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Effect of Extracorporeal Photopheresis on the Mobilisation of Granulocytic Myeloid-Derived Suppressor Cells in Chronic Graft Versus Host Disease

N.C. Matthews, C.E. Barker, A. Alfred and P.C. Taylor

Dept of Photopheresis, Rotherham NHS Foundation Trust, Rotherham, United Kingdom

Introduction

Extracorporeal photopheresis (ECP) is a therapy for treating steroid-refractory chronic graft versus host disease (cGVHD) and facilitating immunotolerance. Recent data suggests that therapeutic response to ECP is associated with a sustained induction of circulating granulocytic myeloid-derived suppressor cells (G-MDSCs), a heterogeneous population of immature and exhausted granulocytes, which exert suppression of proliferative and inflammatory cytokine T-cell responses (1). A relationship between sustained increases in G-MDSC frequency and therapeutic response to ECP was observed in acute GVHD patients, but not in those with cGVHD (1). However, data about G-MDSCs in long-term ECP patients (>6months) have not been reported. Here, we have used immunophenotyping to monitor the effect of ECP on G-MDSC mobilisation in cGVHD patients starting ECP and in a cohort of patients that are established on ECP.

Material (or patients) and Methods

A total of 29 patients: 11 with steroid refractory or steroid-dependent cGVHD before starting ECP (Pre-ECP), 18 established (>6 months; mean treatment duration of 19 months) cGVHD patients receiving ECP (Est-ECP) and 9 healthy controls were recruited. Pre-ECP patients had GVHD affecting skin (10/11), mouth (3/11) and gut (2/11).Est-ECP patients initially had GVHD affecting skin (16/18), mouth (4/18), gut (6/18) and liver (4/18). PBMC were isolated by Ficoll Hypaque separation and immunophenotyped for markers of G-MDSCs by flow cytometry. Statistical analysis was by two-tailed Mann Whitney test using GraphPad 6

Results

High side scatter CD14^{-ve}, CD16, CD66b, HLA-DR^{-ve}, CD33^{int} cells in PBMC were identified as G-MDSCs (Fig 1). Fig 2A shows that Est-ECP patients had substantially greater frequencies of circulating G-MDSCs than healthy controls (P<0.0001; median: 4% and IQR 1.6%-26% vs 0.3% and IQR 0.1%-0.35%, respectively), but a significantly lower frequency than in Pre-ECP cGVHD patients (P<0.02; median 13% and IQR: 1.5%-26%). Monitoring of G-MDSCs in both ECP cohorts (in 6/11 of the pre-ECP and 12/18 of the Est-ECP patients) over 12 weeks of ECP showed marked flux in some patients and relative stability in most others (Fig 3). Fig 4 shows that the frequency of G-MDSCs in Est-ECP patients showed an inverse relationship with time, where, as shown in Fig 5., patients with a complete response to long-term ECP therapy had low G-MDSC frequencies far closer to those of healthy controls (median:0.6% and IQR 0.5%-2.3%). Where analysis over 3 months showed that most Est-ECP patients had relatively stable G-MDSC frequencies, Fig 6. shows that further immunophenotyping revealed that patients with very low levels of, or undetectable, circulating CD19^{+} B-cells (<1% of total lymphocytes), had significantly higher frequencies of G-MDSCs than patients with higher frequencies of B-cells (P<0.0013; median: 31% and IQR 26%-42%; n=4 vs median 3.7% and IQR 1.9%-8.1%; n=14, respectively).

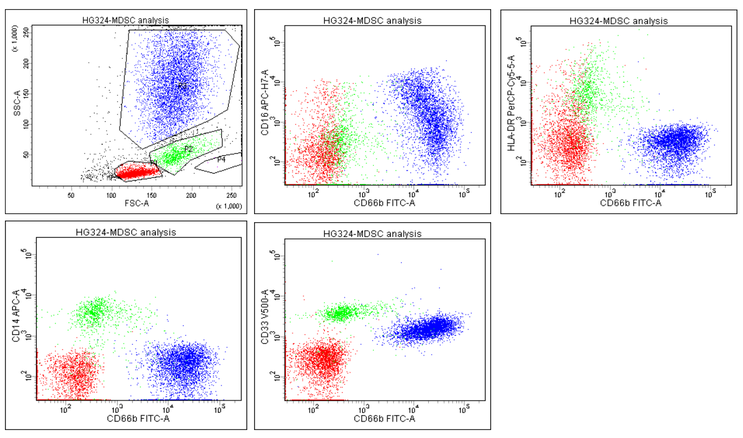

Fig 1. Identification of G-MDSCs (blue region) in PBMC (lymphocytes (red region) and monocytes (green region) from patients with cGvHD (n=29).

