I would not be able to distinguish T_{RM} from other circulating memory CD8 T cells without integrating cell surface protein expression assays as well.

SIGNIFICANCE FOR PSORIATIC DISEASE

Psoriatic arthritis is a multi-modal disease that affects skin and joints, as well as other extra-articular manifestations and co-morbidities. However, biologic therapy does not equally improve all manifestations of disease, and in the case of IL-17 inhibitors and inflammatory bowel disease, may instead aggravate disease. Our knowledge of synovial T_{RM} is limited and underdeveloped compared with that of skin T_{RM}, and inflammatory arthritis remains a major component of the patient experience. This proposal investigating the gene expressions of synovial T_{RM} in a murine model of inflammatory arthritis provides a basis for identifying genes that may regulate T_{RM} survival or tissue retention as possible therapeutic targets for PsA. Supporting this work would accelerate research into the pathophysiology of synovitis and as well as translational projects that would be able to shape future patient care.

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