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NEWS AND COMMENTARY

Ectopic B-helper CD4+ T cells

Pathogenic CD4+ T cells regulating B-cell differentiation in autoimmunity: not exactly Tfh cells

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T follicular helper (Tfh) cells are typically defined by the expression of CXCR5, PD-1 and BCL-6, and their ability to promote the differentiation of B cells into antibody (Ab)-producing plasma cells.[1,2] In a recent article in *Nature*, Brenner and colleagues described a putative pathogenic population of B-helper CD4+ T cells in tissues from patients with rheumatoid arthritis (RA).[3] Curiously, while these cells expressed high levels of PD-1 and promoted B-cell differentiation, they lacked CXCR5 and BCL-6, suggesting they represent a novel subset of effector CD4+ T cells.

A fundamental function of B cells is to produce Abs that recognize and bind specific antigens on microbes, resulting in the neutralization and/or eradication of infectious pathogens.[4] B cells usually require inputs from various cell types to ensure they produce the most appropriate class of Abs. The key cell type involved in this process is the Tfh cell, a subset of CD4+ T cells specialized to provide 'help' to B cells.[1,2] Cardinal features of Tfh cells include expression of the B-cell zone homing chemokine receptor CXCR5, the transcriptional repressor BCL-6, the cytokine interleukin (IL)-21, the chemokine CXCL13 and a suite of surface molecules—CD40L, ICOS, SLAM receptors, PD-1—involved in T-cell/B-cell interactions.[1,2] Critically, Tfh cells localize with B cells in follicles and germinal centers in secondary lymphoid tissues and promote the differentiation of activated B cells into memory cells and Ab-secreting plasma cells.[1,2] It is this ability of B cells to generate memory and plasma cells that underlies long-lived serological memory following pathogen infection, and the success of most currently available vaccines that protect the host against infection for extended periods of time, and in some instances a life time.[4]

However, not all Abs produced by B cells are good—in fact, in the setting of autoimmunity, autoreactive B cells are pathogenic, producing Abs that result in severe tissue damage and ultimately organ failure.[5] Akin to responses against non-self antigens, the production of autoAbs by B cells can be driven by Tfh cells.[5] Indeed, many studies have reported increased proportions of circulating Tfh-like cells (usually defined as CXCR5+PD-1hi or CXCR5+ICOShi CD4+ T cells) in the peripheral blood of patients with a broad range of autoimmune diseases often associated with production of autoAbs, such as systemic lupus erythematosus (SLE), Sjogren's syndrome, RA and diabetes.[2,5–8] Remarkably, the proportions of circulating Tfh-like cells in these immunopathologies often correlates with levels of autoAbs or other readouts of disease, and therapeutic interventions that alleviate disease severity also reduced Tfh-like cells.[2,5–8] Collectively, these studies have defined Tfh cells—along with their partner B cells—as being drivers of autoimmunity in humans, and thus represent targets for potential therapies.

A limitation of many studies performed to date that have investigated the potential role of Tfh cells in human autoimmunity is that analysis has largely been performed on blood cells—when clearly the 'action' in these conditions occurs in affected tissues. Thus, a strength of the study by Rao *et al.*[3] is their access to inflamed tissues and peripheral blood from individuals with RA, coupled with the multidimensional analysis of cells in these sites. Synovial fluid and synovial tissue from patients with seropositive, but not seronegative, RA were found to be enriched for a population of memory CD4+ T cells expressing high levels of PD-1, MHC class II and ICOS. Transcriptional assessment of these PD-1hi CD4+ T cells revealed them to contain higher levels of messenger RNA encoding IL-21, CXCL13, MAF, SAP, BATF, OX2 (CD200) and BTLA than PD-1− CD4+ T cells present in the same sites.[3] Importantly, the synovial PD-1hi CD4+ T cells from RA patient tissues were more efficacious at inducing B-cell differentiation, with respect to generating Ab-secreting plasmablasts *in vitro*, than PD-1− or PD-1intermediate CD4+ T cells present in the same sites. This process required interactions involving the surface receptor SLAMF6 (CD84) and IL-21,[3] as reported previously for murine and human Tfh cells[1,2] (Figure 1).

These phenotypic, molecular and functional features, as well as mechanisms of action, of synovial PD-1hi CD4+ T cells are signatures of Tfh cells detected in human lymphoid tissues.[9,10] However, in contrast to classic Tfh cells, synovial PD-1hi CD4+ T cells from RA patients lacked expression of CXCR5 and BCL-6; they also expressed elevated levels of interferon gamma (IFNγ).[3] Interestingly, BLIMP-1, which is regulated by a balance between BCL-6 and BLIMP-1 inasmuch that Tfh cells are BCL-6hi BLIMP-1lo (refs 1, 10) was also increased in synovial PD-1hi CD4+ T cells[3] (Figure 1). Thus, despite sharing many features with Tfh cells, PD-1hi CD4+ T cells clearly do not belong to the Tfh compartment of effector CD4+ T cells—rather they represent a distinct subset of B-helper CD4+ T cells enriched at inflamed sites, the crucible for the production of pathogenic autoAbs.

