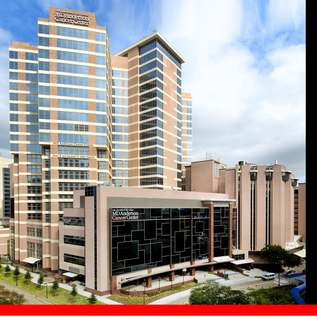

Evaluation of newly generated LRP1 antibodies in different cell types: THP1 cancer cell line, human and mouse immune cells

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Background

- Enhanced understanding of the basic biology surrounding T-cell activation and proliferation has enabled the use of immunotherapy to combat deadly diseases, such as cancer[1].
- In our lab's previous findings, Low Density Lipoprotein Receptor Related Protein 1 (LRP1) has been shown to play a direct role in T-cell proliferation[2]
- The use of anti-LRP1 antibody to target T-cells resulted in a significant and permanent reduction of T-cell inhibition of proliferation [2]

Hypothesis

- Exploiting the similarity in LRP1's homology in mouse and human, we hypothesize that newly-generated anti-human LRP1 antibody (6 clones) will bind to mouse LRP1

Objective

- In this project, 6 newly generated purified anti-human LRP1 antibodies will be tested for binding on a monocyte-like cell line (THP1) and on different hematopoietic cell populations from both mice and human to effectively study LRP1's immunological function

Materials and Methods

- 6 novel purified LRP1 antibodies were generated by GenScript (unconjugated)
- Two commercially available LRP1 antibodies (directly conjugated to AF488)
  - 8G1
  - EPR3724
- Mouse cell surface marker antibodies and secondary antibody
  - 2nd IgG antibody (anti-mouse), CD3, CD45, CD11B, Ly6C, Ly6G
- Human cell surface marker antibodies and secondary antibody
  - 2nd IgG antibody (anti-mouse), CD3, CD14
- THP1 monocyte-like AML cell line
- Human buffy coat
- Mouse splenocytes
- Mouse peripheral blood
- Calculations were performed to ensure use of equal quantities of each antibody
- Flow cytometry was performed to determine binding intensity for each clone

Results

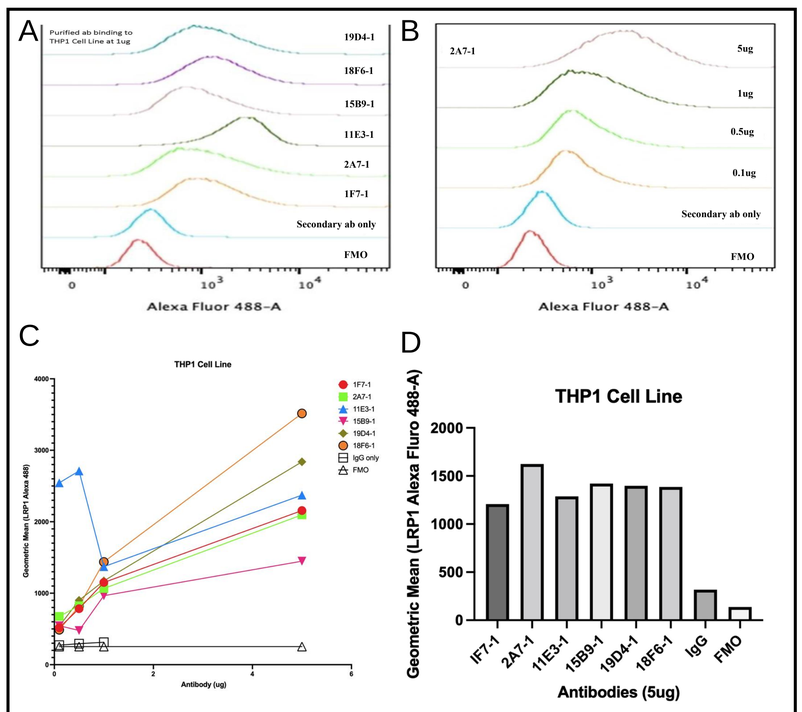
Human Cell Line and Human Peripheral Blood

- The newly generated LRP1 purified antibody clones bind strongly to the THP1 cancer cell line
- Significant binding was seen on human CD14+ (monocytes)
- No significant binding was seen on human CD3+ (T-cells)

Mouse Splenocytes

- Significant binding occurred in mouse T-cells, monocytes, and neutrophils

Figure 1. Purified LRP1 Abs Bind Strongly to THP1 AML Cell Line



A. Flow cytometry histograms at 1.0ug for each antibody clone and negative controls. **B.** Antibody binding intensity is dose dependent for clone 2A7-1. **C**. Line graph of different antibody binding intensity at different concentrations. **D.** Bar graph of antibody binding intensity at 5ug.

