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P1909

TARGET ORGAN TROPISM OF EFFECTOR T CELL POPULATIONS IN CHRONIC GRAFT-VERSUS-HOST DISEASE

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Introduction

- Chronic graft-versus-host-disease (cGVHD) may affect a broad range of organs, including the skin, gut, liver, eyes and lung.
- Patients may exhibit single or multiple organ pathology. Factors which influence particular organ involvement are poorly understood.
- Chemokines are a group of small (8–14 kDa) cytokines that exert key roles in the regulation of cell migration, activation and proliferation
- HSCT conditioning regimes can trigger cytokine production (e.g TNF-α, IFN-γ, IL-1) which consequently up-regulate expression of chemokine receptors, adhesion molecules (such as integrins) and their ligands
- Expression of integrins & specific chemokine receptors have been illustrated to promote allogeneic T cell entry to target organs and the profile of chemokine & chemokine receptor expression varies in GVHD target organs [1]
- We have previously reported associations between differential chemokine expression, cGVHD organ profile, disease severity and correlation with treatment response [2]
- We investigated chemokine receptor & tissue specific adhesion molecule expression in circulating effector T cells of cGVHD patients to determine whether organ involvement is also reflected within these cell populations

Methods

- Peripheral blood lymphocytes from 20 steroid-refractory active cGVHD adult patients (*See Table 1*) were examined using flow cytometry. Effector, naïve and memory CD4+ and CD8+ T cells were identified by CD45RA +/- CCR7 and regulatory T cells by CD4+CD25+ CD127dim/neg phenotype
- Expression of chemokine receptors CXCR3, CXCR6, CCR4, CCR5, CCR7, CCR9 and CCR10 were assessed along with expression of skin homing receptor, cutaneous lymphocyte antigen (CLA), gut tropic β7 integrin vascular addressins & compared with normal controls (NC).

Results

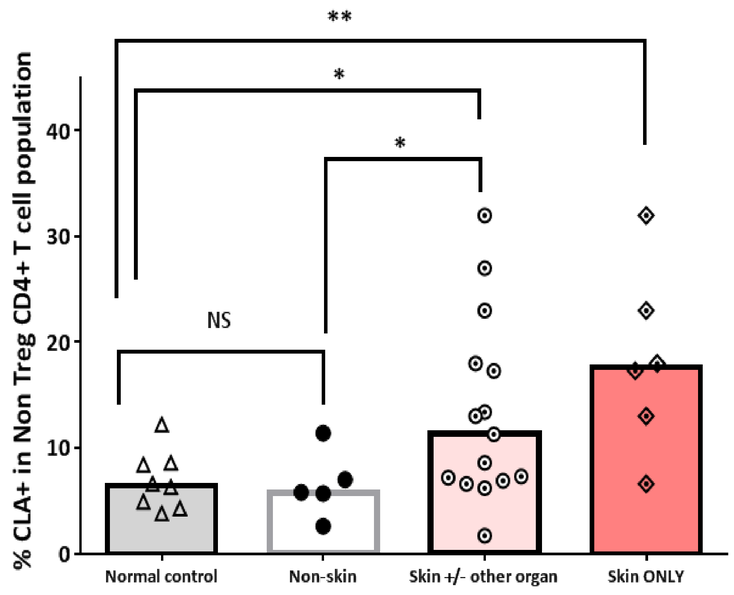

Figure 1. The proportion of circulating effector CD4+ T cells bearing skin homing receptor CLA were significantly elevated in patients with skin disease (n=15), compared to patients without skin disease (n=5) and healthy controls (Median 13% vs 6%, P=<0.05). Patients without skin disease had proportions similar to healthy controls. Median proportion of CLA+ CD4+ effectors was highest in patients with isolated skin disease (n=6, 18%).

Note for all figures; (*P=<0.05, **=<0.01, ***=<0.001, P=>0.05, NS)

Acknowledgements; Thanks to all nursing staff involved in the study and to all patients providing samples

