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INSIGHTS

Tregs tame skin bacteria and IFN-γ–associated pathology

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Mibrobial dysbiosis worsens cutaneous leishmaniasis. In this issue of *JEM*, Singh et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20230558) show that Rorγt⁺ regulatory T cells suppress pathogenic IFN-γ responses to control *Staphylococcus aureus* growth and limit *S. aureus*– and *Leishmamia braziliensis*–associated immunopathology at the skin barrier.

Cutaneous leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania* and transmitted by sandflies. The World Health Organization estimates that ∼1 million new cases occur yearly worldwide, primarily affecting impoverished communities in developing nations (Burza et al., 2018). Cutaneous leishmaniasis features a wide spectrum of clinical presentations ranging from mild, self-resolving skin lesions to severe, chronic, ulcerative infections that can cause scarring, disfigurement, and/or metastatic disease. These infection outcomes are largely shaped by the balance between mounting effective immune responses that restrain parasite replication and preventing the development of uncontrolled inflammation that causes skin damage (Scott and Novais, 2016). Licensed vaccines are not available for leishmaniasis and although antimicrobial treatments exist, they can be costly, lengthy, and toxic, with drug resistance now emerging in some regions (Burza et al., 2018). Therefore, a better understanding of the immunological factors that promote effective anti-*Leishmania* defense and curtail exaggerated cutaneous inflammation could lead to personalized immunotherapeutic, prognostication, and vaccination strategies for vulnerable patients.

Murine studies have shown that the nature of the early immune response to *Leishmania* plays a pivotal role in determining the microbiological outcome of the infection. Type 1 immune responses are crucial for parasite control, predominantly driven by IFN-γ produced by IL-12–primed CD4⁺ Th1 cells (Scott and Novais, 2016). IFN-γ acts synergistically with other type 1 cytokines (TNF-α, GM-CSF) to promote optimal activation of the macrophage respiratory burst that enables intracellular *Leishmania* killing (Scott and Novais, 2016). In agreement, patients with chronic granulomatous disease who exhibit defective phagocyte respiratory burst, those harboring mutations in the IL-12/IFN-γ pathway, and patients receiving TNF-α inhibitors are all at risk for developing severe leishmaniasis (Asensi et al., 2000; Khattak et al., 2021; Zanger et al., 2012). Conversely, IL-4– and IL-13–driven type 2 immune responses impair parasite control by suppressing macrophage activation, thereby facilitating intracellular *Leishmania* survival (Scott and Novais, 2016). Importantly, exaggerated infection-induced immune responses can promote immunopathology leading to more severe cutaneous leishmaniasis. For example, IL-10 signaling, dysregulated overproduction of TNF-α, IL-1β, or IL-17, and increased accumulation of cytolytic CD8⁺ T cells have been shown to drive pathogenic inflammation during leishmaniasis (Scott and Novais, 2016).

Recent studies demonstrated that cutaneous leishmaniasis is associated with a dysregulated skin microbiome, characterized by enrichment of *Staphylococcus aureus* in many patients (Amorim et al., 2023 *Preprint*; Gimblet et al., 2017). This work has also shown the contribution of *Staphylococcus* species in promoting immunopathology, severe disease, and delayed healing during cutaneous leishmaniasis in mice and humans (Amorim et al., 2023 *Preprint*; Gimblet et al., 2017; Naik et al., 2012). However, the factors that promote dysbiosis-driven severe cutaneous leishmaniasis remain elusive; their elucidation could uncover novel targets for immune- and/or microbiome-based therapies. Thus, Singh et al. (2023) set out to investigate how *S. aureus*–associated

