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ANALYSIS OF PERIPHERAL INFLAMMATORY T-CELL SUBSETS AND THEIR EFFECTOR FUNCTIONS IN PATIENTS WITH BIRDSHOT-RETINOCHOROIDITIS

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PURPOSE

To characterize and correlate inflammatory T cell subsets derived from peripheral blood mononucleated cells (PBMC) with clinical parameters of patients with birdshot-retinochoroiditis (BSRC).

METHODS

In our pilot study, we examined 11 patients (22 eyes) with BSRC (HLA-A29 positive). Clinical examination and multimodal standard imaging with spectral domain optical coherence tomography, fluorescein angiography (FA), and indocyanine green angiography (ICGA) were performed to classify disease activity. Peripheral blood-derived CD4+ and CD8+ T cells were analyzed for their naïve, memory, and EMRA status by CD45RA and CCR7 expression using flow cytometry. The memory compartment was further subdivided into diverse functional subsets regarding the surface expression of 4 chemokine receptors (CCR4, CCR6, CCR10, CXCR3) and the functional profile assessed by intracellular cytokine staining.

RESULTS

We identified retinal vascular leakage on FA/ICGA as active disease in 10 eyes of 5 patients, while 12 eyes of 6 patients demonstrated no retinal leakage, but altered vascular architecture and retinal thinning as inactive, end-state disease group (Figure 1). The inactive, end-stage disease group revealed a significant accumulation of peripheral CD8+ effector memory T cells expressing CD45RA (T_{EMRA}) in blood compared to active disease group (Figure 2). In the active disease group, we found decreased frequencies of Th2 (CCR6- CCR4+ CXCR3-) memory CD4+ T-cells accompanied by increased Th1 (CCR6- CCR4- CXCR3+) cell frequencies compared to healthy controls and the inactive disease group. Functional assays demonstrated impaired cytokine production of CD4+ and CD8+ memory T-cells in BSRC patients regardless of their treatment and stage of disease (Figure 3).

CONCLUSION

Our preliminary results revealed a Th1/Th2 imbalance in BSRC which may indicate autoimmune processes. High frequencies of CD8+ (T_{EMRA}) could be an indicator for a poor prognostic outcome. In addition, decreased cytokine levels in the periphery are probably caused by immunosuppression or exhaustion of the T-cell subsets. Accordingly, we propose to distinguish these cells *ex vivo* based on the expression of chemokine receptor instead of functional analyses.

These findings offer new insights into the immunological pathophysiology of BSRC disease and may help in defining new biomarkers for monitoring.

