POSTER 190

The CD6-ALCAM Pathway Selectively Modulates Pathogenic T cell Migration

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Introduction

- CD6 is a costimulatory receptor on T cells that through binding to its ligand "Activated Leukocyte Cell Adhesion Molecule" (ALCAM), modulates both the activity and trafficking of effector T cells (Teff).[1]
- ALCAM expression is reported in several cell types including hematopoietic stem cells, cancer stem cells, intestinal epithelial crypts, the central nervous system and the microvascular endothelium.[2,3]
- ALCAM has been seen to be overexpressed on various tissues during inflammation and it is associated with increased infiltration of pathogenic CD6high CD4+ T cells in patients with autoimmune and inflammatory diseases, including Acute Graft versus host disease (aGVHD).[4,5]
- ALCAM plays an essential role in the recruitment of leukocytes through the Blood Brain Barrier (BBB) and blocking ALCAM prevents the migration of CD4+ T cells and monocytes, reducing the onset on autoimmune encephalomyelitis[6].
- Similarly, expression of CD6 and ALCAM is increased in inflamed mucosa of IBD patients, driving the Th1/Th17 immune response[7].
- However, the exact role of the CD6-ALCAM pathway in the chemotaxis of pathogenic T cells into inflamed tissues is not yet clear.

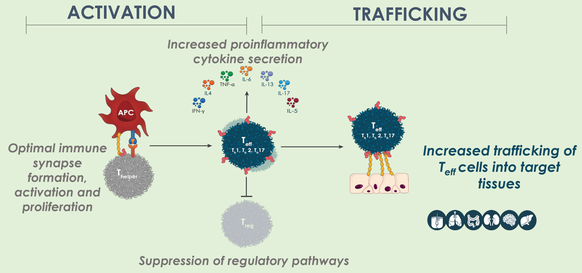


Fig.1 CD6 promotes T cell activation, cell proliferation and trafficking.

- Treatment of T cells with itolizumab (EQ001), a first in class monoclonal antibody targeting CD6, induces the cleavage of the receptor from the surface of T cells in the presence of monocytes[8], making EQ001 the perfect tool to study both the blocking of CD6-ALCAM interaction and the effect of lack of CD6 on the surface of T cells. (Fig.2)

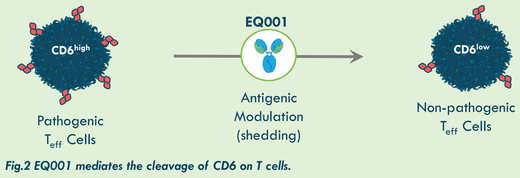
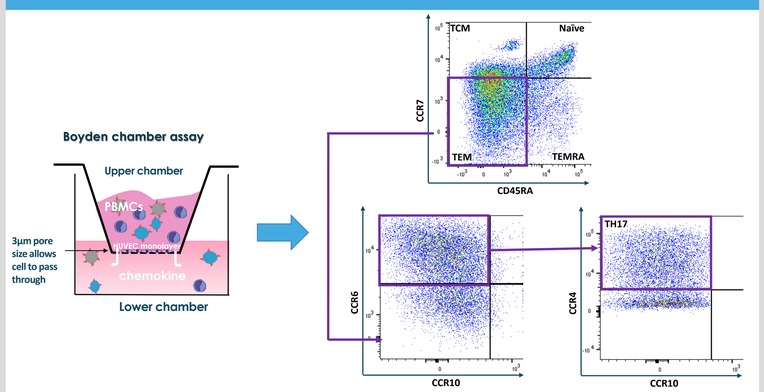


Fig.2 EQ001 mediates the cleavage of CD6 on T cells.