Results

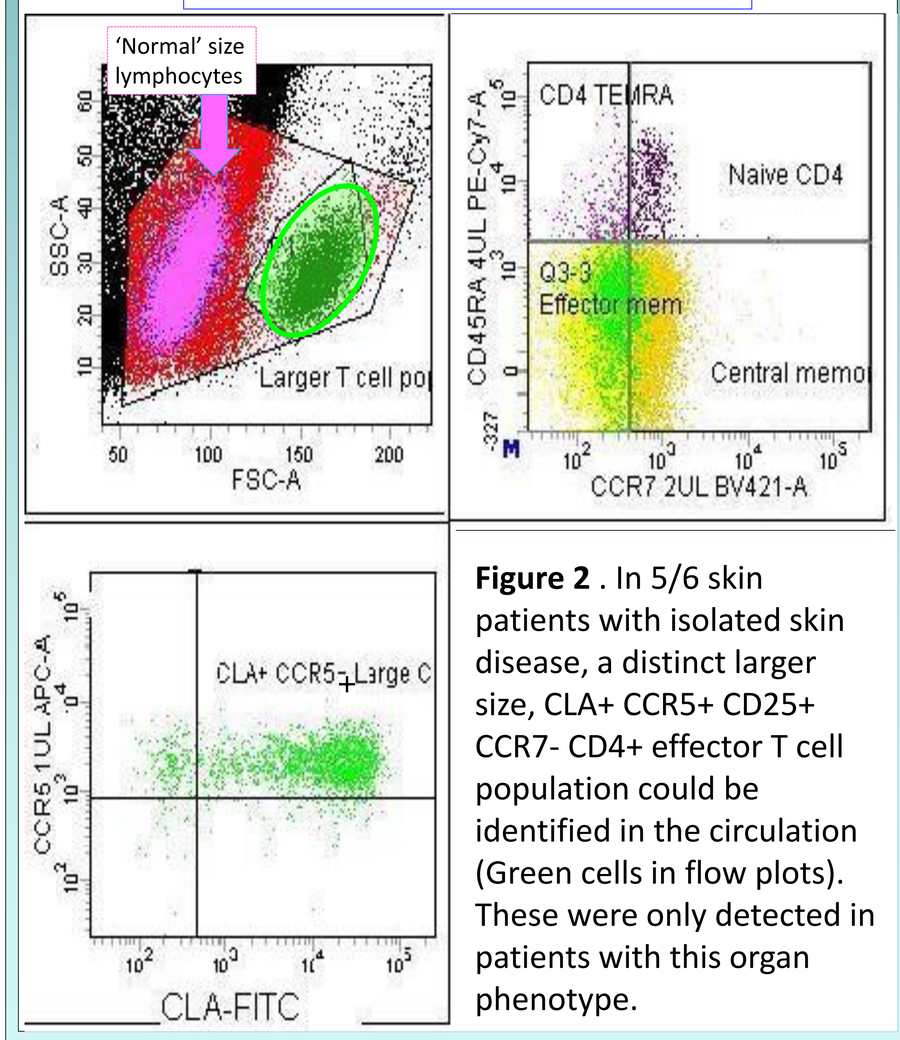

Figure 2 . In 5/6 skin patients with isolated skin disease, a distinct larger size, CLA+ CCR5+ CD25+ CCR7- CD4+ effector T cell population could be identified in the circulation (Green cells in flow plots). These were only detected in patients with this organ phenotype.

- Th1 phenotype effector cells were elevated in cGVHD patients (**Fig 3**).
- CD4+ effector T cells bearing chemokine receptor CCR5 were elevated in skin-only disease patients compared with non-skin (Median 60% vs 20%, P=<0.05).(Data not shown)
- CCR5 expression in circulating effector CD8+ T cells was also significantly elevated in cGVHD patients compared to controls (Median 45 vs 28%, P=<0.05)(Data not shown)