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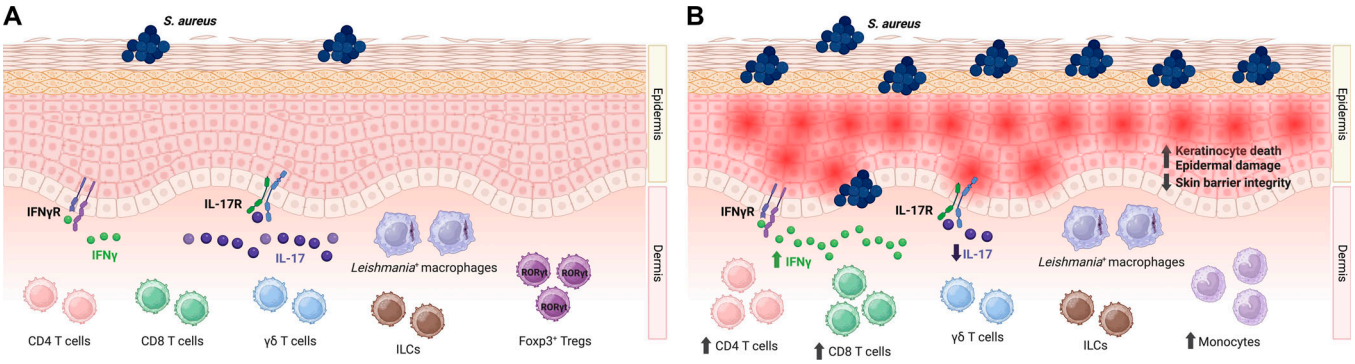

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Model of Treg-mediated control of *S. aureus* and *L. braziliensis*–associated immunopathology in the skin. (A) Topical application of *S. aureus* onto the murine skin promotes the accumulation of IL-17–producing CD4$^+$ T cells, γδ T cells, and ILCs, which mediate the local control of *S. aureus* proliferation, as well as of IFN-γ–producing CD4$^+$ and CD8$^+$ T cells and RORγt-expressing Foxp3$^+$ Tregs. Preexisting *S. aureus* skin colonization exacerbates tissue inflammation during subsequent *L. braziliensis* cutaneous infection. (B) In the setting of depletion of RORγt-expressing Tregs, there is an expansion in the skin of inflammatory monocytes and of CD4$^+$ and CD8$^+$ T cells that produce IFN-γ during *S. aureus* colonization. This excess of IFN-γ results in (i) decreased IL-17 production primarily by γδ T cells, which drives enhanced *S. aureus* cutaneous growth and distal dissemination, and (ii) increased keratinocyte death, which drives epidermal damage and reduces skin barrier integrity, thereby exacerbating immunopathology. *L. braziliensis* infection of *S. aureus*–colonized mice that lack Tregs results in markedly enhanced skin inflammation and immunopathology relative to mice with an intact Treg compartment without significantly altering the parasite tissue burden. The illustration was created with Biorender.com.

dysbiosis promotes severe disease and delayed healing in human cutaneous leishmaniasis. Here, the authors uncover a previously unrecognized role for regulatory T cells (Tregs) in modulating IFN-γ responses to maintain the balance between *S. aureus* control and inflammation at the cutaneous barrier and show that Tregs restrain immunopathology during mouse and human cutaneous leishmaniasis in the setting of *S. aureus*–associated dysbiosis (Singh et al., 2023).

Because *S. aureus* often dominates the microbiota of *Leishmania* skin lesions and is associated with poor leishmaniasis outcomes (Amorim et al., 2023 *Preprint*; Gimblet et al., 2017), the authors first examined how murine skin colonization with *S. aureus* influences local immune responses. They used a clinical *S. aureus* isolate harvested from a patient with *Leishmania braziliensis* skin lesions and found that epicutaneous application led to long-term bacterial colonization with expansion of CD4$^+$ T cells, γδ T cells, and innate lymphoid cells (ILCs) and induction of type 1 and type 17 responses (see figure). Despite the enhanced IL-17 production by CD4$^+$ T cells, γδ T cells, and ILCs, and the increased IFN-γ production by CD4$^+$ and CD8$^+$ T cells, *S. aureus* colonization caused minimal skin thickening and inflammation without tissue injury, implying the presence of factors that regulate the cutaneous response to *S. aureus*. The authors then infected *S. aureus*–colonized mice with *L. braziliensis* and found that bacterial colonization caused exacerbated skin inflammation and damage without affecting the *Leishmania* skin burden. This *S. aureus*–mediated pathogenic response was driven by excess IL-17 and was recapitulated in another murine model of IL-17–dependent skin inflammation following topical application of the TLR7 ligand imiquimod. Collectively, these data indicate that *S. aureus* skin colonization promotes IL-17–driven pathogenic inflammation in cutaneous leishmaniasis.