OBJECTIVES:

1. **To investigate changes in CD6 levels in the migration of lymphocytes in response to the chemokine CXCL12 (SDF-1).**
2. **To study the role of the CD6-ALCAM pathway in the migration of pathogenic effector CD4+ T cells.**
3. **To characterize the effector populations whose migration is enhanced by the CD6-ALCAM pathway.**

Methods



- The migration of T_{eff} cells was tested using a Boyden chamber assay that incorporated human umbilical vein endothelial cells (HUVEC) which express high levels of ALCAM (Fig3).
- HUVEC cells were plated on a PET insert (3µm) in growth media for 48h to form a monolayer.
- Before entering the transmigration assay, PBMCs were pre-treated with EQ001 or an isotype control. In some experiments, PBMCs were stimulated with soluble anti-CD3 5 µg/ml and anti-CD28 1µg/ml overnight.
- 500k PBMCs were introduced in the migration assay for 24h.
- To generate CD6 low cells, total PBMCs were incubated with EQ001 overnight (O/N) and washed twice to remove the antibody, then introduced in the migration assay.
- Th17 were stained from the total PBMCs population using the extracellular markers CCR6, CCR10 and CCR4.
- 2 donors were used for unstimulated and activated PBMCs.

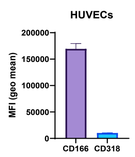
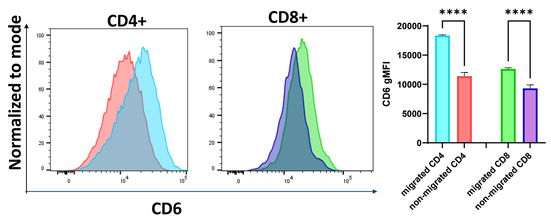


Fig. 3 ALCAM and CD318 (the two main CD6 ligands) expression levels on HUVECs

Results

- **T cells that migrate in response to CXCL12 preferentially express higher levels of CD6**

CD6 expression was investigated in the migrated CD4 and CD8 population in response to CXCL12 compared to non-migrated cells



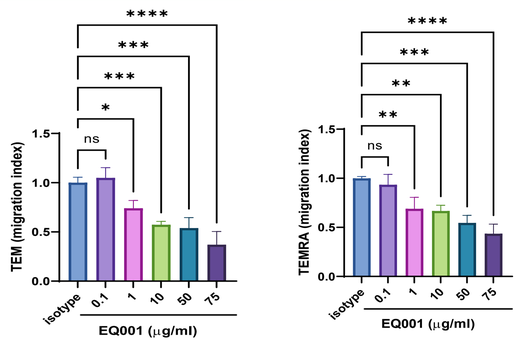
Left: Histogram levels of CD6 on non-migrated CD4+(red) or CD8+(green) vs migrated T cells (respectively blue and purple).

Right: Geometric mean of CD6 MFI on non-migrated vs migrated CD4+ and CD8+ T cells (mean ± SD, p<0.0001, 1-way ANOVA test).

- **Migration of CD4+ T Effector Memory cells (TEM) and Terminally Differentiated Effectors (TEMRA) in response to CXCL12 is prevented by treatment with EQ001**

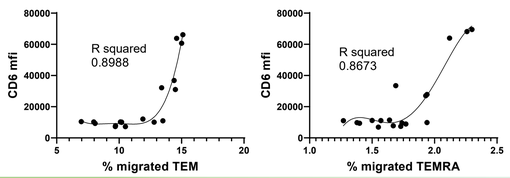
- To assess the role of CD6 in the migration of effector T cells, total PBMCs were treated with different concentration of EQ001, generating a gradient of CD6 levels.

UNSTIMULATED PBMCs: TEM and TEMRA analysis



TEM (CD4+CCR7-CD45RA-) and TEMRA (CD4+ CCR7-CD45RA+) migration index, calculated by dividing the number of migrated cells in the treatment by the number of migrated cells in the control (mean ± SD, p<0.05 *, p<0.01 **, p<0.001 ***, p<0.0001 ****, calculated with One way ANOVA test).

