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Groundbreaking discoveries in Immunology

Title:

Single-cell RNA analysis: guiding the treatment of DIHS/DRESS

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Abbreviations: DEGs, differentially expressed genes; DIHS/DRESS, drug induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms; HHV, human herpesvirus; PBMCs, peripheral blood mononuclear cells; scRNA-seq, single cell RNA-sequencing; SM, smooth muscle; SMX, sulfamethoxazole; TMP, trimethoprim; atopic dermatitis, AD; SCARS, severe cutaneous adverse reactions

Main text:

Drug-induced hypersensitivity syndrome (DIHS) ─also termed as drug reaction with eosinophilia and systemic symptoms (DRESS)─ is a potentially lethal inflammatory disease associated with human herpesvirus (HHV) reactivation. DIHS/DRESS is classified within a group of syndromes named severe cutaneous adverse reactions (SCARs), including acute generalized exanthematous pustulosis and Stevens-Johnson syndrome/toxic epidermal necrolysis. SCARs are delayed type IV hypersensitivity reactions mainly characterized by T-cell activation. The pathophysiology of DRESS is still poorly understood.[1] The proposed mechanisms implicated in its patogenesis include drug detoxification enzyme abnormalities, sequential reactivation of herpesviruses (cytomegalovirus, Epstein-Barr virus, HHV-6 and -7), and genetic predisposition related to certain human leukocyte antigen alleles. The limited knowledge of the mechanisms and pathways underlying the pathology of DRESS present an important treatment hurdle.

Over the last few years, targeting specific immunological pathways has become a promising approach to treat complex inflammatory diseases. In that regard, single-cell RNA sequencing (scRNA-seq) provides individual cell information at unprecedented resolution. This enables the analysis of cell lineage trajectories, cell-cell and cell-microenvironment interactions as well as the identification and discovery of new cell types or phenotypes, which all inform about pathogenic mechanisms.[2,3] For example, He *et al*. conducted a novel study using scRNA-seq analysis in atopic dermatitis (AD) and showed a novel fibroblast population directing type 2 immune responses in lesional skin of AD patients.[4]

Kim *et al*.[5] have recently reported an in-depth study of a DIHS/DRESS patient who was treated succesfully with the guidance of scRNA-seq. A 44-year-old patient presented with trimethoprim/sulfamethoxazole-induced DIHS/DRESS. In addition, the patient had generalized exfoliative dermatitis and uncontrolled systemic symptoms despite prednisone, etanercept, intravenous immunoglobulin, cyclosporine, and mycophenolate mofetil treatment. The analysis of skin-derived cells of this patient, in comparison with those of healthy controls (HCs), revealed an inflammatory state with relative abundance of keratinocytes and immune cells. Lymphocytes exhibited the greatest transcriptomic changes with T cell predominance and upregulation of genes involved in proliferation (MKI67), migration (CCR10), activation (IL2RG) and signaling pathways (JAK3 and STAT1).

The scRNA-seq analysis of peripheral blood mononuclear cells (PBMCs) showed a predominance of $CD4^+$ and $CD8^+$ subclusters and CCR4- and CCR10-expressing T cells.

Moreover, central memory $CD4^+$ T cells were enriched with DNA from HHV6b. In contrast to the observation in the skin, *JAK* and *STAT* genes were not detected in an unsupervised manner when the whole PBMC lymphocyte cluster was compared between DiHS/DRESS and HCs. This shows that the local site of inflammation (skin) was optimal for detecting targetable pathways. Altogether, these results led to the identification of potential therapeutic targets for treating this DIHS/DRESS patient, namely the JAK-STAT pathway and the viral infection. The patient achieved disease control after 2 weeks of treatment with tofacitinib (a JAK1 and JAK3 inhibitor) plus valgancyclovir (an antiviral).

These findings indicate that single-cell omic-based approaches as scRNA-seq might be a powerful means for directing patient care in diseases with a complex inflammatory pathophysiology as SCARs. Drug allergy apart, scRNA-seq has been also applied in allergic asthma. For example, Tibbit *et al.* showed distinct T helper cell subsets in a mouse model of house dust mite-induced asthma (REF). In another study, conducted by Vieira Braga *et al.* in asthmatic patients and HCs, novel subsets were identified of tissue-resident memory T cells and mucus ciliated cells (REF).

Although scRNA-seq has been progressively optimized (reduction of costs and number of cells required for analysis),[2,3] there are still difficulties that preclude its implementation in the clinical practice such as the need for tissue sampling (REF-soumelis) and the complexity of data analysis and interpretation (REF- by Rostom). The increasing use of techniques such as scRNA-seq, or alike, will likely enlighten the understaning of the pathophysiology of allergic/immunological diseases, thus enabling the development of targeted therapies.

