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Melanocytes in psoriasis: convicted culprit or bullied bystander?

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A recent study published in the *Journal of Experimental Medicine* reports that melanocytes may be targets of autoreactive T cells in psoriasis. Psoriasis is a common inflammatory disease of the skin that affects 1–3% of people worldwide. It presents with well-demarcated, scaly plaques that reduce the quality of life of affected patients (Lowe et al. 2014).

Historically, psoriasis was thought to be a disorder of keratinocyte hyperproliferation, but most investigators now agree the driving force behind the disease is a dysregulated immune signaling cascade that results in the characteristically inflamed psoriatic lesions (Lowe et al. 2014). There is an ongoing debate whether the disease is truly autoimmune in origin, and if it is, it remains unclear what specific antigens, let alone which cell types, are the targets of this autoimmune response.

The pathogenesis of psoriasis is mediated by several immune populations in the skin, and many of the inflammatory pathways involved are now well described. These pathways lead to continued immune cell recruitment, inflammation, and the unrestrained proliferation of keratinocytes, which gives rise to the thickened, scaly plaques (Lowe et al. 2014). In the chronic inflammatory phase of the disease, T cells play a prominent role, which may produce Th1, Th17 or Th22-specific cytokines. Of these, the IL-23/IL-17/TNF- α axis appears to be central to disease pathogenesis, as targeting this axis for treatment results in vast improvement of the disease (Lowe et al. 2014). Classically, IL-17 producing cells were thought to be $\alpha\beta$ T cells, but more recent studies of psoriasis in both humans and mice have revealed an emerging role of $\gamma\delta$ T cells as additional contributors (Lowe et al. 2014). This has important implications for antigen targeting in the disease, as $\alpha\beta$ T cells recognize specific antigens in the context of HLA presentation, while $\gamma\delta$ T cells do not.

Despite advancements in our understanding of the key role T cells play in the self-amplifying inflammatory signaling loop, it is not known if some of these T cells are responding to an autoantigen. Several potential antigens have been identified, but thus far none are reportedly cell-specific. For example, psoriasis patients have autoreactive T cell clones against the antimicrobial peptide LL37, which is also implicated in initiation of psoriasis (Lande et al. 2014). Likewise, experimental animal models have implicated a number of other potential autoantigens (Lowe et al. 2014). Important evidence in support of an autoantigen includes preferential V α 3S1/V β 13S1 chain rearrangement in the TCR among the CD8+ T cell clones present in psoriatic skin (Kim et al. 2012). This suggests that the specific antigen of this prevalent TCR is present in the skin, which could lead to the continued recruitment and retention of these cells. Coupled with the fact that a prominent risk allele for psoriasis, HLA-C*06:02, is involved in self-antigen display (Lowe et al. 2014), it is conceivable that an autoantigen leads to activation of T cells with a V α 3S1/V β 13S1 TCR, which then promotes psoriatic inflammation.

Based on this hypothesis, Arakawa and colleagues sought to identify potential psoriatic autoantigens by using a T cell hybridoma reconstituted with a V α 3S1/ V β 13S1 TCR derived from a HLA-C*06:02 positive psoriatic patient. This particular T cell hybridoma reported TCR signaling by robust GFP expression under the control of NFAT, which allowed the authors to detect low-level TCR stimulation by TCR-transfected cell lines. This became a powerful tool through which they could investigate potential skin resident cells as the source of psoriatic autoantigen. TCR signaling, indicated by GFP signal, only occurred during co-culture with melanocyte cell lines, and only in the context of HLA-C*06:02. Histologic studies of psoriatic plaques revealed a higher frequency of CD8⁺ T cell-melanocyte contacts. This also revealed that CD8⁺ T cells directed lytic granules toward contact sites with melanocytes. Taken together, these results support the hypothesis that some of the recruited CD8⁺ T cells in psoriatic skin recognize and respond to a melanocyte-specific antigen.

The authors then sought to identify the antigen recognized by their T cell clone. They screened plasmid-encoded combinatorial peptide libraries to determine which antigens were recognized by their reconstituted TCR in the context of HLA-C*06:02. They identified several mimotopes with overlap in sequence and positional residue preference. They then performed bioinformatic searches against the human proteome and transcriptome to identify naturally occurring self peptides. These experiments allowed the authors to narrow nearly a billion peptides to just six full-length, naturally occurring potential autoantigens. They then tested if HLA-C*06:02 positive cells expressing the full protein of each of these potential autoantigens would trigger TCR activation in their hybridoma line. They found that only full-length protein of a disintegrin-like and metalloprotease domain containing thrombospondin type 1 motif-like 5 (ADAMTSL5) yielded GFP signal when expressed in target cells during cocubation experiments with the T cell hybridoma. Thus, ADAMTSL5 became the first autoantigen in psoriasis found by examining a predominant T cell clone derived from psoriatic skin.

