CD6 Inhibition Prevents Skin and Kidney Disease in a Spontaneous Murine Model of SLE

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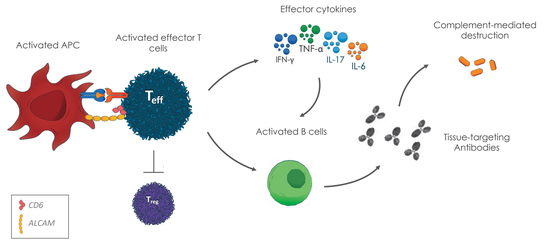
Introduction

- T cells are an important contributor to the pathogenesis of systemic lupus erythematosus (SLE) and its various end organ manifestations, including lupus nephritis (LN). Consequently, they present themselves as attractive therapeutic targets. However, the optimal approach to targeting T cells in SLE still remains under investigation.
- CD6 is a co-stimulatory receptor, predominantly expressed on T cells, that binds to activated leukocyte cell adhesion molecule (ALCAM), a ligand expressed on antigen presenting cells and various epithelial and endothelial tissues.
- The CD6-ALCAM pathway plays an integral role in modulating T cell activation, proliferation, differentiation, and trafficking (diagram below).

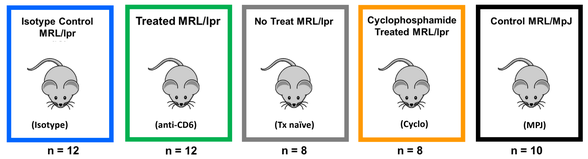
Role of CD6+ T cells in SLE/LN



- In this study, we assessed the expression of CD6 and ALCAM within the context of a spontaneous murine model of SLE and LN, and then targeted this signaling pathway to determine its role in the pathogenesis of disease.

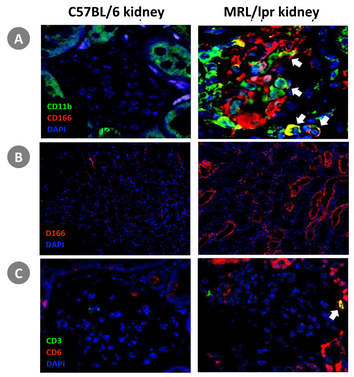
Experimental Design

Study groups



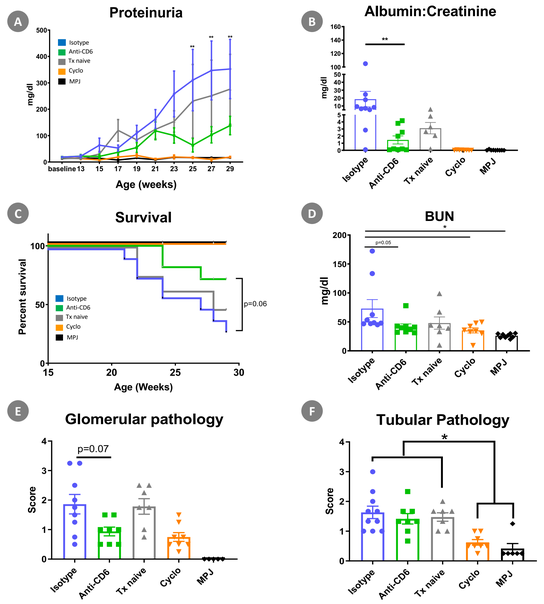
- The MRL/lpr mouse is a murine model of SLE with many similarities to human disease, including the spontaneous development of systemic anti-nuclear autoimmunity, nephritis and skin disease.
- Female MRL/lpr mice were aged to 9-10 weeks of age, at which point treatment was begun with either anti-CD6 antibody (10D12, 60 ug/dose, intraperitoneally twice per week), an irrelevant polyclonal rat IgG isotype control (60 ug/dose, twice per week), or cyclophosphamide (25 mg/kg, once per week). We also included a no treatment group and a group of MRL/MpJ mice, a congenic healthy control strain.
- Baseline levels of anti-DNA antibodies, weight and proteinuria in the MRL/lpr groups were similar. Mice were monitored weekly for proteinuria, lymph node enlargement, and macroscopic skin lesions.

Figure 1: ALCAM & CD6 renal expression



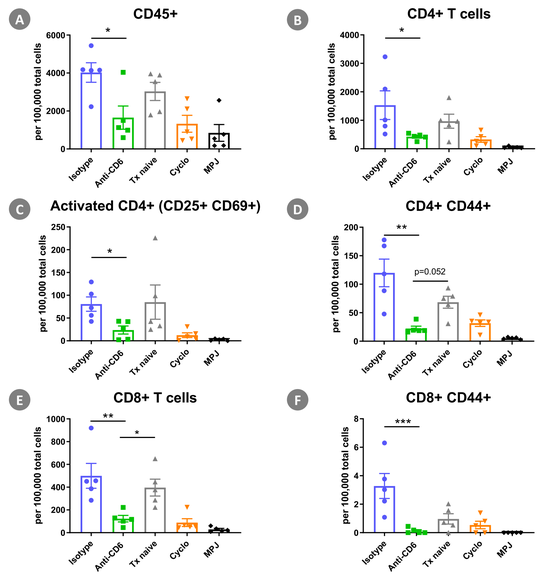
Kidneys harvested from MRL/lpr mice (+nephritis) and C57BL/6 mice (no renal disease) at 6 months of age were stained for ALCAM (CD166, red, A and B) and CD6 (red, C). MRL/lpr mice show increased levels of renal ALCAM expression, both within their tubules (B) and glomeruli (A) compared to C57BL/6 healthy control mice. Additionally, macrophages infiltrating into the glomeruli of MRL/lpr mice were ALCAM+ (white arrows, A). CD6+ T cell infiltration was also increased in the lupus mouse strain (white arrows, C). Images are representative of 3 mice per group.

