Variable Levels of Suppressor Function in Low Density Neutrophils from Patients Receiving Extracorporeal Photopheresis for Chronic Graft Versus Host Disease

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Introduction

Extracorporeal photopheresis (ECP) is a therapy for steroid-refractory chronic graft versus host disease (cGvHD). Therapeutic response to ECP has been linked with a progressive increase in circulating granulocytic myeloid-derived suppressor cells (G-MDSC) in acute GvHD, but not in cGvHD[1].

1. Low density neutrophils (LDN) phenotypically resembling G-MDSC (putative G-MDSC) show marked flux in cGvHD patients receiving ECP, and a reduction in their frequency is associated with a sustained therapeutic response to ECP[2].

2. Recent data has identified Lectin–type oxidized LDL receptor-1 (LOX-1) as a specific marker of LDN with T-cell (Tc) suppressive activity[3].

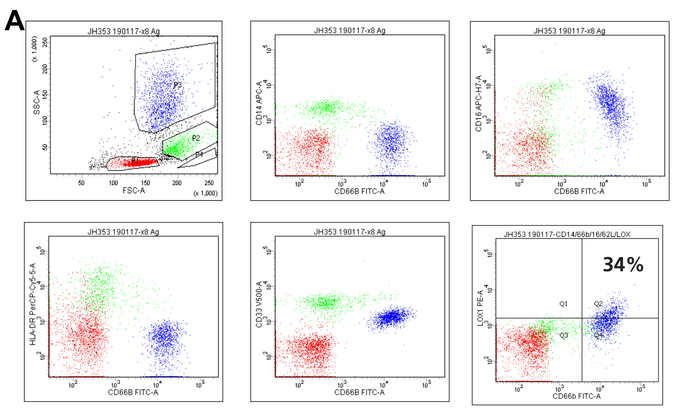
3. Using this marker we have conducted a cross-sectional study to assess whether putative G-MDSCs in this patient cohort have suppressive activity.

Materials & Methods

15 patients with steroid refractory or steroid-dependent cGVHD (mean treatment duration of 9 months) receiving ECP and 8 healthy controls were recruited. Patients had GVHD affecting skin (15/15), liver (3/15) and gut (2/15). PBMC were isolated and immunophenotyped by flow cytometry for markers of G-MDSCs (CD14-ve, CD16, CD66b, HLA-DR-ve, CD33int) and LOX-1 expression. Suppressive function was determined by measuring the inhibition of proliferation of anti-CD3/CD28-activated purified CD3 Tc from healthy donors by 4-day co-culture with G-MDSCs from patients. Statistical analysis was conducted using GraphPad 6.

Results

ECP patients had substantially greater frequencies of circulating putative G-MDSC than healthy controls (Fig 1A and summarised in Fig 1B: median: 13% and IQR 2%-32% vs 0.2% and IQR 0.1%-0.6%, respectively; P<0.0001). While there were substantially greater frequencies of circulating LOX-1+ cells in PBMC from ECP patients than healthy controls (Fig 2A and summarised in Fig 2B: median: 1.5% and IQR 0.39%–9.2% vs 0.053% and IQR 0.029%–0.062%, respectively; P<0.0001), these were mainly the minority population within the putative G-MDSC fraction with no significant difference between ECP patients and healthy controls in the proportion of LOX-1+ cells within LDN (Fig 2C: 29% +/- 16% vs 21% +/- 9%, respectively). Fig 3A shows that ECP had no significant effect on circulating putative G-MDSC frequency measured before and the day after treatment (median: 8.4% and IQR 4%-44% vs 16% and IQR 6%-25%; n=11, respectively) nor on LOX-1 frequency (Fig 3B: median: 1% and IQR 0.29%–12% vs 2.8% and IQR 0.88%–7.3%; n=9, respectively). Analysis of suppressive capacity by functional assay shown in Fig 4 demonstrates that at a T-cell:G-MDSC ratio of 1:1, isolated G-MDSCs from ECP patients suppressed CD3 Tc proliferation (mean +/- SD: 52%+/- 23 %; n=14). However, the potency of suppression was highly variable (min-max: 18%-82%).



