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Letter to the Editor

Transcriptome mining and B cell depletion support a role for B cells in psoriasis pathophysiology

Introduction

Increasing evidence suggests that certain subpopulations of B cells can have regulatory functions. Herein, we report immunohistochemical and clinical evidence of psoriasis following B cell depletion with rituximab, administered as therapy for pemphigus vulgaris. We also mine RNA-Seq datasets to help elucidate a role for B cells in psoriasis pathophysiology, an understudied but potentially important area of further investigation.

Case report

A 50-year-old man with recently diagnosed pemphigus vulgaris (PV) presented to clinic for medical management. Five months prior, he had begun experiencing difficulty swallowing and underwent laryngoscopy evaluation. Biopsy of a laryngeal ulcer revealed histology consistent with pemphigus vulgaris, which was later confirmed by detection of desmoglein 3-specific antibodies by ELISA. The patient responded well to a regimen of low dose prednisone, doxycycline, niacinamide and one cycle of rituximab.

However, 6 months after completion of rituximab therapy, he returned with bilateral erythematous, scaly plaques on the elbows and knees (Fig. 1a–c), coinciding with morning joint pain and stiffness. Histologic H&E evaluation revealed classic features of psoriasis (Fig. 1d–f) and immunohistochemistry revealed clusters of IL-17A positive cells (Fig. 1g–h). The patient was started on adalimumab with improvement of his skin disease and modest improvement of his joint symptoms.

Transcriptome mining

To explore the relationship between B cells and other cells in psoriasis, we applied a machine-learning, nonlinear dimensionality reduction strategy to map the entire psoriasis transcriptome as a 2-dimensonal (2D) image (Fig. 2) [1]. The RNA-Seq data was obtained from 99 patients with psoriasis and 90 healthy controls [2]. This visual representation illustrates the gene co-expression network between skin-expressed genes in healthy and psoriasis skin. Analysis revealed that in psoriasis, gene clusters corresponding to T cells (e.g. *CD3E*, *CD4*, *CD8*, *ICAM1*, *ITK*, *LCK*, *TRA*, *TRB*, *TRD*, *TRG*), B cells (e.g. *BTK*, *CD19*, *IGH*, *IGK*, *IGL*, *MS4A1* (CD20)), and monocytes (*CD14*, *ITGAM*, *CSF1R*, *TLR2*, *FCGR3A*, *CD86*) are located in close proximity to one another. Of particular interest, *IL10* is located centrally between these clusters in psoriatic but not healthy skin. This suggests that in psoriasis, IL-10 is likely produced by multiple cell types, including B cells, but its peripheral location in the 2D image of the healthy skin transcriptome indicates that these same cell types poorly express *IL10* in healthy skin. Likewise, in healthy skin, psoriasis-associated cytokines such as *IL17A, IL1B, IL12,* and *IL36,* are distributed across the 2D image, possibly due to their poor expression. However, in psoriasis, these pathogenic cytokines cluster closely together but away from *IL10*, demonstrating that IL-10 and psoriasis-associated cytokines are produced separately, likely by distinct subpopulations of cells (Fig. 2a, b).

Finally, multiple gene-gene correlative studies revealed a close association between B cells and T cells. Specifically, the expression of inducible T cell kinase (*ITK*) and the corresponding B cell-associated kinase, Bruton's tyrosine kinase (*BTK*), correlated significantly with one another ($p = 0.00016$). Similarly, T cell receptor (e.g. *TRA* and *TRB*) expression positively correlated with B cell receptor (e.g. *IgH*, *IgK*, and *IgL*) expression ($p = 0.00028$, 0.0012, 0.002 for *TRB* vs *IGH*, *TRA* vs *IGK*, *TRA* vs *IGL*, respectively) (Fig. 2c–f).

Discussion

Rituximab, an anti-CD20 B cell-depleting monoclonal antibody, has demonstrated efficacy in the treatment of B cell malignancies and B cell-mediated diseases. Its relevance in autoimmunity is highlighted by the increased use of rituximab to treat a variety of autoimmune diseases, including multiple sclerosis. In psoriasis, the opposite seems to be true, as at least 12 cases of rituximab-induced de novo psoriasis have been reported [3].