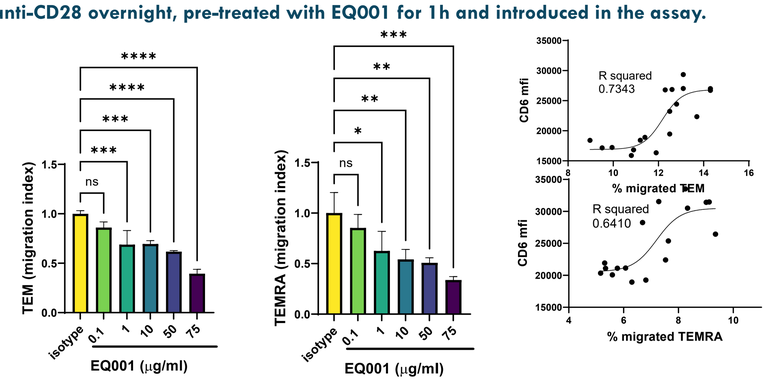
- **Reduced migration of CD4+ TEM and TEMRA correlates with CD6 levels**



Correlation between CD6 levels on TEM CD4+ cells and their % in the migrated cell (left panel). Correlation between CD6 levels on TEMRA CD4+ cells and their % in the migrated cell (right panel).

ACTIVATED PBMCs: TEM and TEMRA analysis

- To boost the activity of T cells, total PBMCs were activated with soluble anti-CD3 and anti-CD28 overnight, pre-treated with EQ001 for 1h and introduced in the assay.

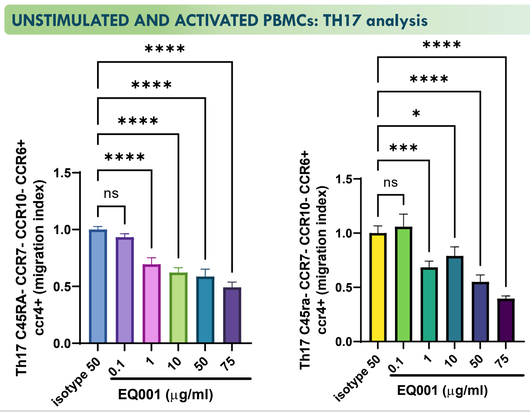


Migration index of TEM and TEMRA in activated PBMCs treated with isotype or EQ001 at different concentrations (left) and corresponding correlation with CD6 levels (right), (mean ± SD, p<0.05 *, p<0.01 **, p<0.001 ***, p<0.0001 ****, calculated with One way ANOVA test).

- **Migration of TH17 is reduced by treatment with EQ001**

- We next investigated specifically the different effectors populations and identified TH17 cells as main one that migrates less when blocking the CD6-ALCAM pathway

UNSTIMULATED AND ACTIVATED PBMCs: TH17 analysis

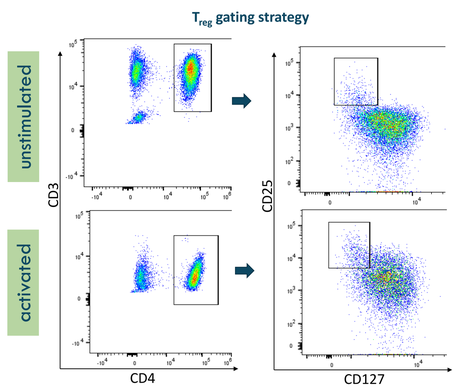


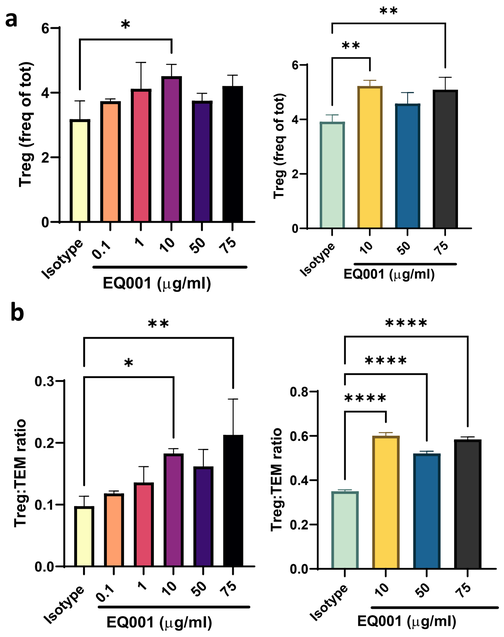
TH17 effectors migration index from unstimulated PBMCs and activated PBMCs, calculated by dividing the number of migrated cells in the treatment by the number of migrated cells in the control (mean ± SD, p<0.05 *, p<0.01 **, p<0.001 ***, p<0.0001 ****, calculated with One way ANOVA test).
Left: unstimulated PBMCs
Right: activated PBMCs

- **Migration of T_{reg} cells is not affected by EQ001 treatment**

- As Treg migration in inflamed tissues is important for restoration of a healthy environment, we investigated whether EQ001 treatment affected the migration of this cell subtype.