However, to rule out the possibility that the recognition of ADAMTSL5 was limited only to the one patient donor in this study, the authors isolated PBMCs from psoriatic and healthy patients to perform ex vivo simulation using ADAMTSL5 and irrelevant peptides. They found that CD8⁺ T cells from psoriatic, but not healthy individuals, made more IL-17a and IFN- γ in response to ADAMTSL5. Moreover, PBMCs from HLA-C*06:02-positive and HLA-C*06:02-negative psoriatic individuals responded to the peptide, suggesting that HLA-C*06:02 is not a requirement for the development of activated ADAMTSL5-specific clones in psoriasis. Finally, the authors searched for the source of ADAMTSL5 and found it highly expressed in melanocytes and melanocyte cell lines compared with whole skin by RT-qPCR. Similarly, immunofluorescence analysis revealed that only melanocytes expressed ADAMTSL5 and, in psoriatic lesions, CD8⁺ T cells were observed to be in direct contact with ADAMTSL5⁺ melanocytes. Taken together, this would suggest that melanocyte-specific T cell clones exist in psoriatic individuals and infiltrate psoriatic lesions.

This report suggests that psoriasis may be an autoimmune attack on melanocytes, although the findings are surprising and lead to many more intriguing questions. As the authors discuss, the discovery of the autoantigen ADAMTSL5 was based on working from a single clone isolated from a single individual; therefore, this finding does not preclude a role for

additional autoreactive T cell clones in psoriasis pathogenesis. Other clones could react to different skin cell specific antigens, including keratinocyte antigens, a possibility that has been proposed by the same group and others. It also remains unclear whether these autoreactive, ADAMTSL5-specific T cells are required for any particular psoriatic phenotype, of which there are many (e.g. plaque versus guttate). The authors discovered that evidence of CD8⁺ T cell degranulation occurs in the presence of ADAMTSL5-positive melanocytes, yet it does not result in melanocyte death. This is consistent with recent literature describing a role for the psoriatic axis cytokine TNF- α and IL-17 in interfering with melanogenesis. Intriguingly, these cytokines actually cause an increase in melanocyte number in psoriatic skin; although psoriatic lesions are commonly hypopigmented during inflammation, after resolution areas can become hyperpigmented. This is in stark contrast to vitiligo, in which IFN- γ , and not IL-17, drives autoreactive CD8⁺ T-cell attack of melanocytes, leading to melanocyte loss and subsequent depigmentation (Rashigi et al. 2014). In this context, psoriatic IL-17 driven inflammation may temporarily inactivate or 'stun' melanocytes, resulting in hypopigmentation that can later resolve or overcompensate, while vitiligo IFN- γ -driven inflammation destroys melanocytes within lesions, resulting in long-lasting depigmentation.

A related question involves the age-old chicken and egg problem – do the ADAMTSL5-specific, autoreactive T cells present in psoriatic individuals lead to psoriatic inflammation, or does the dysregulated immune network in psoriasis promote the recruitment or expansion of self-reactive T-cell clones? It is feasible that psoriatic inflammation precedes the recruitment of ADAMTSL5-specific T cells. If so, these cells could be unrelated to the pathogenesis of psoriasis, but recruited to psoriatic skin where self-tolerance is then lost in the inflammatory milieu.

Additionally, if ADAMTSL5 is an autoantigen in psoriasis, it would raise the question of why psoriatic inflammation is limited to the skin. ADAMTS family proteins are involved in microfibril biogenesis and formation, and they are widely expressed in multiple tissues. ADAMTSL5, although enriched in melanocytes in skin compared to other skin cell populations, is expressed in all tissues tested in mice (Bader et al., 2012). Human expression data is scarce, but expression profiles of ADAMTSL5 produced by the Genotype-Tissue Expression (GTEx) project and the Human Protein Atlas suggests ADAMTSL5 is more highly expressed in female reproductive tissues, the heart, and esophagus compared to other sites, including the skin, and immunohistochemical studies suggest the ADAMTSL5 is enriched in melanocytes and skin fibroblasts. If ADAMTSL5-specific T cells are responsible for psoriatic inflammation, it would be important to understand why other tissue sites do not become involved in disease pathogenesis.

Whether or not psoriasis is an autoimmune disease remains under debate; however, Arakawa and colleagues have provided evidence for the involvement of ADAMTSL5-specific autoreactive T cells and have indicated melanocytes as the target cell type, a novel, thought-provoking hypothesis. Whether melanocytes are the culprits that initiate inflammation in psoriasis or simply innocent bystanders that get bullied during the melee will require further study. It is unknown what functional role these cells play in psoriasis, but future studies may provide the roadmap to understand how ADAMTSL5-specific cells contribute to psoriatic

inflammation (Figure 1). Beyond psoriasis, the techniques used by Arakawa and colleagues could be utilized to identify target antigens and cell types in other autoimmune skin diseases, such as alopecia areata.

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Figure 1.

The potential role of a melanocyte antigen within the complex psoriatic inflammatory pathway. T cells encounter the autoantigen ADAMTSL5 (yellow circle) in the skin, via melanocyte (MC) presentation on MHC I. TCR stimulation in the context of the psoriatic pro-inflammatory milieu may lead to enhanced IL-17 production by these cells; however, the functional significance of ADAMTSL5 specific T cells remains unknown. Higher IL-17 levels contribute to dysregulated KC proliferation and release of LL37, cytokines and chemokines (red arrows) that perpetuate the inflammatory response.