Figure 2. Human Buffy Coat Flow Cytometry Gating Strategy

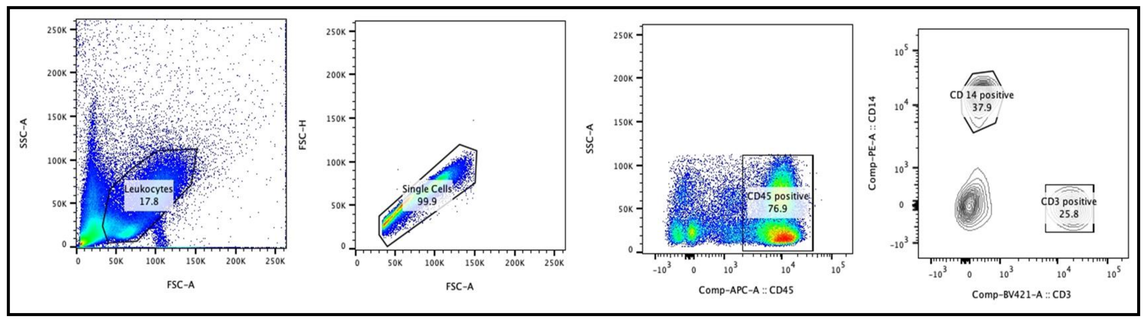
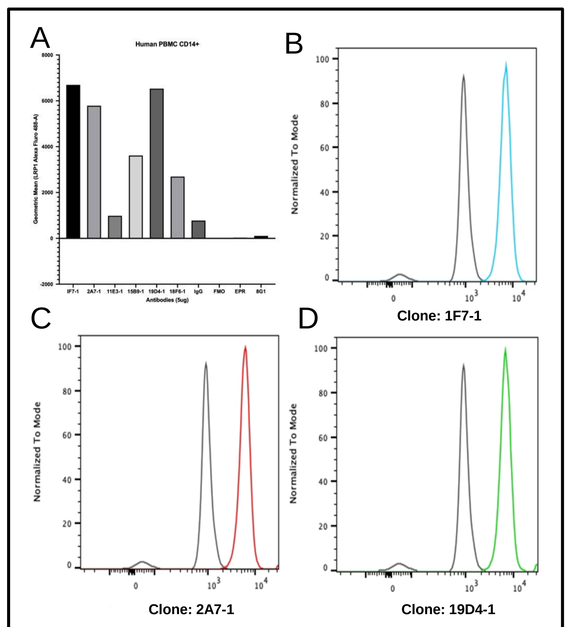
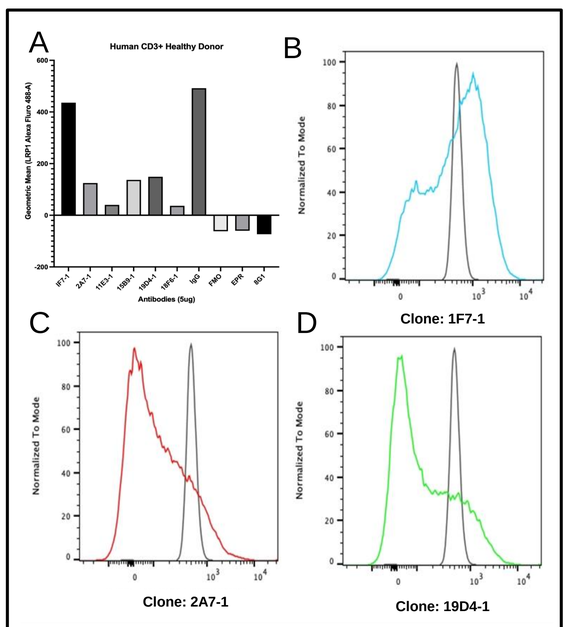


Figure 3. Purified LRP1 Antibodies Bind Strongly to Human Monocytes



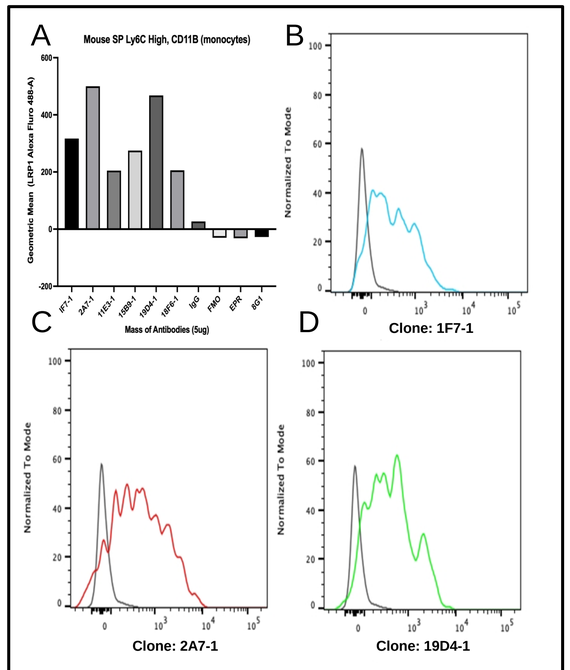
A. Flow cytometry fluorescence intensity bar graph at 5.0ug for each antibody clone and negative controls. **B, C, D** . Comp-Alexa Fluor 488-A:LRP1 is shown on the X axis and has a significant shift compared to the negative control for clone 1F7-1, 2A7-1, and 19D4-1. Grey is the secondary antibody only negative control.