The lack of CXCR5 on these effector cells perhaps is not surprising. The first detailed description of pathogenic B-helper CD4+

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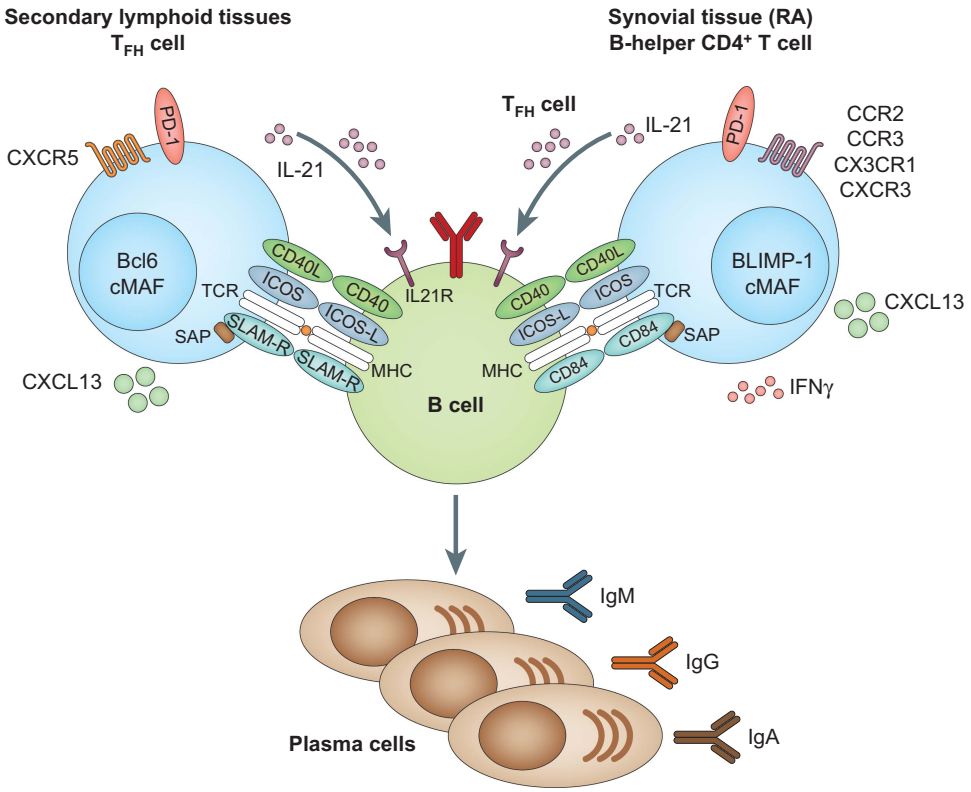

Figure 1 Pathogenic B-helper CD4+ T cells in synovial tissues in rheumatoid arthritis. Conventional Tfh cells, detected in human secondary lymphoid tissues, are characterized by the elevated expression of CXCR5, BCL-6, PD-1, production of IL-21 and CXCL13, and a potent ability to induce B-cell differentiation into plasma cells via pathways dependent on CD40L, ICOS and IL-21. Similar cells were found in inflamed synovial tissues of patients with RA, however, marked differences included a lack of expression of CXCR5 and Bcl-6, and paradoxically increased expression of BLIMP-1. These cells exhibited other features of inflammatory cells such as expression of chemokine receptors to facilitate migration to sites of inflammation and production of IFNγ.

T cells was by Joe Craft's lab who studied the MRL^{lpr} mouse model of SLE.[11] Here, extrafollicular CD4$^+$ T cells lacking CXCR5 induced pathogenic autoAbs via an ICOS/IL-21 dependent pathway.[11] More recently, using a murine model of airway inflammation and tissue damage, it was found that lung infiltrating CXCR5$^-$ Bcl-6$^-$ CD4$^+$ T cells could promote B-cell differentiation.[12] These cells were also enriched for IFNγ production.[12] Thus, CXCR5 is not a prerequisite for functionality of B-helper CD4$^+$ T cells. Rather, CXCR5 is required for localization of Tfh cells to B-cell zones of conventional lymphoid tissues, while in the setting of autoimmunity, alternative guidance cues are utilized to recruit effector cells to sites of inflammation. Indeed, synovial PD-1^{hi} CD4$^+$ T cells in RA patients expressed high levels of chemokine receptors including CCR2, CX3CR1 and CCR5[3] (Figure 1), which are typically associated with trafficking to inflammatory sties. Thus, there is a clear shift in expression from homeostatic—CXCR5—to inflammatory chemokine receptors by B-helper CD4$^+$ T cells in cases of autoimmunity.

