Amelioration of Immune Complex-Mediated Glomerulonephritis by CD6 Modulation

Samantha A. Chalmers¹, Sayra J. Garcia¹, Leal Herlitz², Jeannete Ampudia³, Cherie Ng¹, Stephen Connelly³, Chaim Putterman¹

¹Albert Einstein College of Medicine, Bronx, NY  ²Cleveland Clinic, Cleveland, OH  ³Equilibrium, 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 immune-inflammatory.
- 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 nephrotropic 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 bi per week with an anti-CD6 monoclonal antibody (mAb), 1D012 (60μg/dose, n=23), or with vehicle control (n=23). Healthy mice (immunized with rabbit IgG, but not given nephrotropic 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

- Nephrotropic 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 nephrotropic 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 bi per week with an anti-CD6 monoclonal antibody (mAb), 1D012 (60μg/dose, n=23), or with vehicle control (n=23). Healthy mice (immunized with rabbit IgG, but not given nephrotropic 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 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) Nephrotropic serum, containing the nephrotropic rabbit anti-IgM 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

- 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 of 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

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 isotopic and vehicle control mice (A). Further analysis showed decreases in inflammatory monocytes (CD14+) 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).

Figure 5: Anti-CD6 treatment decreases cellular infiltration

- Inhibiting the CD6-ALCAM pathway with an anti-CD6 treatment attenuates the nephritis associated with nephrotropic 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

Conclusion