Effect of Missense Somatic Mutations on Surface HLA Expression in Patients with Immune-mediated Aplastic Anemia

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INTRODUCTION

Acquired aplastic anemia (aAA) is an autoimmune blood disease caused by the immune attack of early hematopoietic cells in the bone marrow. Because the mechanism behind aAA is not well understood, disease prognosis remains poor. Treatments target general immune processes and are not very effective. However, upon observing patients responding positively to T cell-directed immunosuppressive therapies, scientists hypothesize that the autoimmune attack may be mediated by cytotoxic T cells. Babushok et al and Zaimoku et al recently identified recurrent somatic mutations in Human Leukocyte Antigen (HLA) class I alleles among the surviving hematopoietic stem cells of aAA patients (Babushok et al. 2017, Zaimoku et al. 2021). HLA molecules present intracellular peptides on the surface of cells for immune surveillance. The observed survival advantage of hematopoietic stem cells with mutated HLA class I alleles leads us to hypothesize that the mutated HLA allele may have a key role in mediating autoantigen presentation in the autoimmune attack, and that the mutations allow the cells to survive. While most mutations in patients with aAA cause complete loss of HLA expression, some missense mutations result in partial or reduced surface expression. Additionally, the mutations selected for this study all result in amino acid substitutions in the peptide binding region of the HLA molecule. These mutations have the potential to be informative about autoantigen binding in aplastic anemia. We compiled a list of mutations bearing these characteristics and sought to evaluate how each mutation affected HLA surface expression.

STUDY AIM

To evaluate how missense somatic mutations in HLA class I alleles affect HLA surface expression levels.

PROPOSED AUTOIMMUNE ATTACK MECHANISM

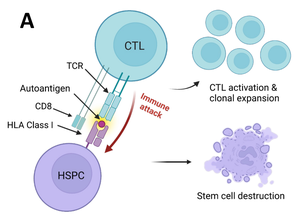


Figure A shows the proposed autoimmune attack mechanism in aAA. The autoantigen is presented by the HLA class I molecule on the surface of the HSPC. The cytotoxic T cell recognizes the autoantigen and destroys the HSPC.

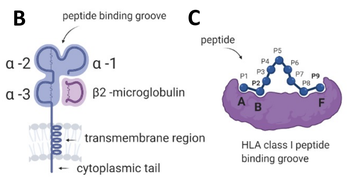
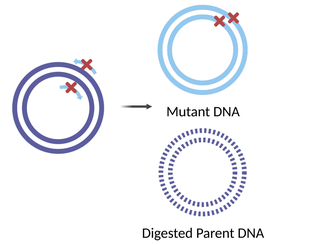
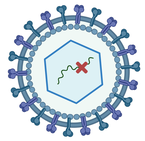


Figure B shows the structural domains of the HLA molecule.
Figure C illustrates how a peptide binds to the peptide binding groove of the HLA molecule.

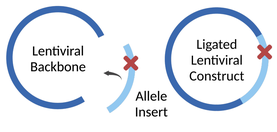
METHODS



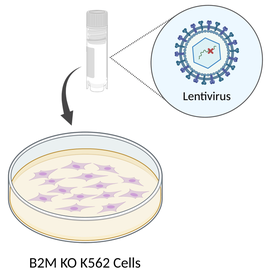
1. Site-Directed Mutagenesis



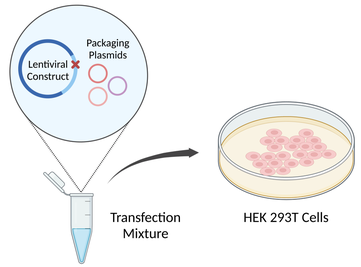
4. Lentivirus Synthesized



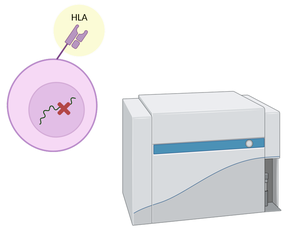
2. Lentiviral Cloning



5. Transduction



3. Transfection



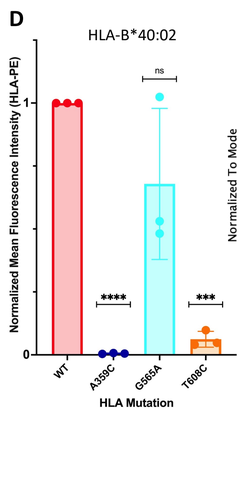
6. Flow Cytometry

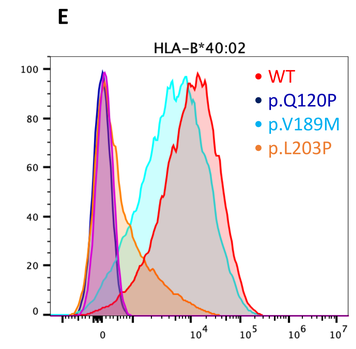
Figures created using BioRender.