UNSTIMULATED AND ACTIVATED PBMCs: Treg analysis





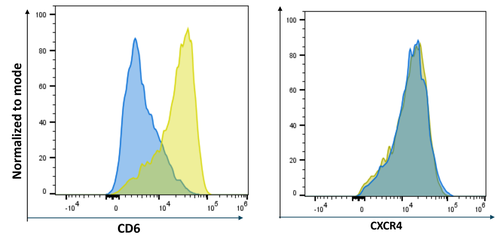
Frequency of T_{reg} in the migrated and non-migrated population for unstimulated (a) and activated (b) PBMCs. Data are shown as frequency of total cells and Teff/Treg ratio. (mean ± SD, p<0.05 *, p<0.01 **, p<0.001 ***, p<0.0001 ****, calculated with two-way ANOVA test).

- Blocking the CD6-ALCAM pathway does not affect the migration of T_{reg} through the endothelial layer and, thus, increases the ratio of Treg to effector CD4+ T cells in the lower chamber.

ACTIVATED PBMCs TREATED WITH EQ001: CXCR4 analysis

- **Changes in the migration of Effector T cells are specific to CD6 and not related to changes on the CXCL12 receptor, CXCR4**

- CXCR4 levels in migrated effector cells treated with isotype or EQ001 were investigated in order to show that the effect on migration is specific to CD6



Isotype
EQ001

Histograms of CD6 and CXCR4 levels on migrated CD4+ TEM treated with isotype (yellow) or EQ001 50µg/ml (blue).

- Blocking the CD6-ALCAM pathway in unstimulated and activated PBMCs reduces the migration of CD4+ TEM and TEMRA cells by ~60% and this correlates with CD6 expression levels on these cells, while it is not due to a reduction in CXCR4 expression.

Conclusions

- **Higher level of CD6 are associated with migration of CD4 and CD8 in response to CXCL12 and the amount of migration correlates with levels of CD6 expression, suggesting that CD6 is engaged during T cell migration across the endothelial monolayer.**
- **Decreasing CD6 expression led to decreased migration of TEM and TEMRA cells while migration of T_{reg} cells was unaffected, thus suggesting targeting CD6 in autoimmune and inflammatory diseases would decrease infiltration of pathogenic T cells while still permitting modulation of the immune response by T_{reg} cells.**
- **Th17 cells were stained from the total PBMCs population that migrated in response to CXCL12. Our data showed a reduction of the migration of those cells after blocking the CD6-ALCAM pathway with EQ001.**
- **Altogether, this data suggests that CD6-ALCAM pathway is involved in the movement of T cells through the endothelial tissues and confirms CD6 as a target to prevent pathogenic T cell recruitment into inflamed organs, supporting evaluation of EQ001 in subjects with aGVHD in our ongoing clinical study (NCT05263999).**

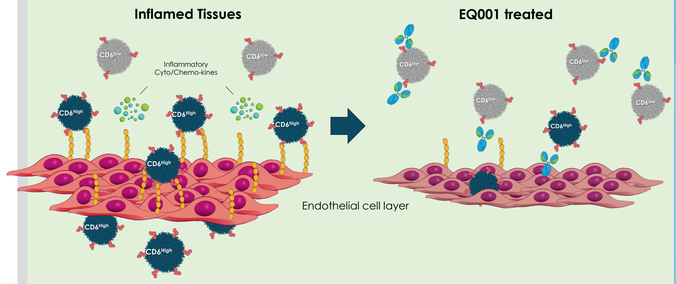


Fig. 4 Blocking the CD6-ALCAM pathway prevents the migration of pathogenic CD6high T cells.

Acknowledgments

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Disclosures

This study was funded by Equillium, Inc. Valeria Marrocco, Jeanette Ampudia, and Cherie Ng are currently employees and stockholders of Equillium. Stephen Connelly is currently an employee, stockholder, and officer of Equillium.

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