Given that autoreactive antibodies are not implicated in psoriasis pathogenesis [4], the role of B cells in psoriasis has not been well explored. Nevertheless, previous reports [5,6] and this current case of rituximab-induced psoriasis highlight a putative role for B cells in psoriasis. Although current evidence is insufficient to categorize B cells as pathogenic or regulatory in psoriasis, both are intriguing possibilities. Supporting the former, studies have shown that appropriately activated B cells – those having received CD40 co-stimulation after B cell receptor (BCR) engagement – produce a proinflammatory environment that further activates T cells and thereby promotes the psoriasis phenotype [7]. In contrast, B cells activated with CD40 alone

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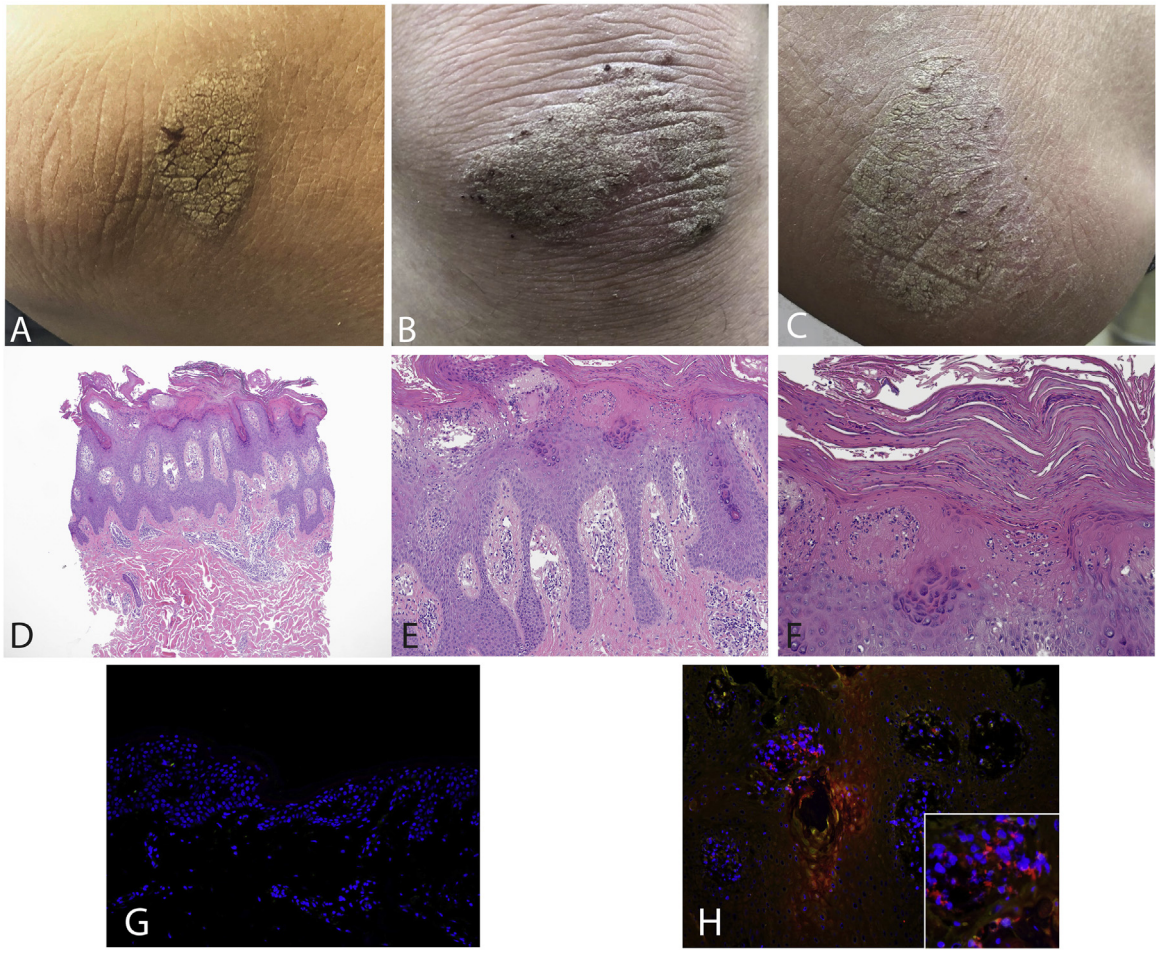