The authors then examined why *S. aureus* colonization alone induced minimal skin thickening and inflammation despite the induction of local type 17 responses. They hypothesized that Tregs, which are known to mitigate tissue damage and promote skin barrier repair in other settings (Boothby et al., 2020) may curb IL-17–dependent inflammation in this model. *S. aureus* skin colonization increased the accumulation of proliferative, ICOS$^+$CTLA4$^+$CD25$^+$ Foxp3$^+$ Tregs, particularly those expressing the transcription factor Rorγt. Temporary Treg depletion using Foxp3-DTR transgenic mice caused increased *S. aureus* burden across superficial and deep cutaneous layers, extracutaneous bacterial dissemination, compromised skin barrier integrity, and enhanced skin thickness and inflammation and epidermal damage compared to Treg-sufficient mice. These findings were confirmed across multiple *S. aureus* isolates recovered from leishmaniasis patients and collectively uncover a crucial role for Tregs in controlling cutaneous colonization by opportunistic bacteria and limiting the resulting skin injury.

The authors then examined how Tregs limit *S. aureus* growth and skin damage. They found that Treg-depleted, *S. aureus*–colonized mice exhibited increased accumulation of monocytes and αβ T cells without a change in γδ T cells relative to Treg-sufficient mice (see figure). Although prior studies had shown that blockade of the Treg-producing cytokine IL-10 increased IL-17 in cutaneous leishmaniasis (Gonzalez-Lombana et al., 2013), Singh et al. (2023) found that Treg depletion decreased IL-17 production by CD4$^+$ T cells and γδ T cells, and markedly increased IFN-γ production by T-bet–expressing CD4$^+$ and CD8$^+$ T cells in *S. aureus*–colonized mice, while an increase was also noted in IL-5$^+$ and IL-13$^+$ T cells. Of interest, these Treg effects were driven by their Rorγt expression as conditional deletion of Rorγt in Tregs phenocopied total Treg depletion in *S. aureus*–colonized mice, thereby promoting impaired bacterial control, decreased IL-17 production by CD4$^+$ T cells and γδ T cells, enhanced IFN-γ production by αβ T cells, and increased skin inflammation and injury.

Whether GATA3-expressing Tregs, the most common cutaneous Treg subset at steady-state, also contribute to limiting bacteria and skin damage merits investigation.

To shed light on the mechanisms by which Treg depletion impaired bacterial control and compromised skin barrier integrity, the authors then focused on the potential pathogenic role of excess IFN-γ production in Treg-depleted mice, as prior studies have shown that enhanced IFN-γ responses can decrease the production of the critical anti-*Staphylococcus* mediator, IL-17 (Mills, 2023), and can disrupt the mucocutaneous barrier (Break et al., 2021; Shao et al., 2019). Indeed, neutralization of IFN-γ in Treg-depleted, *S. aureus*–colonized mice restored the accumulation of IL-17–producing γδ T cells, increased local IL-17 responses, and reduced *S. aureus* skin burden and extracutaneous dissemination. Furthermore, blockade of IFN-γ in Treg-depleted, *S. aureus*–colonized mice decreased skin thickening, cellular infiltration, inflammation, and tissue injury and increased epidermal cell survival. Moreover, IFN-γ was toxic to *S. aureus*–exposed primary human keratinocytes in vitro. Collectively, these findings show that Tregs suppress pathogenic type 1 responses in *S. aureus*–colonized mice, thereby boosting an effective, IL-17–dependent anti-staphylococcal response and protecting the skin barrier from bacterial invasion and damage.

