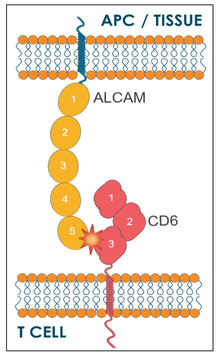
Amelioration of Immune Complex-Mediated Glomerulonephritis by CD6 Modulation

Samantha A. Chalmers[1], Sayra J. Garcia[1], Leal Herlitz[2], Jeanette Ampudia[3], Cherie Ng[3], Stephen Connelly[3], Chaim Putterman[1]

[1]Albert Einstein College of Medicine, Bronx, NY [2] Cleveland Clinic, Cleveland, OH [3]Equillium, San Diego, CA

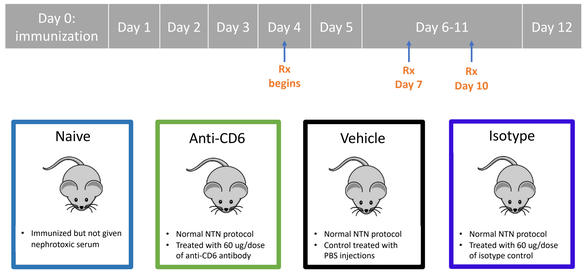
Introduction

- CD6 is a co-stimulatory receptor expressed predominantly 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 and trafficking, making this an integral pathway in immuno-inflammation.
- The CD6-ALCAM pathway may contribute to disease pathogenesis, as increased levels of CD6 are associated with pathogenic T cell responses.
- T cells are a key cellular mediator in both systemic lupus erythematosus (SLE) and lupus nephritis (LN), as they amplify the autoimmune response and inflammation and contribute to end organ damage.



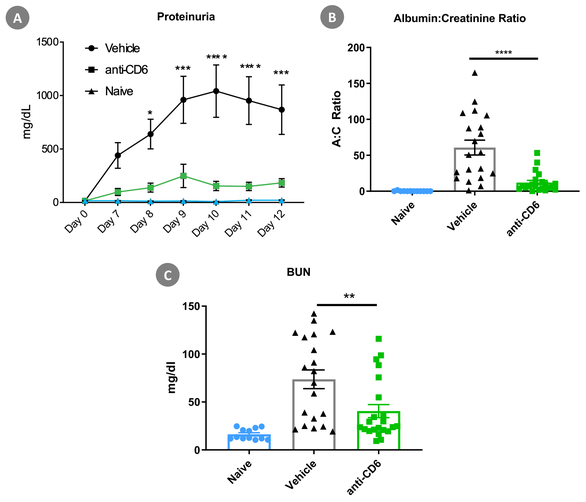
- Th1, Th2 and Th17 effector T cells are implicated in the pathogenesis of autoimmune nephropathies.
- Thus, using an anti-CD6 mAb we designed an experiment to probe the role of CD6-ALCAM in the pathogenesis of LN using a murine model of glomerulonephritis.

Experimental Design



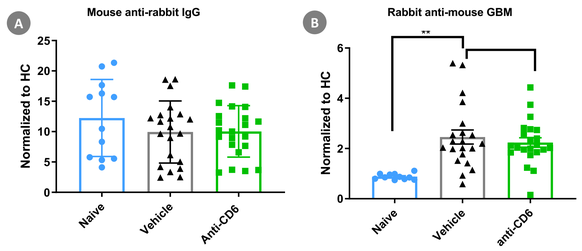
- Nephrotoxic serum nephritis (NTN) is a validated mouse model that manifests antibody mediated immune glomerulonephritis that is mechanistically and histologically like that observed with LN, and consequently is commonly used to test pharmacologic agents for this specific complication of SLE.
- Disease was induced in two separate cohorts of female 129/svJ mice, both aged to 10 weeks. Mice were immunized with rabbit IgG and CFA on day 0 to stimulate mouse anti-rabbit antibodies, which then cross-reacted with nephrotoxic rabbit serum given on day 5, causing an antibody-mediated nephritis similar in pathology to LN.
- To assess the importance of the CD6-ALCAM pathway in LN pathogenesis, mice were treated 3x per week with an anti-CD6 monoclonal antibody (mAb, 10D12) (60ug/dose, n=23), or with vehicle control (n=23). Healthy mice (immunized with rabbit IgG, but not given nephrotoxic serum) were also included as a control (n=12). In a second cohort, we also included an isotype control group, as shown in Figures 4 and 5.

Figure 1: Anti-CD6 improves nephritis



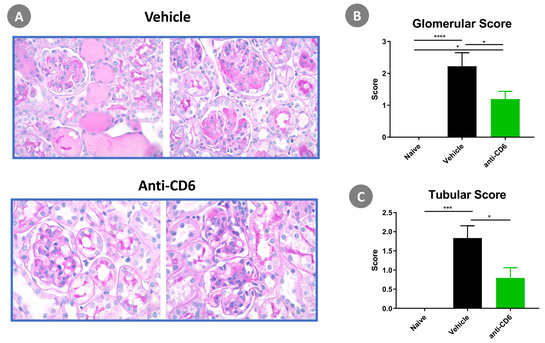
(A) Treatment with the anti-CD6 antibody, 10D12, significantly improved proteinuria levels compared to vehicle control mice, as measured by uristix. Significant differences were noted as early as day 8 and continued for the remainder of the experiment. (B) These results were confirmed by measuring albumin:creatinine (A:C) ratios in terminal urine. (C) In addition to improving the filtration barrier, treatment also improved terminal blood urea nitrogen (BUN) levels, indicating preserved kidney function. (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)

