Beware of wolves in sheep's clothing: immune cell plasticity and instability in health and disease

We read with great interest the article by Watad *et al* that characterised for the first time T lymphocytes in normal human enthesis.¹ The authors demonstrated that enthesal resident CD4⁺ and CD8⁺ T cells show increased expression of immunomodulatory genes, including interleukin (IL)-10 and transforming growth factor- β compared with the circulating counterpart and that on stimulation they acquire the capability to produce tumour necrosis factor (TNF)- α and IL-17. Of note, normal enthesal T cells do not express FoxP3 as peripheral blood regulatory T cells (Treg), but its expression can be induced in specific conditions (eg, in the presence of phosphodiesterase type 4 inhibitor).

The plasticity and instability of immune cells in response to the stimuli of the local microenvironment are a highly interesting field of research under investigation in rheumatic and musculoskeletal diseases (RMDs).² The article by Watad *et al* provides important insights that will help to better understand pathogenic mechanisms underlying the spectrum of spondyloarthritis (SpA). However, in light of data from experimental models and patients with RMDs, including SpA, some aspects should be remarked.

The dawn of regulatory T cells (Treg) dates back in 1990s,³ but the understanding that Treg can transdifferentiate into an effector Th17 phenotype⁴ and that Th17 cells can be redirected to acquire regulatory features is recent history.⁵ The balance between phenotypical plasticity and stability of Treg is orchestrated at the molecular level via the action on regulatory sequences, such as enhancers and promoters, of FoxP3, the master transcription factor of Treg.² In rheumatoid arthritis, TNF- α is able to interfere with the phosphorylation of FoxP3 thereby hampering Treg function, and this can be reverted on treatment with TNF- α inhibitors.⁶

TNF- α may also modulate Treg aberrant transdifferentiation providing yet another rationale for its blockade for therapeutic purposes. However, some aspects on this matter need to be fully addressed due to differences between experimental models and humans, along with the peculiar effects of TNF- α signalling, and likely other cytokines through different receptors.⁷ It would be instrumental to clarify whether the capability of normal enthesal T cells to induce FoxP3 expression may be affected by the concomitant presence of other soluble factors, such as TNF- α in the local microenvironment and whether enthesal T cells from patients with SpA still own this capability.

On the other hand, immunomodulatory factors like those secreted by umbilical cord mesenchymal stem cells may facilitate the switch of pathogenic Th17 cells into Treg cells, as demonstrated in healthy subjects, primary Sjögren's syndrome (pSS) and experimental type 1 diabetes.^{8,9}

The evidence that also CD3⁺ T lymphocytes lacking both CD4 and CD8 on the cell surface (double negative, DN) may display either a regulatory or a Th17-like phenotype in health and disease adds another layer of complexity to this matter.^{10,11} IL-17-producing DN T cells have been identified in the cutaneous inflammatory infiltrate of experimental models and patients with psoriasis.¹²⁻¹⁴ In inflamed skin, these cells seem to predominantly express the $\alpha\beta$ T-cell receptor (TCR), while in humans, normal and injured enthesal cells expressing the $\gamma\delta$ -TCR account for the majority of local IL-17 production.¹⁵ It would be therefore interesting to dive into the topic of DN T cells in normal

human enthesis in order to reinforce and broaden the rationale for their assessment in SpA. It has been postulated that DN T cells may arise from lymphocytes losing surface CD4 or CD8 on chronic activation¹⁶; hence, low number or even absence of DN T cells in normal enthesis should not discourage a more detailed assessment of their phenotype and pathogenic role when affected in SpA. In this regard, it is worth mentioning that while being inhibited by corticosteroids in normal subjects, DN T cells display a certain degree of resistance in patients with pSS, and it would be of great relevance to confirm or rule out whether enthesal DN T cells display this feature in light of possible therapeutic purposes in SpA.¹⁰

Finally, as recently demonstrated by Wade *et al*, psoriatic arthritis (PsA) synovial tissue is enriched of exTh17 cells, also known as nonclassical Th1 cells since they are Th17 cells that no longer produce IL-17 but rather IFN- γ .^{17,18} Whether exTh17 cells can be also found in normal and/or affected enthesis would shed additional light on this complex scenario. Interestingly, also DN T cells may produce IFN- γ , for instance, in the inflamed skin of psoriasis-like inflammation, thereby suggesting that a Th17-like towards a Th1-like transdifferentiation may occur also within the DN T cell compartment.¹³

In conclusion, we acknowledge that the study by Watad *et al* represents a major advancement in the understanding of enthesal pathophysiology, and we believe that further research aimed at addressing the above-mentioned open issues will help elucidate SpA pathogenesis and ultimately improve the care of patients with this disease.

