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# Science in the News - Using CRISPR/Cas9 to Remove the HIV-1 Genome from T-Cells

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# CRISPR and HIV Therapy

Most HIV medications today are very good at controlling the spread of HIV within an infected person and can even help restore some of the T-cells that were damaged, however, HIV can stay dormant in host T-Cells. Rafal Kaminki et al. explored the possibility of using CRISPR/Cas9 gene editing to remove the dormant HIV genome from infected T-Cells. This is an attractive hypothesis as if the HIV-1 genome is removed completely, HIV can no longer spread as it relies on the T-cells to replicate. First of all, CRISPR is a technique that uses Cas9, a naturally occurring enzyme found in bacteria, and guide RNA (gRNA) to find and cut out a portion of undesirable DNA. The group modified CRISPR to recognize the DNA sequence of the HIV-1 promoter through the 5' terminal where there seems to be very little to no opportunity for a mistake to be made that could compromise the T-cell. To test this, they found "a HIV-1 genome in a human T-lymphatic cell line..." that had the full length of the inserted Long Terminal Repeats which are used by HIV and other viruses to insert themselves in other cells. They paired its removal of the HIV1 gene with its replacement of the Cas9 gene as well as the GFP gene. The Cas9 gene was inserted so that the T-cell could make them to then go and attack other HIV1 genomes, while the GFP gene was inserted so that they could see if the insertion worked as it would glow green when present. With the HIV promoter still present, if the GFP gene is active, then the HIV1 sequence was removed and replaced. In cells that had Cas9 genes, there was a green glow, meaning that the HIV1 gene was successfully replaced. Now that they

knew that their control experiment worked, they inserted a gRNA gene along with the Cas9 and GFP genes. Upon this insertion, there was very little green glow, meaning that everything had been removed properly. Kaminski et al. then had to decide on a delivery system for the genes. They chose to use a lentivirus, which is only fitting, since HIV is itself a lentivirus. They first delivered a control of just Cas9 to make sure their insertion method worked and then inserted both Cas9 and gRNA genomes. Again, the group saw a large reduction in the amount of HIV replication after the transduction of Cas9 and gRNA. The next steps would be to do a long-term study to see how well these modified T-cells control and eliminate HIV over time. A possible drawback to using gene editing in any case is that we do not know how this would affect future generations like whether this change would be passed down or an error would harm the development of the child. Also, while Kamini et al. were able to precisely remove only the HIV-1 genome, we do not know how well these T-cells would duplicate or transfer their information or if the production of other important macromolecules would be affected. While these first results seem promising, more long term testing has to be done.

Works Cited

Kaminski, R.; Chen, Y.; Fischer, T.; Tedaldi, E.; Napoli, A.; Zhang, Y.; Karn, J.; Hu, W.; Khalili, K. Elimination of HIV-1 Genomes from Human T-lymphoid Cells by CRISPR/Cas9 Gene Editing. https://www.nature.com/articles/srep22555#ref11 (accessed Dec 14, 2018).

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