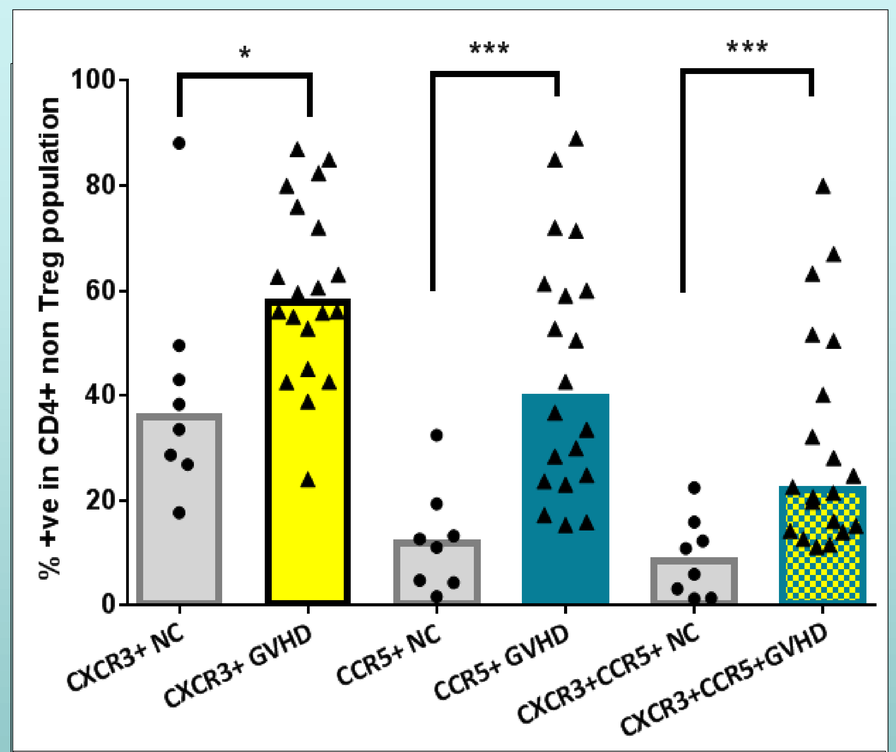

Figure 3. Proportions of CXCR3+, CCR5+ & dual CXCR3+ CCR5+, CD4+ effector T cells of a Th1 phenotype were all elevated compared to NC, however no association with particular differential organ involvement of CXCR3+ cells was evident in the cohort. *(Mann Whitney, * P=<0.05, **=<0.01, *** =<0.001)*

Table 1. Organs affected by cGVHD within cohort

cGVHD organ involvement	n (Cohort size = 20)
Skin +/- and other organ	15
Skin only	6
Liver	6
Mucous membranes	6
Gut	5
Eyes	5
Lung	2

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Conclusions

The frequency of chemokine receptors CCR5, CXCR3, CXCR6 and cutaneous lymphocyte antigen (CLA) expression on circulating effector CD4+ T cells is elevated in cGVHD patients compared to controls.

Circulating effector CD4 T cells bearing CLA and or CCR5 are preferentially elevated in skin disease patients.

For patients with isolated skin disease, a distinct CLA+CCR5+ effector cell population was identified.

Th1 effector phenotype cells (CCR5+ CXCR3+) are prevalent in the circulation of cGVHD patients.

For patients with liver and or gut cGVHD, CXCR6+ non Tregs were significantly more frequent in circulation than in patients without liver or gut disease.

This study identifies associations between the phenotype of specific chemokine receptor & adhesion molecule expression in circulating effector T cells and the organs affected by cGVHD. However it is unclear whether these observations are cause or effect

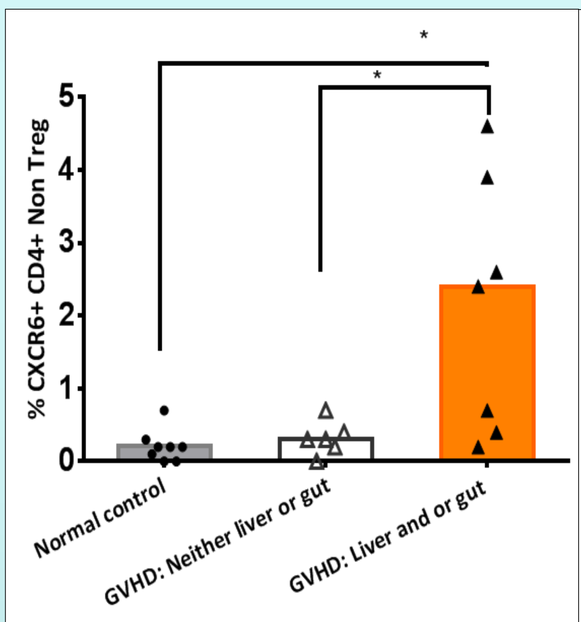

Figure 4. Patients with either liver or gut cGVHD (n=7) had increased frequency of CXCR6+ T cells amongst non Treg CD4 T cells compared to those without liver or gut involvement and controls (2.4 % vs 0.3%. P=0.04, *Fishers exact)*

Discussion

The skin homing receptor CLA and its interaction with its ligand, E-selectin, represent an important axis in the ingress of T cells into the skin. The majority of CLA+ T cells are resident in the skin under resting conditions so whilst recruitment of additional T cells from circulation may be a feature of cutaneous inflammatory conditions, it may not be pre-requisite.

However, the presence of elevated proportions of CLA+ effector T cells in skin cGVHD patients, and in skin disease only patients, the existence of an activated phenotype CLA+ effector T cell population, lacking in CCR7, (unlikely to be retained in the lymph node), is supportive of skin-tropic effector cells which may be contributing to the target organ pathology.

The identification of CCR5+ CD4+ and CD8+ effector populations in skin disease patients is consistent with allogeneic expansion & cellular migration / tissue entry studies in acute GVHD [3]. Our reported lack of association of CXCR3+ CD4+ effector T cells with specific organ involvement may reflect the diverse tissue production of CXCR3 ligand chemokines which may promote multiple target-organ T cell entry [2,4].

In contrast, the expansion of CXCR6+ T cells in liver / gut cGVHD parallels our previous data reporting specific elevation of the CXCR6 ligand, CXCL16, in liver/ gut disease patients [2]. Expansion of cell populations bearing receptors specific for tissue differential chemokines reinforces our previous data illustrating specific modulations of reciprocal chemokine signals in cGVHD[2] & may further understanding of differential organ involvement and prediction of clinical response.