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REFERENCES

- 1 Watad A, Rowe H, Russell T, *et al*. Normal human enthesis harbours conventional CD4⁺ and CD8⁺ T cells with regulatory features and inducible IL-17A and TNF expression. *Ann Rheum Dis* 2020. doi:10.1136/annrheumdis-2020-217309. [Epub ahead of print: 13 May 2020].

- 2 Yang P, Qian F-Y, Zhang M-F, *et al.* Th17 cell pathogenicity and plasticity in rheumatoid arthritis. *J Leukoc Biol* 2019;106:1233–40.
- 3 Sakaguchi S, Sakaguchi N, Asano M, *et al.* Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25). breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995;155:1151–64.
- 4 Hua J, Inomata T, Chen Y, *et al.* Pathological conversion of regulatory T cells is associated with loss of allotolerance. *Sci Rep* 2018;8:7059.
- 5 Esplugues E, Huber S, Gagliani N, *et al.* Control of Th17 cells occurs in the small intestine. *Nature* 2011;475:514–8.
- 6 Nie H, Zheng Y, Li R, *et al.* Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF- α in rheumatoid arthritis. *Nat Med* 2013;19:322–8.
- 7 Tseng W-Y, Huang Y-S, Clanchy F, *et al.* TNF receptor 2 signaling prevents DNA methylation at the *Foxp3* promoter and prevents pathogenic conversion of regulatory T cells. *Proc Natl Acad Sci U S A* 2019;116:21666–72.
- 8 Alunno A, Montanucci P, Bistoni O, *et al.* In vitro immunomodulatory effects of microencapsulated umbilical cord Wharton jelly-derived mesenchymal stem cells in primary Sjögren's syndrome. *Rheumatology* 2015;54:163–8.
- 9 Montanucci P, Alunno A, Basta G, *et al.* Restoration of T cell subsets of patients with type 1 diabetes mellitus by microencapsulated human umbilical cord Wharton jelly-derived mesenchymal stem cells: an in vitro study. *Clin Immunol* 2016;163:34–41.
- 10 Alunno A, Bistoni O, Bartoloni E, *et al.* IL-17-Producing CD4-CD8- T cells are expanded in the peripheral blood, infiltrate salivary glands and are resistant to corticosteroids in patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2013;72:286–92.
- 11 Tian D, Yang L, Wang S, *et al.* Double negative T cells mediate Lag3-dependent antigen-specific protection in allergic asthma. *Nat Commun* 2019;10:4246.
- 12 Sherlock JP, Joyce-Shaikh B, Turner SP, *et al.* IL-23 induces spondyloarthritis by acting on ROR- γ t+ CD3+CD4-CD8- enthesal resident T cells. *Nat Med* 2012;18:1069–76.
- 13 Brandt D, Seron M, Abraham S, *et al.* Tcr + CD3 + CD4 – CD8 – effector T cells in psoriasis. *Clinical Immunology* 2017;181:51–9.
- 14 Ueyama A, Imura C, Fusamae Y, *et al.* Potential role of IL-17-producing CD4/CD8 double negative $\alpha\beta$ T cells in psoriatic skin inflammation in a TPA-induced STAT3C transgenic mouse model. *J Dermatol Sci* 2017;85:27–35.
- 15 Cuthbert RJ, Watad A, Fragkakis EM, *et al.* Evidence that tissue resident human enthesal $\gamma\delta$ T-cells can produce IL-17A independently of IL-23R transcript expression. *Ann Rheum Dis* 2019;78:1559–65.
- 16 Grishkan IV, Ntranos A, Calabresi PA, Gocke AR, *et al.* Helper T cells down-regulate CD4 expression upon chronic stimulation giving rise to double-negative T cells. *Cell Immunol* 2013;284:68–74.
- 17 Hirota K, Duarte JH, Veldhoen M, *et al.* Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol* 2011;12:255–63.
- 18 Wade SM, Canavan M, McGarry T, *et al.* Association of synovial tissue polyfunctional T-cells with DAPSA in psoriatic arthritis. *Ann Rheum Dis* 2019;78:350–4.