Figure 4. Purified LRP1 Antibodies Do Not Bind Strongly to Human T-Cells



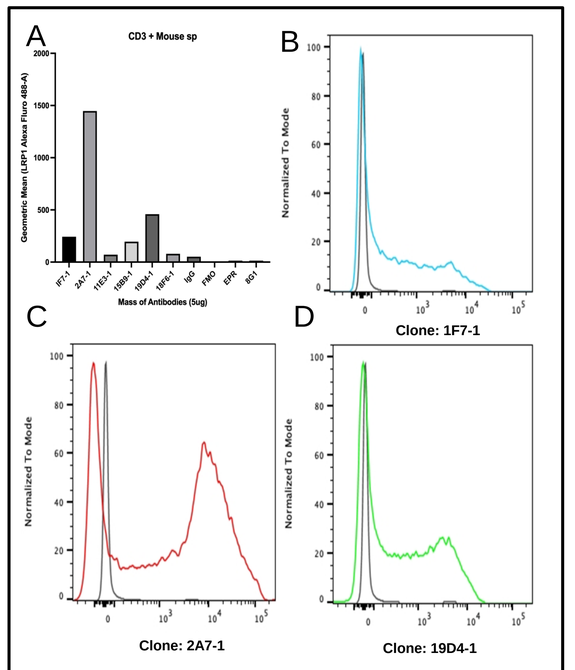
A. Flow cytometry fluorescence intensity bar graph at 5.0ug for each antibody clone and negative controls. **B, C, D** . Comp-Alexa Fluor 488-A:LRP1 is shown on the X axis and does not have a significant shift compared to the negative control for clone 1F7-1, 2A7-1, and 19D4-1. Grey is the secondary antibody only negative control.

Figure 5. Purified LRP1 Antibodies Bind to Mouse Monocytes



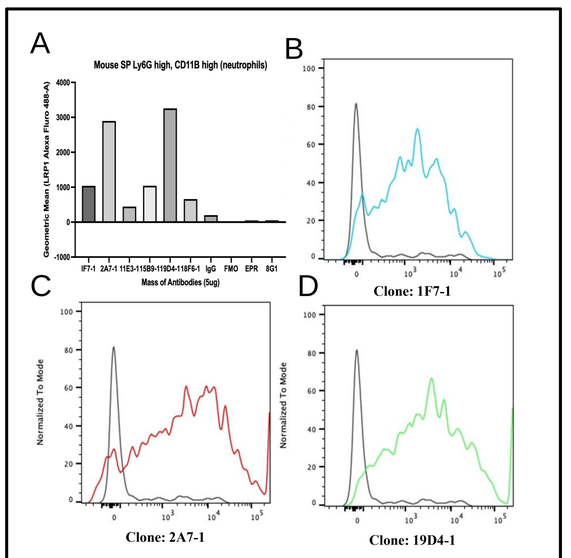
A. Flow cytometry fluorescence intensity bar graph at 5.0ug for each antibody clone and negative controls. **B, C, D** . Comp-Alexa Fluor 488-A:LRP1 is shown on the X axis and has a significant shift compared to the negative control for clone 1F7-1, 2A7-1, and 19D4-1. Grey is the secondary antibody only (negative control).

Figure 6. Purified LRP1 Antibodies Bind to Mouse T-Cells



A. Flow cytometry fluorescence intensity bar graph at 5.0ug for each antibody clone and negative controls. **B, C, D** . Comp-Alexa Fluor 488-A:LRP1 is shown on the X axis and has a significant shift compared to the negative control for clone 1F7-1, 2A7-1, and 19D4-1. Grey is the secondary antibody only (negative control).

Figure 7. Purified LRP1 Antibodies Bind to Mouse Neutrophils



A. Flow cytometry bar graph at 5.0ug for each antibody clone and negative controls. **B, C, D** Antibody fluorescence intensity is having a significant shift compared to the negative control for clone 1F7-1, 2A7-1, and 19D4-1.

Conclusion and Future Directions

- Three antibody clones exhibit strong binding to LRP1 in the THP1 cancer cell line, human monocytes, and mouse splenocytes (T-cells, monocytes, and neutrophils)
  - 1F7-1
  - 2A7-1
  - 19D4-1
- The next steps include the following:
  1. Utilizing various assays, determine which antibody has the strongest affinity to mouse and human LRP1
  2. Directly conjugate the antibody with fluorochrome to exclude using the secondary antibody
  3. Exploiting the ability of LRP1 to increase surface expression upon T-cell stimulation, test the LRP1 ab clones on CD3/CD28-stimulated human and mouse T-cells
  4. Validate LRP1 KO mouse T-cells model (negative control) with the chosen LRP1 ab clones

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