RESULTS

Table 1. HLA Mean Surface Expression Values

HLA Allele	DNA Change	Protein Change	Previous Results	Mean Expression
HLA-B*14:02	c.403C>A	p.R135S	N/A	1.19
HLA-B*14:02	c.403C>T	p.R135C	N/A	0.15
HLA-B*14:02	c.490G>A	p.A164T	N/A	0.12
HLA-B*14:02	c.453C>G	p.N151K	N/A	0.36
HLA-B*14:02	c.244G>A	p.E82K	N/A	0.34
HLA-B*14:02	c.575T>A	p.L192H	N/A	0.056
HLA-B*14:02	c.421G>A	p.A141T	N/A	0.09
HLA-B*14:02	c.409T>G	p.Y137D	N/A	0.542
HLA-B*14:02	c.376G>T	p.D126Y	Expressed	0.4
HLA-B*14:02	**WT**		**Expressed**	**1**
HLA-B*40:02	c.608T>C	p.L203P	Expressed	0.062
HLA-B*40:02	c.359A>C	p.Q120P	N/A	0.0051
HLA-B*40:02	c.565G>A	p.V189M	N/A	0.68
HLA-B*40:02	**WT**		**Expressed**	**1**
HLA-B*40:01	c.305T>A	p.L102Q	Expressed	0.028
HLA-B*40:01	c.171C>A	p.F57L	Expressed	0.27
HLA-B*40:01	**WT**		**Expressed**	**1**
HLA-B*08:01	c.110C>T	p.S37F	Expressed	0.41
HLA-B*08:01	c.421G>A	p.A141T	N/A	0.047
HLA-B*08:01	**WT**		**Expressed**	**1**
HLA-B*50:02	c.325T>A	p.Y109N	N/A	0.25
HLA-B*50:02	**WT**		**Expressed**	**1**
HLA-B*27:05	c.449T>C	p.L150P	N/A	0.02
HLA-B*27:05	**WT**		**Expressed**	**1**
HLA-B*13:02	c.504G>C	p.Q168H	N/A	0.347
HLA-B*13:02	**WT**		**Expressed**	**1**
HLA-B*49:01	c.351C>G	p.H117Q	N/A	0.052
HLA-B*49:01	**WT**		**Expressed**	**1**
HLA-A*74:01	c.323A>G	p.Y108C	N/A	0.041
HLA-A*74:01	**WT**		**Expressed**	**1**





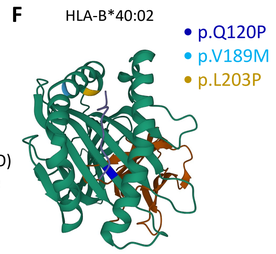


Table 1 lists the mean HLA surface expression level for each mutated allele compared to the wildtype expression level (n=3 replicates).
Figures D & E Mutant and wildtype alleles were transduced into cells with beta2-microglobulin knockout (KO) that lack endogenous HLA. Then, the cells were stained with anti-HLA antibody in order to check for surface HLA expression. The data here, for the HLA-B*40:02 allele, is a representative example for flow cytometry data. Mean fluorescence intensity was normalized to account for differences in transfection efficiency. The data is also displayed in histogram format.
Figure F This protein model shows where the mutations occur on the HLA molecule for HLA-B*40:02.

CONCLUSIONS

- HLA-B*14:02 p.R135S, HLA-B*14:02 p.Y137D, HLA-B*14:02 p.D126Y, HLA-B*40:02 p.V189M, and HLA-B*08:01 p.S37F showed preserved expression (mean expression level of 0.4 or higher).
- HLA-B*14:02 p.R135C, HLA-B*14:02 p.A164T, HLA-B*14:02 p.N151K, HLA-B*14:02 p.E82K, HLA-B*40:01 p.F57L, HLA-B*50:02 p.Y109N, and HLA-B*13:02 p.Q168H showed reduced expression (mean expression level between 0.1 and 0.4).
- The remaining mutations led to loss of expression (mean expression level less than 0.1).

FUTURE DIRECTIONS

- For all mutated alleles, we will perform flow cytometry with an alternative antibody to confirm lack of surface expression as well as qPCR to check for HLA allele transcription and mRNA expression.
- We will perform a time-course experiment with Brefeldin, a cellular transport-inhibitor, to investigate the HLA processing pathway of the mutated HLA molecules.
- The mutations that led to preserved expression are good candidates for autoantigen peptidome analysis.

REFERENCES

- Babushok et al. (2017). Somatic HLA Mutations Expose the Role of Class I-Mediated Autoimmunity in Aplastic Anemia and its Clonal Complications. *Blood advances*, *1*(22), 1900–1910.
- Zaimoku et al. (2021). HLA associations, somatic loss of HLA expression, and clinical outcomes in immune aplastic anemia. *Blood*, *138*(26), 2799–2809.

ACKNOWLEDGEMENTS

Special thanks to the Babushok Lab, the College Alumni Society, CURF, the Penn DNA Sequencing Core, and the Flow Cytometry Core at CHOP for making this research possible.