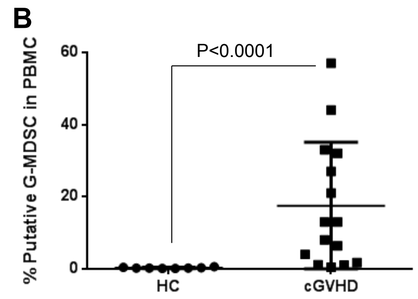
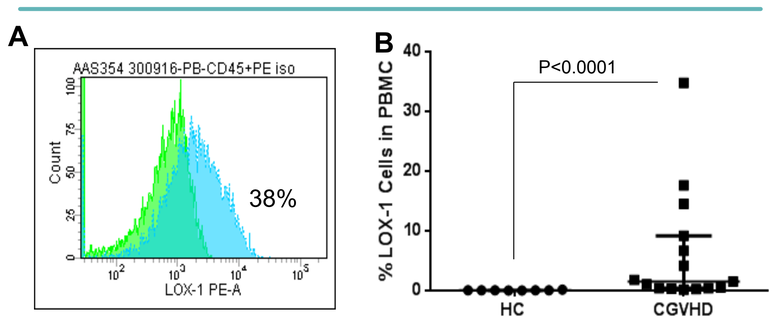


Fig 1. (A) Identification of putative G-MDSCs (blue region) in the LDN fraction of PBMC (lymphocytes (red region) and monocytes (green region) from patients with cGvHD. (B) ECP patients (n=15) have significantly elevated frequencies of putative G-MDSCs compared to healthy controls (n=8). P values calculated by Mann Whitney.



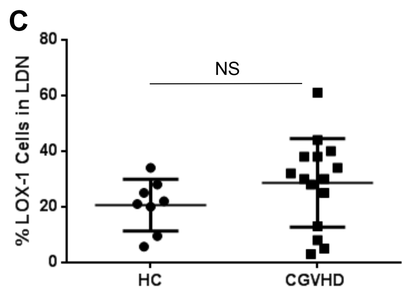


Fig 2 (A) Expression of LOX-1 by LDN from a cGvHD patient undergoing ECP. (B) ECP patients (n=15) have elevated frequencies of LOX-1 cells compared to healthy controls (n=8). (C) ECP patients (n=15) have similar proportions of LOX-1+ cells within LDN compared to healthy controls (n=8). P values calculated by Mann Whitney.

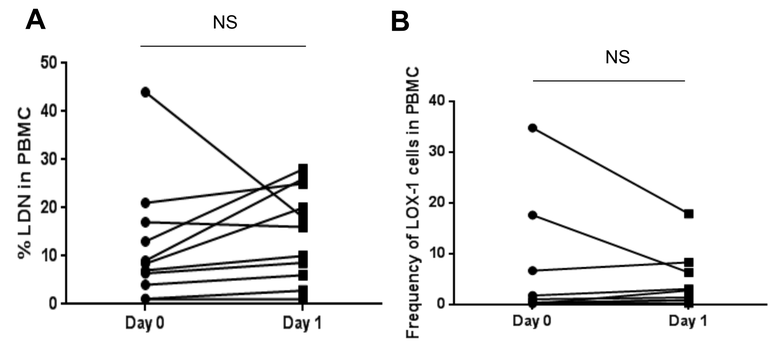


Fig 3. No significant effect of ECP on proportions of either (A) LDN in PBMC or (B) LDN expressing LOX-1 (n=11 and 9, respectively). Calculated by Wilcoxon's Signed Rank test.

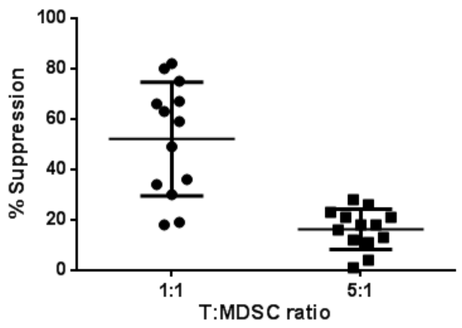


Fig 4. Putative G-MDSCs from ECP patients (n=14) have a wide range of ability to suppress activated CD3 T-cells

Conclusion

The pattern of LOX-1 expression suggests that only a subset of putative G-MDSCs in ECP patients are suppressive and may explain why suppressive function in this cell fraction is so highly variable. However, the relatively high frequency of LOX-1 cells in this patient cohort might contribute to overall immunosuppression. This requires further investigation.

References

1. Rieber, N. et al. 2014. Bone Marrow Transplantation 49:545-552
2. Matthews, N.C. et al. 2016. Bone Marrow Transplantation 51:S375
3. Condamine, T. et al. 2016. Sci. Immunol 1: aaf8943

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