Some interesting questions regarding PD-1^{hi} CD4$^+$ T cells in the synovium of RA patients remain unanswered. The transcriptional network underpinning pathogenic PD-1^{hi} CD4$^+$ T cells in RA is enigmatic. The absence of BCL-6 implies roles for additional molecular regulators—it would be important to determine whether ASCL2, which is upstream of BCL-6 in murine Tfh cells,[1] is expressed by these cells. Alternatively, BCL-6 expression may be downregulated in PD-1^{hi} CD4$^+$ T cells as they migrate to ectopic non-immunological sites.[1,2,5] Expression of BLIMP-1 is also striking; BLIMP-1 can restrain Tfh formation via IL-2/STAT5 signaling.[1] However, PD-1^{hi} CD4$^+$ T cells exhibit reduced production of IL-2.[3] Thus, elucidating the mechanism inducing and maintaining BLIMP-1 expression in PD-1^{hi} CD4$^+$ T cells in RA will inform our understanding of the molecular circuitry of these pathogenic cells. Another interesting observation was that there are distinct CCR2$^-$ and CCR2$^+$ subsets of synovial PD-1^{hi} CD4$^+$ T cells, and the CCR2$^-$ subset could give rise to CCR2$^+$ cells *in vitro*.[3] But it is unclear whether these cells represent a precursor/progeny relationship *in vivo* and if one subset is more pathogenic than the other. These questions notwithstanding, the identification by Rao *et al.*[3] of potent B-helper cells in the reactive sites in human autoimmunity is an important advance, and further analysis of these cells may identify approaches of targeting them to reduce their pathogenicity and improve outcomes for individuals affected by autoimmunity.

CONFLICT OF INTEREST

The author declares no conflict of interest.

1 Crotty ST. Follicular helper cell differentiation, function, and roles in disease. *Immunity* 2014; **41**: 529–542.
2 Tangye SG, Ma CS, Brink R, Deenick EK. The good, the bad and the ugly-TFH cells in human health and disease. *Nat Rev Immunol* 2013; **13**: 412–426.
3 Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y *et al.* Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* 2017; **542**: 110–114.
4 Tangye SG, Tarlinton DM. Memory B cells: effectors of long-lived immune responses. *Eur J Immunol* 2009; **39**: 2065–2075.
5 Craft JE. Follicular helper T cells in immunity and systemic autoimmunity. *Nat Rev Rheumatol* 2012; **8**: 337–347.

6 Choi JY, Ho JH, Pasoto SG, Bunin V, Kim ST, Carrasco S *et al.* Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. *Arthritis Rheumatol* 2015; **67**: 988–999.
7 Kenefeck R, Wang CJ, Kapadi T, Wardzinski L, Attridge K, Clough LE *et al.* Follicular helper T cell signature in type 1 diabetes. *J Clin Invest* 2015; **125**: 292–303.
8 Zhang X, Lindwall E, Gauthier C, Lyman J, Spencer N, Alarakhia A *et al.* Circulating CXCR5$^+$CD4$^+$helper T cells in systemic lupus erythematosus patients share phenotypic properties with germinal center follicular helper T cells and promote antibody production. *Lupus* 2015; **24**: 909–917.
9 Chtanova T, Tangye SG, Newton R, Frank N, Hodge MR, Rolph MS *et al.* T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *J Immunol* 2004; **173**: 68–78.
10 Ma CS, Suryani S, Avery DT, Chan A, Nanan R, Santner-Nanan B *et al.* Early commitment of naive human CD4(+) T cells to the T follicular helper (T(FH)) cell lineage is induced by IL-12. *Immunol Cell Biol* 2009; **87**: 590–600.
11 Odegard JM, Marks BR, DiPlacido LD, Poholek AC, Kono DH, Dong C *et al.* ICOS-dependent extrafollicular helper T cells elicit IgG production via IL-21 in systemic autoimmunity. *J Exp Med* 2008; **205**: 2873–2886.
12 Vu Van D, Beier KC, Pietzke L-J, Al Baz MS, Feist RK, Gurka S *et al.* Local T/B cooperation in inflamed tissues is supported by T follicular helper-like cells. *Nat Commun* 2016; **7**: 10875.