Human T lymphocytes dynamically change their activational status post stroke and interact with Microglia cells *ex vivo*

Lars Peglau[1]; Johanna Ruhnau, MD[1]; Thomas Krüger[2], MD; Alexander Dressel, MD[3]; Oliver Otto, PhD[4]; Juliane Schulze, PhD[1]; Antje Vogelgesang, PhD[1]

1 Department of Neurology, University Medicine, Greifswald, Germany; 2 Department of Neurology, AMEOS Clinic, Ueckermünde, Germany; 3 Department of Neurology, Carl-Thiem Clinic, Cottbus, Germany; 4 Zentrum für Innovationskompetenz: Humorale Immunreaktionen bei kardiovaskulären Erkrankungen, University Greifswald, Germany

Background

Post stroke, T lymphocytes exert differential effects both at the infarction site and systemically: pro-inflammatory cascades, subsequent T cell infiltration into the brain and persisting interaction of infiltrating T lymphocytes with brain resident Microglia cells deteriorate the parenchymal damage. The acute peripheral immune response is characterised by lymphopenia and persisting activation of circulating T lymphocytes. In the temporal course, it is supposed that anti-inflammatory mechanisms in general and especially anti-inflammatory M2-phenotype of Microglia cells help recover functionality of brain parenchyma, whereas inflammatory M1-phenotype contributes to long-term deterioration of network connectivity.

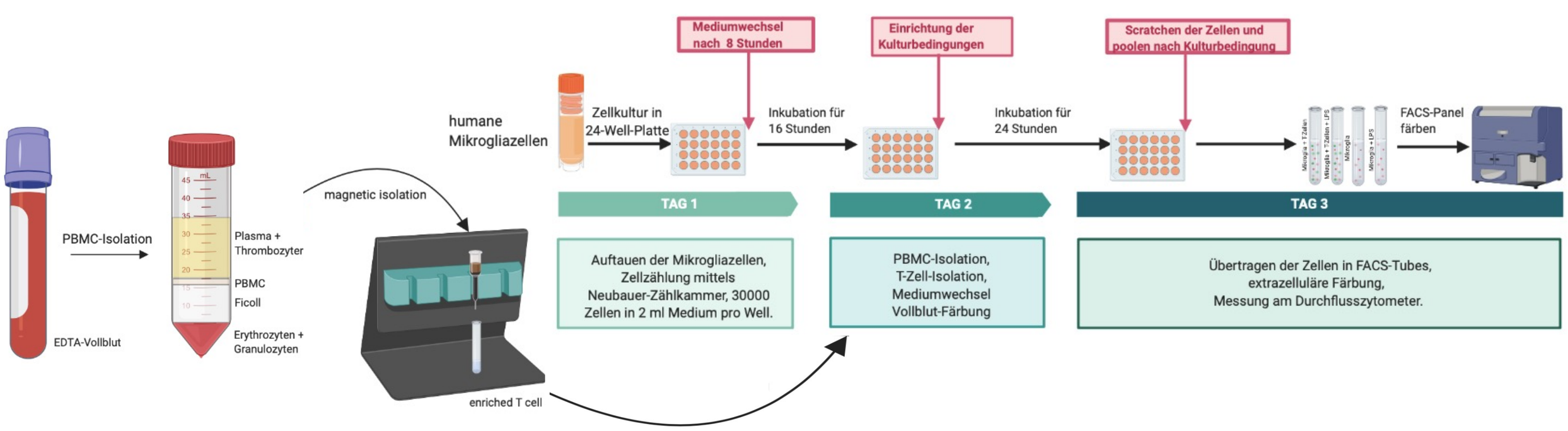
Aim

We elucidated the temporal course of peripheral blood T cell activation post stroke. Furthermore, we assessed the interaction of pan-T cells and DNTs respectively with Microglia cells *ex vivo*.

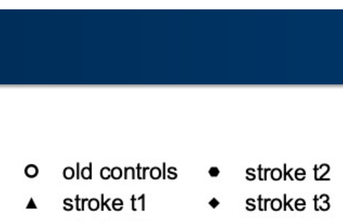
Methods

Samples were gained from a total of 10 patients (mean age ± SD: 68.8 ± 12.6 years). Patients were recruited if their stroke resulted in a National Institute of Health Stroke Scale (NIHSS) score of ≥ 4 and no signs of systemic infection or hyperthyreosis were detected on admission. Patients receiving immunosuppressive drugs or diagnosed with malignoma or an autoimmune disease were not recruited. 8 sex- and age-matched (mean age + SD: 70.3 + 9.8 years) and 20 young (mean age + SD: 23.9 + 3.2) neurologically and immunologically healthy control individuals were recruited.

Full blood from stroke patients and controls was analysed regarding T cell subpopulations and T cell activation (CD4, CD8, CD25, CD27, CD28, CD57, CTLA-4 and PD-L1) by flow cytometry. Pan-T cells and DNTs were isolated magnetically from stroke patients and controls and incubated with or without Microglia Cells ex vivo. Microglia activation (HLA-DR, CX3CR1, CD32, CD86, CD206 and CD209) was assessed by flow cytometry staining. Samples were acquired 3 days (t_1), 1 month (t_2) and 3 months (t_3) post stroke. For all analyses a p-value <0.05 was regarded significant.