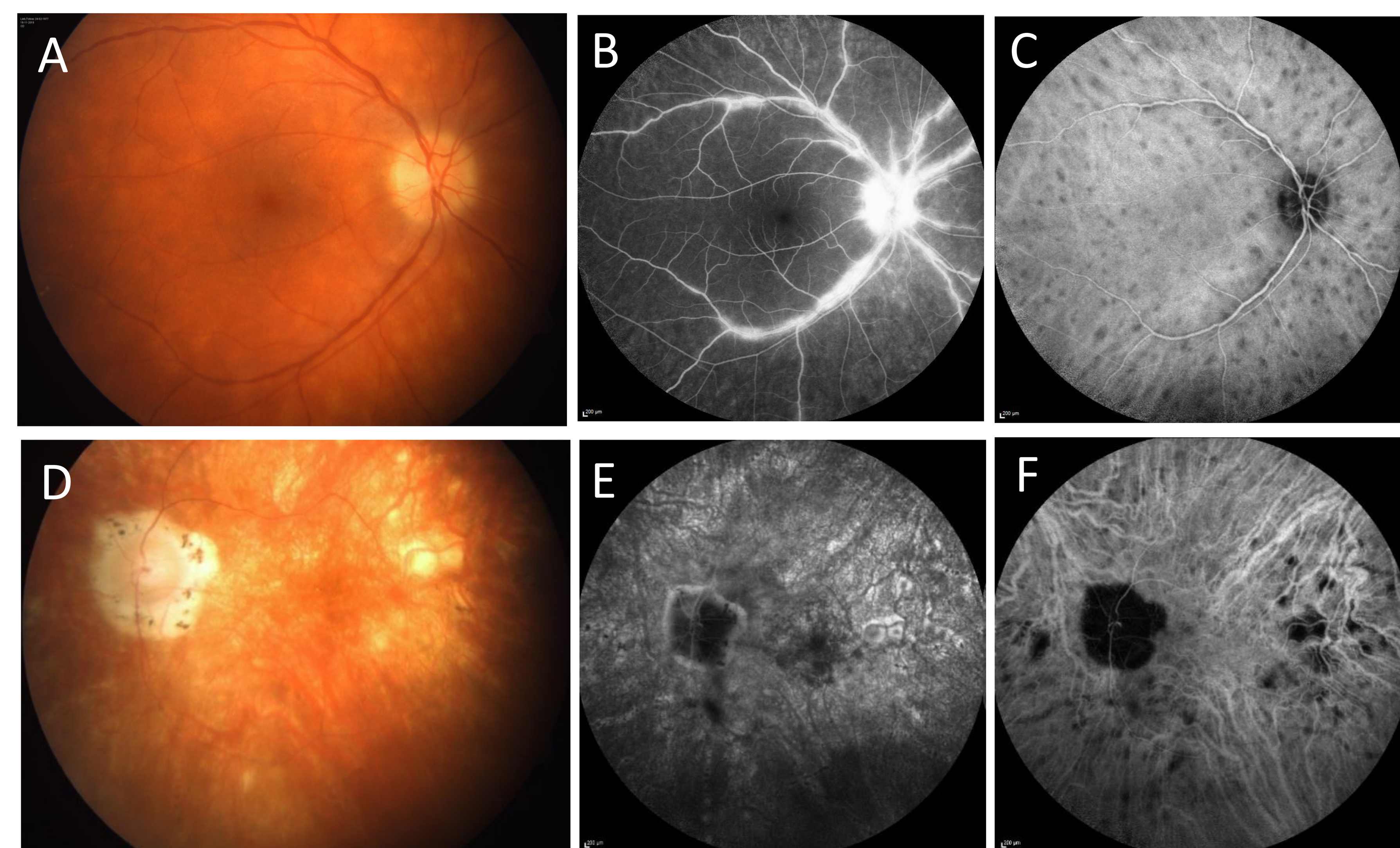

Figure 1. Fundus photography **(A)** presents characteristic choroidal lesions in a birdshot-retinochoroiditis. Fluorescence angiogram **(B)** showing retinal vascular leakage and optic disc hyperfluorescence and indocyanine green angiogram **(C)** showing multiple hypofluorescent spots (dark dots) which represent an active disease. The second fundus photography **(D)** demonstrates brightened fundus, atrophic optic nerv, and choroidal scars. Fluorescence angiogram **(E)** showing no retinal vascular perfusion and indocyanine green angiogram **(F)** showing hypofluorescent areas as sign of atrophy.

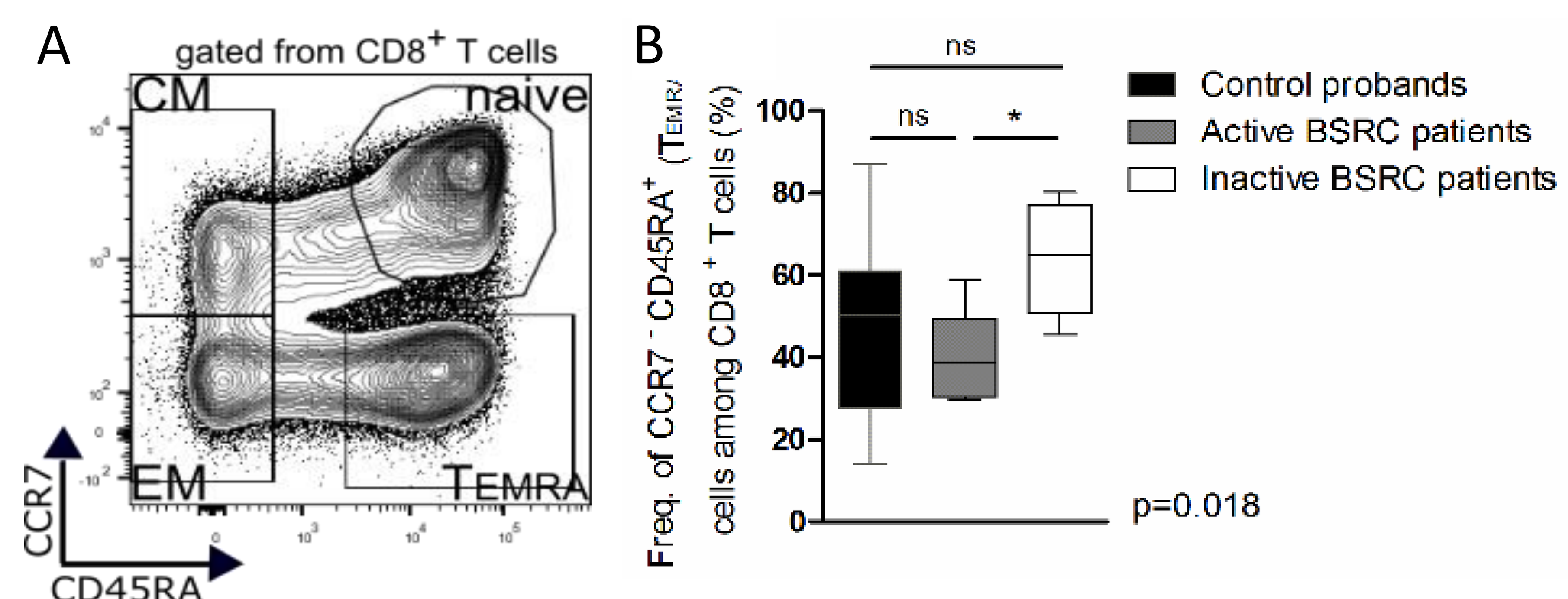

Figure 2. Exemplary analysis and gating strategy of CD8+ T-cell stages **(A)** and analysis of the proportion of peripheral CD8+ TEMRA (CCR7-CD45RA+) T cells **(B)** active and inactive disease, and healthy controls.