Fig. 1. Psoriasis, distribution and histology. (a–c) clinical images of our patient demonstrating well-demarcated, silver-scaly plaques six months after his last rituximab infusion on the right elbow, left knee, and left elbow respectively. (d–f) Hematoxylin and eosin stained biopsy of the left knee revealed (d) psoriasiform epidermal hyperplasia with elongated, clubbed rete ridges (4x), (e) rete ridges are evenly elongated and associated with hypogranulosis (10x) and (f) confluent parakeratosis with neutrophils, as well as secondary superficial necrosis (20x). (g–h) Immunohistochemical IL-17A staining of (g) healthy skin versus (h) our patient's paradoxically-induced psoriatic skin demonstrates a cluster of IL-17A positive epithelial cells located in the dermal papillae.

produce inhibitory cytokines, such as IL-10 [7]. This information aligns with the concept that a healthy immune system suppresses aberrant, self-stimulated immune activity.

IL-10 is of particular interest in psoriasis, as conventional therapies are thought to induce its expression, implicating it as a regulator of disease [8]. IL-10 is also thought to be elevated in pemphigus vulgaris blisters, with some considering it a marker of disease severity and activity [9]. It is also known to be down-regulated by rituximab [10,11]. As a result, it is possible that a large IL-10-competent subset of B cells are present at baseline, and that B cell depletion therapy can tip the scales from a pro-IL-10 or IL-10 neutral environment to an IL-10 depleted state. Without IL-10, psoriasis-associated cytokines from T cells, and other immune populations may become unregulated and induce psoriasis. Models of experimental autoimmune encephalomyelitis (EAE) in mice have also demonstrated the necessity of IL-10 expression in B cells, as mice without them do not recover from EAE [12]. Like psoriasis [13], EAE is considered to be a Th17-mediated disease.

Evidence at the transcriptome level presented herein reveals a close association between B cells and T cells in psoriasis patients, with these two cell populations being proportionately linked to one another (Fig. 2c–f). The 2D representation of the psoriasis transcriptome also supports IL-10 expression by multiple cell types, including B cells (Fig. 2a). Whereas the same analysis performed on healthy skin suggests that *IL10* is not closely associated with any of these same cell types, (Fig. 2b) indicating its inducibility in the setting of inflammation. Lastly, we demonstrated that psoriasis-associated cytokines are likely not expressed by the same cell subpopulations that express IL-10 (Fig. 2a, b), supporting the possibility of a separate B cell population that secretes this cytokine. Although IL-10 secreting B cells likely exist in psoriasis as a regulatory mechanism, clearly the summation of the entire immune response is pro-inflammatory likely due to pathogenic T cells overriding the regulatory B cell-mediated suppression.

We conclude that regulatory B cell subpopulations are a distinct entity proportional to T cells in psoriasis. Based on previously reported rituximab-induced de novo psoriasis and our current case, we propose that depleting regulatory B cells may induce new-onset psoriasis.