Figure 2: Anti-CD6 ameliorates renal disease



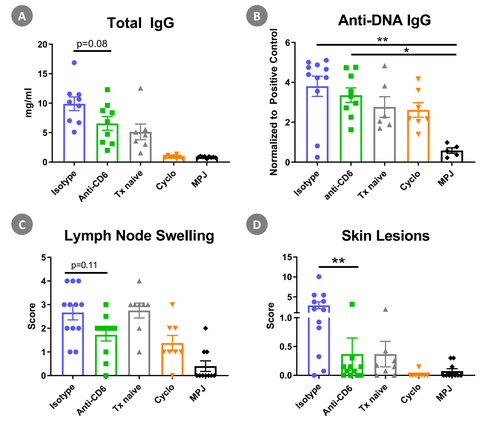
(A) Treatment with anti-CD6 antibody significantly improved proteinuria compared to isotype control treated mice (as measured by uristix). (B) These results were confirmed by measuring albumin:creatinine ratios in terminal urine. (C) Treatment with anti-CD6 significantly improved the survival of MRL/lpr mice, as well as kidney function (D) as measured by blood urea nitrogen (BUN) levels in terminal serum. There was a near significant improvement in glomerular histology (E), although tubular histology (F) was unaffected.

Figure 3: Anti-CD6 modulates renal T cell infiltration



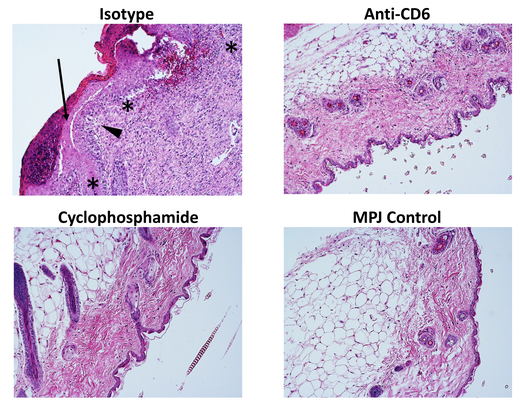
Flow cytometry was performed to determine the effect of anti-CD6 treatment on various T cell subsets infiltrating the kidneys. CD45 staining revealed an overall decrease in immune cell infiltration in anti-CD6 versus isotype control treated mice. CD4+ and CD8+ cells were also both reduced, as were activated CD4+ T cells (CD25+ CD69+) and effector CD4 and CD8 T cells (CD44+).

Figure 4: Anti-CD6 affects systemic disease



(A) Treatment with anti-CD6 resulted in a near significant decrease in total circulating levels of IgG, as measured in terminal serum. (B) Despite lower total IgG levels, levels of anti-DNA antibodies remained unchanged. (C) Isotype and Tx naïve groups showed significant enlargement of their lymph nodes, while anti-CD6 treated mice showed a trend towards decreased lymphadenopathy. (D) Furthermore, mice treated with anti-CD6 showed significant improvement in the severity of macroscopic skin lesions..

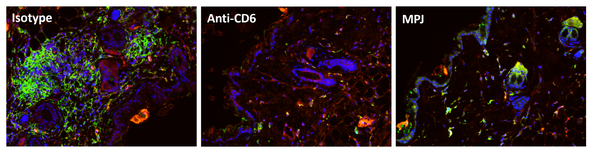
Figure 5: Anti-CD6 improves skin disease



Isotype control treated mice displayed abnormal skin histopathology, including hyperkeratosis (thickening of the epidermis), damage to the dermal-epidermal junction and large cellular infiltrates into the dermis. Anti-CD6 treatment ameliorated many of these pathologies. Epidermal thickening is reduced, as were cellular infiltrates. The histology of the sections from anti-CD6 treated mice is more similar to healthy control sections from MPJ mice than to the isotype control treated mice.

Figure 6: Anti-CD6 decreases immune infiltrates in skin

To further assess how anti-CD6 treatment may be affecting the development of skin disease in the MRL/lpr mice, skin sections were stained for macrophages (green), C3 (red) and IgG (orange). There is a noticeable decrease in the number of accumulating macrophages in anti-CD6 compared to isotype control treated mice. C3 and IgG deposition were similar between both treatment groups, but greater than the healthy control MPJ mice.



Conclusion

Blockade of the CD6-ALCAM pathway using an anti-CD6 antibody is a promising therapeutic target. Administration of anti-CD6 decreased kidney damage and mortality as well as multiple disease manifestations, including cutaneous lesions. Targeting of CD6 with itolizumab (EQ001) is currently being tested in a clinical trial for the treatment of lupus nephritis. Clinical Trial Identifier: NCT04128579