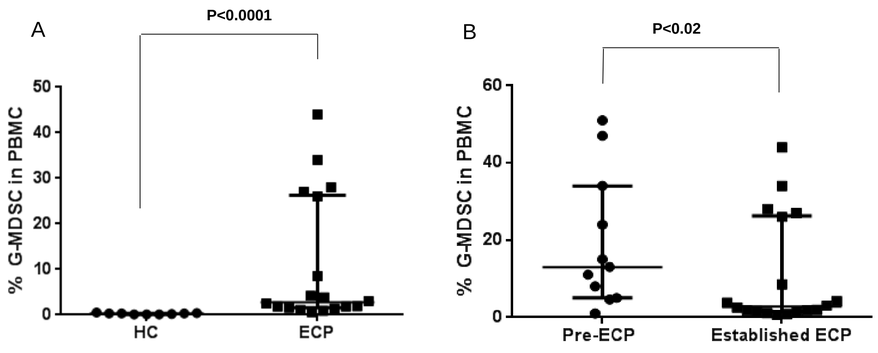

Fig 2. (A) Established ECP patients (n=18) have elevated frequencies of G-MDSCs compared to healthy controls (n=9) . (B) Established ECP patients have significantly lower frequencies than patients newly recruited for treatment with ECP (Pre-ECP; n=11).

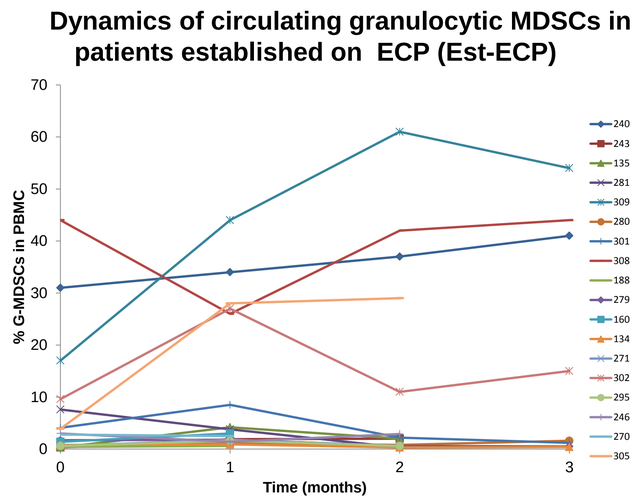

Fig 3. Marked inter-individual variation in flux of frequencies of G-MDSCs in cohorts of both newly-recruited (n=11) and those established on ECP (n=18)

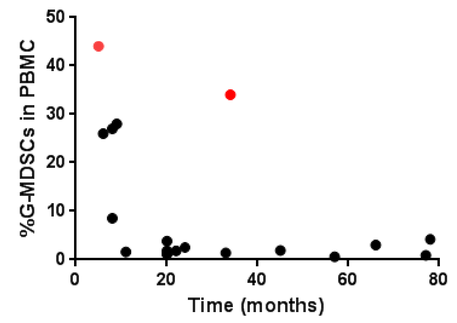

Fig 4. Relationship of G-MDSC frequency to duration of treatment with ECP in established ECP patients (n=18). The red symbol denotes those patients on Rituximab.

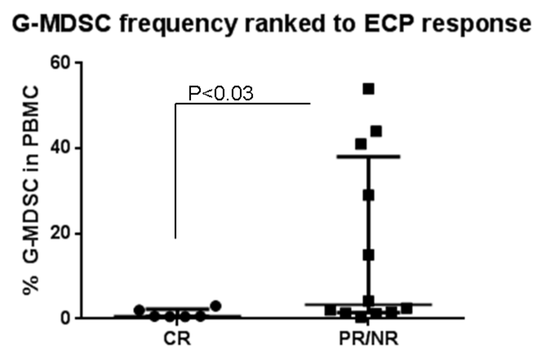

Fig 5. Complete response (CR) to ECP is linked with normalisation of G-MDSC frequency in established ECP patients compared to those with partial (PR) or no response (NR).

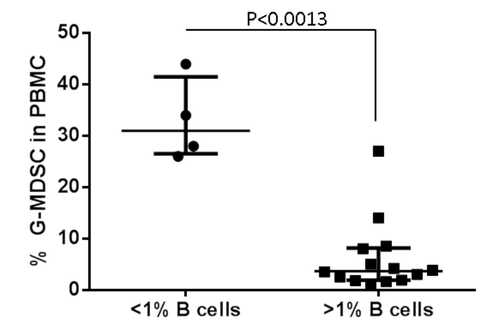

Fig 6. Highest frequencies of G-MDSC frequency in established ECP patients is associated with ultra low B-cell frequency (% of lympho-cytes) (n=18)

Conclusion

Consistent with Rieber et al (1), there was no pattern of sustained G-MDSC induction in ECP-treated cGVHD patients. In contrast, the data suggest that high frequency of circulating G-MDSCs is associated with a state of markedly dysregulated immunity. We conclude that the immunomodulatory therapeutic response to ECP in cGVHD patients is indicated by a decrease in circulating G-MDSC frequency.

References

Rieber, N. et al. 2014. Bone Marrow Transplantation 49:545-552

Acknowledgements

We thank the patients who kindly donated blood samples for this study, the nursing team – Julie Ball, Maggie Foster, Tracy Maher, Janet Mayo, Leeah Robertson, Cherie Rushton and Cheryl Swift, Carron Bilton for patient data collection and the Medical Statistics Group, ScHARR, University of Sheffield