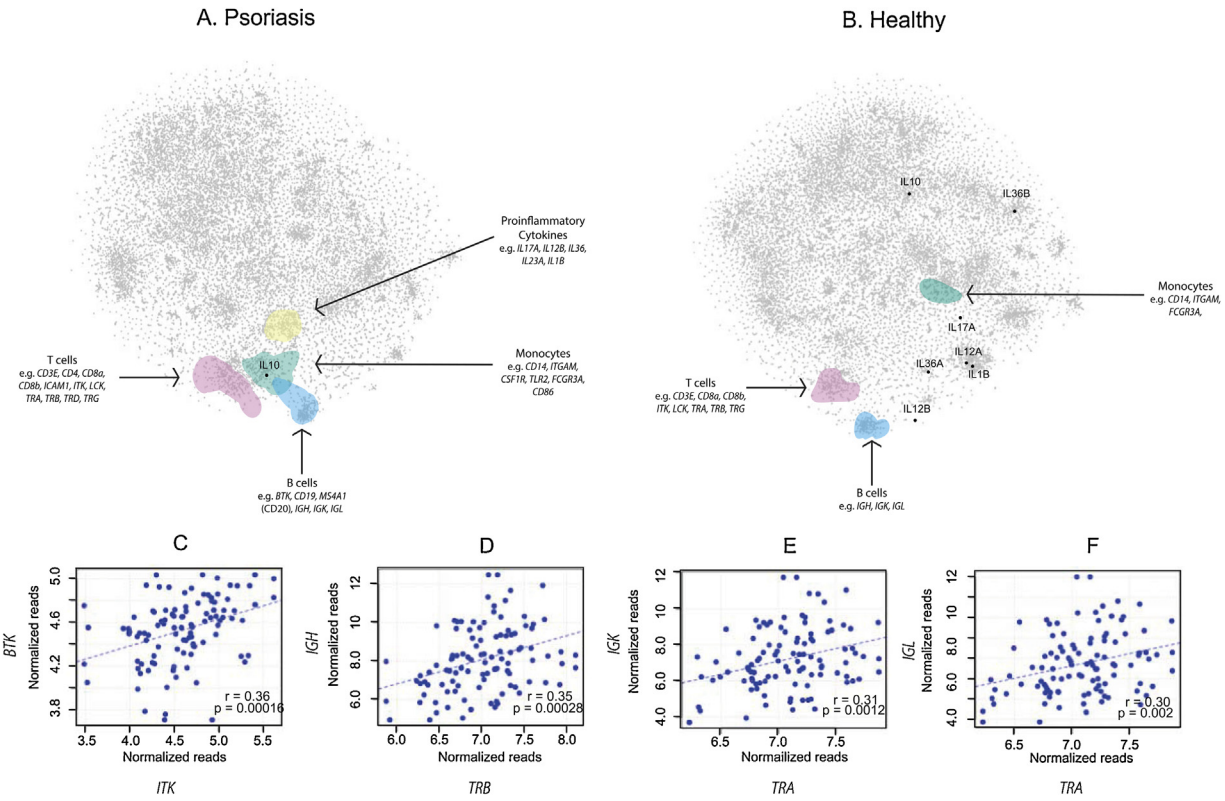

Fig. 2. Distribution and correlation of cytokines and cell populations illustrated by nonlinear dimensionality reduction strategy. (a, b) Gene expression clusters were displayed using the t-Distributed Stochastic Neighbor Embedding (t-SNE) method where distance was calculated as (d) = 1-r^2, where r equals Pearson's correlation coefficient. The RNA-Seq dataset used for this analysis consisted of 99 psoriasis vulgaris skin biopsy samples obtained from patients washed out of all systemic and topical therapies and 90 healthy controls. (a) *IL10* is located between T cells, monocytes and B cells clusters in psoriasis skin. Proinflammatory cytokines, such as *IL17A*, *IL23A*, *IL1B*, *IL36*, *IL12B* cluster together and away from *IL10*. (b) *IL10* maps away from T cells, monocytes and B cells in healthy skin. Proinflammatory cytokines distribute sporadically throughout the transcriptome, possibly due to their low-level expression in healthy patients. (c–d) Correlation analyses of gene expression in psoriasis. Read counts were normalized with the DESeq2 package. Values were log transformed and winsorized. Pearson's correlation coefficients were calculated in R, using the median ratio method. (c) Expression of T cell kinase (*ITK*) correlates strongly to B cell kinase (*BTK*) expression. (d–f) Additionally, T cell receptor (*TRA* and *TRB*) expression correlates with B cell receptor expression (*IGH*, *IGK*, *IGL*). Correlation coefficients and p values displayed in figure.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.11.006.

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