These mouse observations have direct clinical relevance. IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked) is a life-threatening monogenic syndrome caused by *FOXP3* mutations, which cause Treg deficiency or dysfunction. IPEX patients present with early-onset severe autoimmune manifestations including enteropathy, dermatitis, thyroiditis, and type 1 diabetes, but also exhibit susceptibility to *S. aureus* infections (Gambineri et al., 2018). Although autoimmune dermatitis contributes to barrier disruption and staphycoccal susceptibility in this setting, the work by Singh et al. (2023) supports a direct role for Treg deficiency in impairing bacterial immune surveillance in the skin of IPEX patients by dysregulating type 1 and type 17 responses. Thus, future studies should define IFN-γ and IL-17 responses in situ in the skin of IPEX patients in the presence or absence of *S. aureus* colonization.

Based on these findings, the authors then examined whether a decrease in Tregs would cause greater skin *S. aureus* burden and augmented IFN-γ–associated pathology in cutaneous leishmaniasis. To avoid the development of autoimmunity in mice with chronic complete Treg depletion, they used female heterozygous Foxp3-DTR mice in which ∼50% of Tregs are depleted upon diphtheria toxin administration. After colonizing mice with *S. aureus* and infecting them with *L. braziliensis*, the authors found that Treg reduction led to amplified skin thickness, cellular infiltration, inflammation, and epidermal injury, increased T cell–derived IFN-γ production, decreased local IL-17 responses, and increased *S. aureus* burden relative to mice with intact Tregs, while parasite levels remained unchanged. Thus, these data extend the role of Tregs in regulating *S. aureus* burden and IFN-γ–associated pathology in the setting of cutaneous leishmaniasis.

Finally, to assess the role of Tregs in human cutaneous leishmaniasis, the authors examined a dual RNA-sequencing dataset from biopsies of *L. braziliensis*–infected patients (Amorim et al., 2023 *Preprint*). Lesions with low *FOXP3* expression displayed distinct transcriptome profiles compared to those with high *FOXP3* expression and were characterized by increased expression of *IFNG* and of genes encoding for cytolytic activity (*PRF1*, *GZMB*), which have been previously associated with treatment failure in *L. braziliensis*–infected patients (Amorim et al., 2019). Conversely, *FOXP3*-high lesions exhibited enrichment for genes associated with skin repair. The authors also investigated the relationship between Tregs, *S. aureus* colonization, and IFN-γ levels. They found that lesions with high *S. aureus* levels had reduced *FOXP3* and increased *IFNG* expression and demonstrated greater levels of genes associated with cell death and cytolysis and decreased levels of genes linked to wound healing. Importantly, *L. braziliensis*–infected patients with high lesional *FOXP3* levels had faster healing, indicating that Tregs may play a key role in mitigating tissue damage, excessive IFN-γ levels, and *S. aureus* colonization in human cutaneous leishmaniasis, mirroring the mouse findings. Future work will be needed to determine the generalizability of these findings in patients infected by non-*braziliensis Leishmania* species. Intriguingly, one could speculate that Tregs may also play a role in regulating *S. aureus* skin burden and IFN-γ–associated pathology in other cutaneous conditions, such as psoriasis or atopic dermatitis.

In conclusion, the translational findings of this study, together with data from another recent study by the authors that revealed an IL-1β–driven, Treg-independent, pathogenic program in *S. aureus*–colonized, *L. braziliensis*–infected mice and humans (Amorim et al., 2023 *Preprint*), provide the foundation for devising personalized, immune-based strategies for risk stratification and prognostication of leishmaniasis patients. The objective moving forward will be to identify immune biomarkers to guide individualized, mechanism-based therapeutic interventions with targeted FDA-approved biologics that will ameliorate tissue immunopathology and improve patient outcomes.

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