Figure 2: Anti-CD6 does not interfere with disease induction



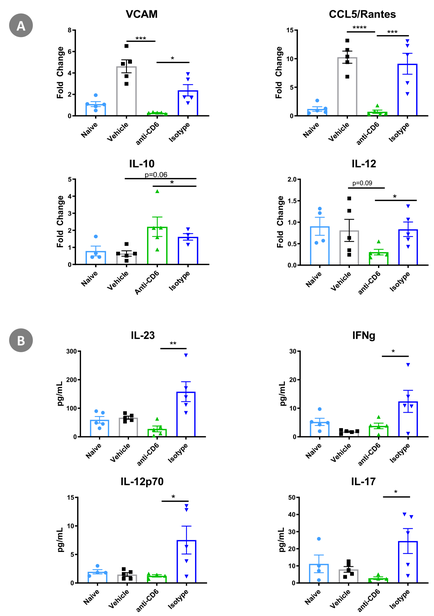
To ensure that treatment with anti-CD6 antibodies does not interfere with induction of the NTN model, we measured the disease mediating antibodies. (A) First, we looked at the level of mouse anti-rabbit antibodies generated from the day 0 immunization. Levels were measured in terminal serum of all three groups of mice. As each group is immunized, we expected, and found, similar levels in each treatment group. (B) Nephrotoxic serum, containing the nephrotoxic rabbit anti-GBM antibodies, was given to both the anti-CD6 treated and vehicle control groups on day 5, but not the healthy control mice. As shown, vehicle control and anti-CD6 treated had significantly higher levels, confirming proper induction of NTN.

Figure 3: Anti-CD6 treatment improves renal histology



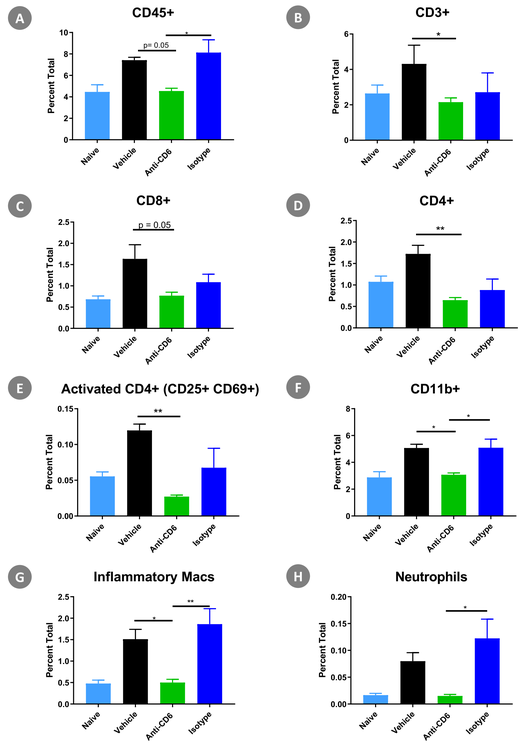
(A) Histological sections of renal tissue were scored blindly by an experienced nephropathologist. (B) Glomerular sections were assessed by scoring endocapillary proliferation, crescent and deposits on a scale from 0-4. Anti-CD6 treatment significantly attenuated glomerular pathology vs. vehicle control mice. (C) Tubular scores were determined by scoring tubular casts and interstitial inflammation on a scale 0-4. Like the glomerular scores, anti-CD6 treated mice were significantly improved compared to vehicle control.

Figure 4: Anti-CD6 treatment reduces inflammatory cytokine expression



(A) We assessed expression levels of inflammatory proteins via RT-PCR of whole kidney RNA extracts. The levels were measured in cohort 2, which also included an isotype control group. (B) We assessed protein levels of inflammatory cytokines in the kidneys via a multiplex, flow cytometry-based system from BioLegend (LEGENDplex). Both at the RNA and protein level we can observe a reduction in inflammatory mediators in mice treated with anti-CD6.

Figure 5: Anti-CD6 treatment decreases cellular infiltration



Flow cytometry was performed on kidneys to assess the effect of anti-CD6 treatment on immune cell infiltration. We noted an overall decrease in immune cell accumulation (CD45+) in anti-CD6 treated mice vs. both isotype and vehicle control mice (A). Further analysis showed decreases in inflammatory myeloid cells (F-G) and in T cell populations (B-D). Kidney CD4+ T cells were decreased in anti-CD6 treated mice (D), with a significant difference noticed in activated CD4+ T cells, defined as CD25+, CD69+ cells (E).

Conclusion

- Inhibiting the CD6-ALCAM pathway with an anti-CD6 treatment attenuates the nephritis associated with nephrotoxic antibody administration, an inducible model of lupus nephritis.
- CD6 blockade improves kidney function via reductions in renal inflammatory cytokine expression and immune infiltration of myeloid and T cells in the kidney.
- These results highlight the CD6-ALCAM pathway as a promising therapeutic option which is more selective than the immunosuppressive therapies currently offered.
- Targeting of CD6 is currently being tested in a clinical trial for the treatment of lupus nephritis. Clinical Trial Identifier: NCT04128579