The CD6-ALCAM Pathway Selectively Modulates Pathogenic T cell Migration

Valeria Marrocco1, Jeanette Ampudia1, Stephen Connelly1, Cherie Ng1
1Equilibrium, Inc. San Diego, CA

Introduction

CD6 is a co-stimulatory 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 (Ref1).

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.

ALCAM has been shown to be overexpressed on various tissues during inflammation and it is associated with increased infiltration of pathogenic CD6high T cells in patients with autoimmune and inflammatory diseases, including Acute Graft versus Host disease (GvHD).1,5

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 of an 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.

Methods

- Treatment of T cells with hizumab (EQ001), a first in class monoclonal antibody targeting CD6, induces the clearance of the receptor from the surface of T cells in the presence of monocytes, 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)

- To study the role of the CD6-ALCAM pathway in the migration of pathogenic effector CD4+ T cells.

- To characterize the effector populations whose migration is enhanced by the CD6-ALCAM pathway.

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

- Migration of CD4+ T Effector Memory cells (TEM) and Terminally Differentiated Effectors (TEMRA) correlates with CD6 levels

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 ***), 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).

- Migration of Treg cells is not affected by EQ001 treatment

Migration of Treg cells is not affected by EQ001 treatment (Fig 4)

- Block the CD6-ALCAM pathway does not affect the migration of Treg cells through the endothelial layer

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

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 TREG cells while migration of TEMRA cells was unaltered, 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 Treg cells.

- Th17 cells were stained from the total PBMCs population using the extracellular markers CCR6, CCR10 and CCR4.

- 2 donors were used for unstimulated and activated PBMCs.

Acknowledgments

We would like to acknowledge the whole research team for constant feedbacks and suggestions on the project, particularly to Maya Rieder for her contributions in experiments that are not presented in this context.


Disclosures

This study was funded by Equilibrium, Inc., Valeria Marrocco, Jeanette Ampudia, and Cherie Ng are employees of Equilibrium and stockholders of Equilibrium.

Stephen Connelly is currently an employee, stockholder, and officer of Equilibrium.

To request permission or to ask any questions about the poster, please contact Valeria Marrocco at equilibriumbio.com.

© Equilibrium Inc.