Establishing Rag1 Overexpressed pro-B Cell Line by Retroviral Transduction

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ABSTRACT

A cell line with an overexpression of Rag1 protein is needed in order to be able to analyze V(D)J recombination *in vitro*. This line was established by introducing the Rag1 transgene to a pro-B cell line through retroviral vector transduction. Rag1-expressing cells were selected for by using Blasticidin. The quantitative PCR (qPCR) experiment showed that the expression levels of Rag1 transgene are comparable to endogenous Rag1 expression in Imatinib-stimulated cells. Thus, we successfully created Rag1 pro-B cells that can be used for future V(D)J recombination analysis.

PURPOSE

The purpose of this project is to create a Rag1-expressing pro-B cell line, which lacks the genes under study in the Murre lab (H3f3a and H3f3b). The long-term goal is to be able to analyze V(D)J recombination in the absence of the genes of interest.

BACKGROUND

V, D and J segments in antibody genes are cut and shuffled by Rag1 and Rag2 proteins, to create antibody diversification. This makes a range of B cells receptive to many different antigens, forming an adaptive immune system.

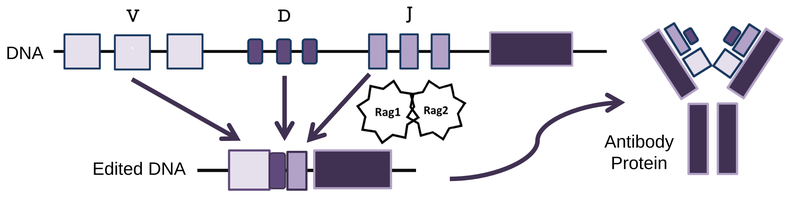

Fig. 1: The Rag1 and Rag2 complex creates the sequences for forming diversified antibodies.

Lack of Rag1 arrests B cell development at the pro-B cell stage and completely blocks V(D)J recombination.

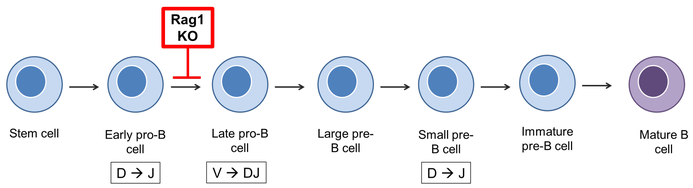

Fig. 2: B cell development and VDJ recombination throughout. Knockout of Rag1 causes arrest at pro-B cell stage.

EXPERIMENTAL PROCEDURES

Step 1: Preparing a Retroviral Supernatant

The Rag1-IRES-Bsr transgene was introduced to the pro-B cells by a retroviral vector system. The virus capsule containing the Rag1 transgene was created using the cellular machinery of 293T human embryonic kidney cells.

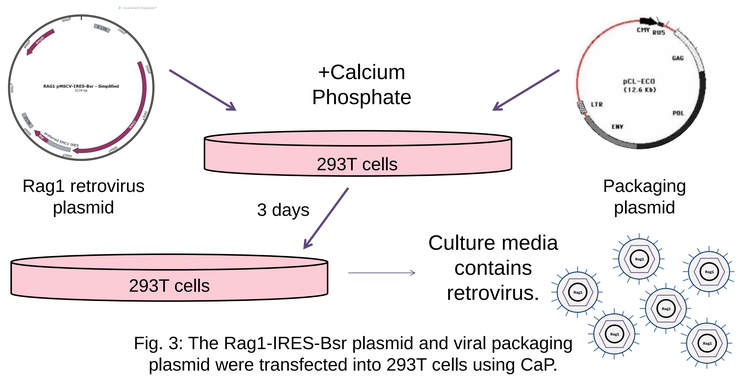

Fig. 3: The Rag1-IRES-Bsr plasmid and viral packaging plasmid were transfected into 293T cells using CaP.

Step 2: Infection and Selection

Scheme:

pro-B Cell + Rag1 virus media → Spin Infection → 48 hours → Selection w/ Blasticidin (20 ug/mL)

Fig. 4: Process of transduction of Rag1 plasmid into pro-B cells.

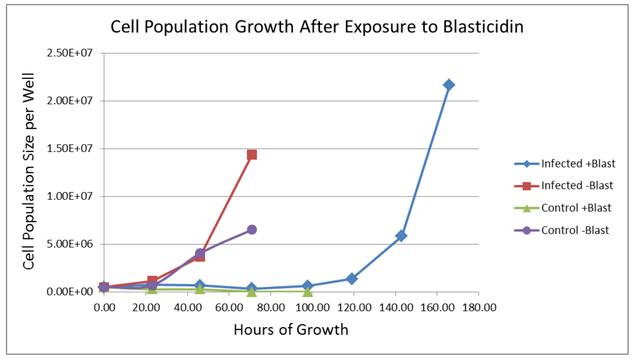

Fig. 5: Infected and uninfected cell growth with and without exposure to Blasticidin.

Growth Rate Analysis of Blasticidin Resistant Cells:

Fitted Growth Curve: $Ct=1951e^{(0.0561t)}$
Doubling Time: 12.36 hours
Estimated Infection Efficiency: 0.4%

Step 3: Quantifying Rag1 mRNA Expression

Imatinib (3uM) →
- Inhibits Bsr-Ab1 kinase
- Upregulates endogenous Rag1 and Rag 2 production

We can induce V(D)J recombination in pro-B cells expressing the Rag1 transgene by adding Imatinib.

Scheme:

Imatinib treatment (24h) → Total RNA collected → mRNA converted to cDNA → qPCR

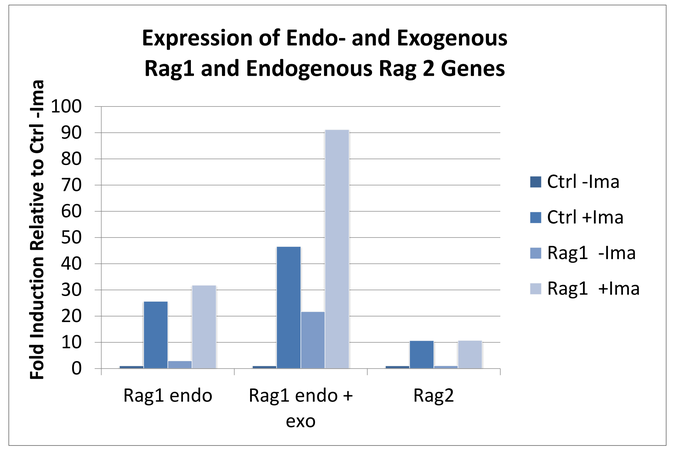

Fig. 6: The relative expression levels of indicated mRNA were measured by qPCR.

"Endo" primers amplified 3' UTR of Rag1 mRNA, whereas "Endo + Exo" primers amplified the Rag1 ORF. Note that endogenous mRNA is mutated and does not produce functional Rag1 proteins.

CONCLUSIONS

1. We have established Rag1-expressing H3f3a+/- H3f3b-/- pro-B cells.
2. The expression level of the Rag1 transgene is comparable to the endogenous Rag1 expression in Imatinib-stimulated cells.
3. The cells are suitable for V(D)J recombination assays in the future.

ACKNOWLEDGEMENTS

This was an amazing experience, and I am grateful to so many people! Thank you to Professor Komivez for making the effort to place me in such an excellent lab, to Professor Murre for being willing to take me in and say hello whenever he saw me, to my mentor, Toshi Aida, for having the patience and kindness to work with a high school senior, and to the entire Murre lab for being so welcoming and supportive!