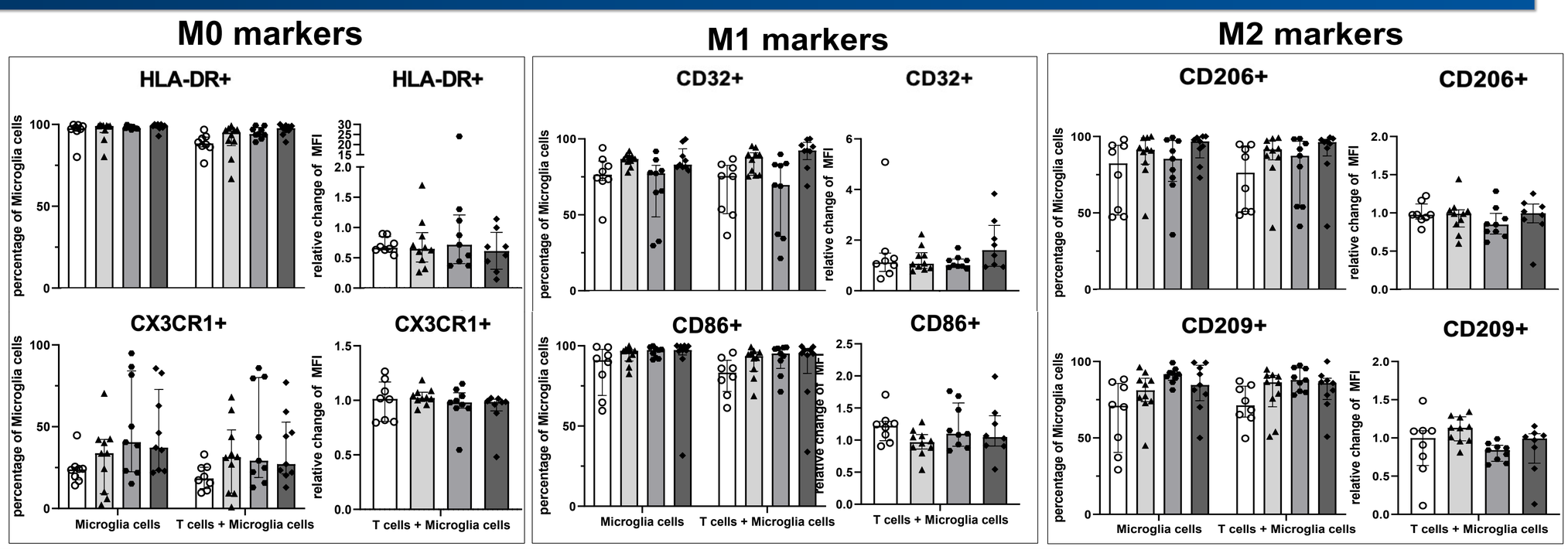


Results

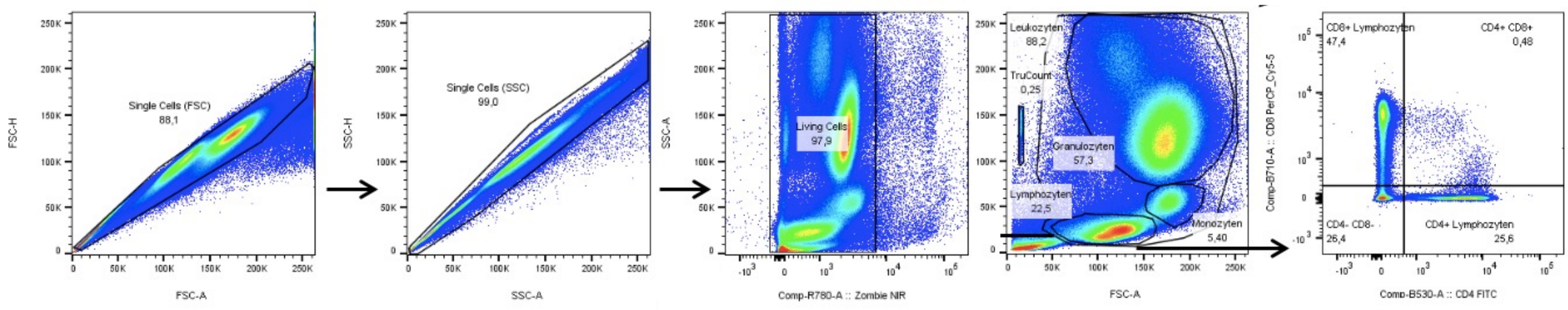


Flow cytometric M0-, M1- and M2-marker expression on Microglia cells after 24 hours of pure or coculture with pan T cells.

Displayed are percentage and expression density measured as relative change of median fluorescence intensity (MFI) calculated as $MFI_{coculture}$ / $MFI_{pure\ culture}$. Samples were acquired 3 days (t_1), 1 month (t_2) and 3 months (t_3) post stroke



Flow cytometric measurement of T cells in whole blood.



Conclusions

Stroke acutely leads to strong activation (CD25) of both CD4+ and CD8+ T cells. This activation persists in the subacute post stroke phase and only declines as early as 3 months post stroke. A role of PD-L1 and CTLA-4 in terminating the persistent T cell activation seem feasible and should be investigated in further studies.

Pan T Cells show little modulatory effects on Microglia cell activation status ex vivo.