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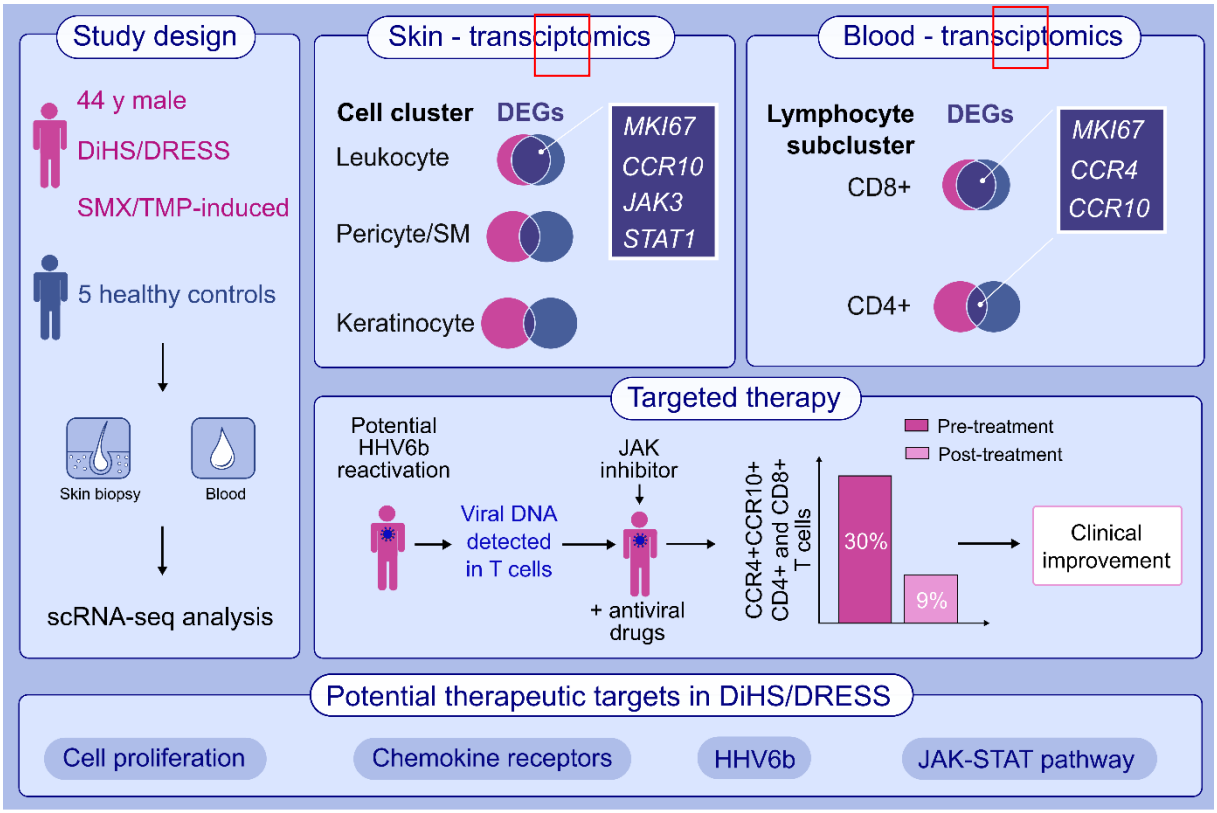

Figure 1: scRNA-seq analysis guided successful therapeutic intervention in a patient with refractory DIHS/ DRESS. A 44 year-old male patient with TMP/SMX-induced DIHS/DRESS and progression to TEN-like rash and subsequent generalized exfoliative dermatitis and uncontrolled systemic symptoms was evaluated to find a potential therapeutic target. Five age- and sex- matched healthy volunteers were enrolled as a control. Both skin biopsy specimens and PBMCs were used for scRNA-seq analysis. Four immunological pathways were revealed at the end of a complex set of analysis: cell proliferation, JAK-STAT pathway, chemokine receptors and HHV6b proliferation in T lymphocytes with central memory type. Targeting JAK-STAT pathway with tofacitinib and HHV6b proliferation with valgancyclovir achieved disease control after 2 weeks of treatment. Pre- and post- treatment scRNA seq analysis of PBMCs revelaed a reduction of CCR4- and CCR10-expressing CD4+ and CD8+T cells with proliferative gene signatures consistent with flow cytometry analysis.

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