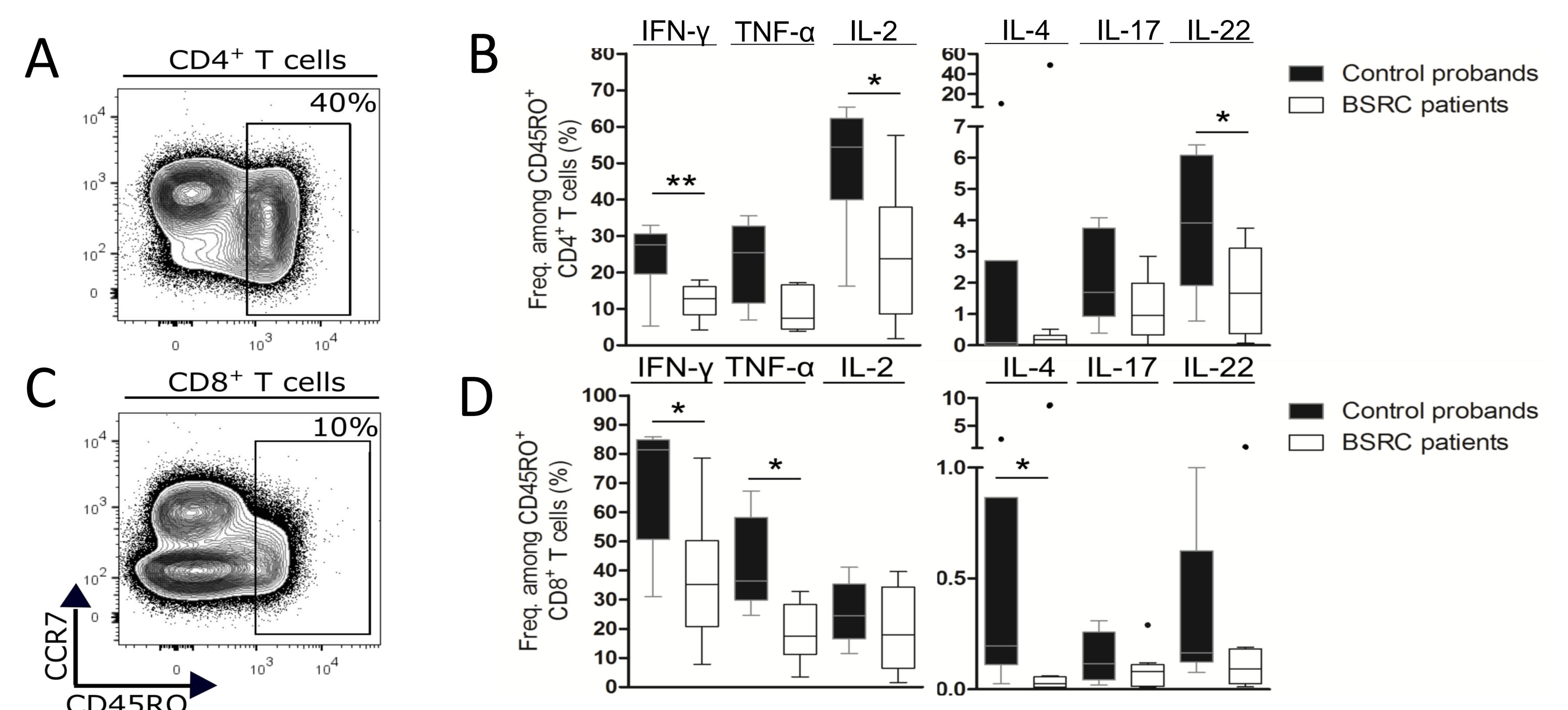

Figure 3. Memory T-cells show reduced potential to exert effector function. The frequencies of IFN-γ, TNF-α, IL-2, IL-4, IL-17 and IL-22-producing CD45RO+ T-cells were determined after mitogenic stimulation (for 6h) *via* intracellular antibody labelling. **(A)** Shows the exemplary gating strategy of parental CD45RO+ T cells, of which the calculation of intracellularly stained cytokines is depicted in **(B)** and **(C)** among CD4+ and CD8+ memory T cells, respectively.

1. nein, 2. nein, 3. Allergan, Bayer, 4. nein, 5. nein