DR SADHNA JOSHI

HIV genetic strategies

In this discussion, **Dr Sadhna Joshi** from the Department of Molecular Genetics at the University of Toronto outlines her team's unique and promising work on HIV prevention and treatment

Could you provide an overview of your work on preventative and therapeutic agents for HIV-1 infection?

Our approach consists of a secreted bifunctional protein that would neutralise the virus by preventing its interaction with both the receptor (CD4) and the co-receptor (CCR5 or CXCR4). Since the protein would be secreted from all gene-modified progeny cells, high levels of it would lead to systemic inhibition of HIV infection. Furthermore, local secretion from gene-modified cells would also inhibit HIV infection in organs that drugs or recombinant proteins fail to reach.

Because it is not mandatory to modify HIV target cells in this approach, other cell types could function as factories to secrete the anti-HIV proteins, making hematopoietic stem cell therapy more attractive and opening the road to a more direct *in vivo* HIV gene therapy approach based on secretion from tissues that are easier to access, such as muscle or skin.

Sexual transmission may also be inhibited if the antiviral protein could be secreted into the vagina and rectum. Organisms that naturally inhabit these sites may be used to secrete the antiviral proteins to develop microflora defence, as gene therapy of healthy individuals is not an option for prophylaxis.

What advantages does your HIV gene therapy strategy have over existing strategies?

For gene therapy to be effective, a significant number of gene-modified HIV target cells with a selective advantage would have to be introduced, which is technically very challenging and not cost-effective. Since not all stem cells will be modified, the unmodified target cells will continuously be replenished, allowing for continued HIV-1 replication.

We are using a unique approach to protect both gene-modified and unmodified target cells. The immune system comprising the gene-modified cells will be capable of protecting the unmodified target cells and hopefully the person will be cured of HIV.

How do microbicides work in HIV prevention and why isn't an effective microbicide available at present?

Microbicides for HIV prevention are designed to inhibit HIV transmission at the primary site of infection. Broad-spectrum microbicides create a harmful environment for the virus, while newer microbicides specifically target the interaction between HIV and host cells.

The challenge in the development of a microbicide lies in the difficulty of finding sophisticated preclinical model systems, which allow for the effective testing of microbicides. We have learned now that the efficacy of a microbicide is not only dependent on the potency of the antiviral compound, but also affected by many other factors.

In the human genital tract, the local pH, presence of biological substances, such as semen or cervicovaginal fluid, composition of the natural bacterial microbiota and stimulation of immunomodulatory molecules can all impact the success of a microbicide.

What is the function of *Lactobacillus jensenii* in the human body and why have you targeted it as a potential preventative agent in HIV-1 infection?

Lactobacillus was chosen as it is part of a healthy vaginal microbiota. Patients without a healthy microbiota have an increased risk of infection. Therefore, it might be beneficial on its own because it strengthens the natural barrier. *Lactobacillus* can persist for prolonged periods and secrete high levels of antiviral proteins.

How do you plan to use your antiviral protein for preventing HIV transmission?

In proof-of-concept studies, genetically engineered lactobacilli have been used to secrete a variety of anti-HIV proteins with a single antiviral moiety. However, inhibition of HIV transmission was not reported.

The success of our strategy in reducing sexual transmission of HIV would depend on whether the genetically-engineered strain of *L. jensenii* could efficiently deliver sufficient quantities of sCD4-17b to the vaginal mucosa, and how well this protein would inhibit HIV infection. This study would be the first to express a bifunctional protein in *Lactobacillus* and to assess the feasibility of this approach in preventing HIV transmission in a humanised mouse model.

In light of your research, how would the genetically engineered strain of *L. jensenii* be administered?

The genetically engineered strain of *L. jensenii* may be delivered to humans in capsules or as a cream. Alternatively, since *Lactobacillus* is commonly used in producing fermented dairy products (eg. yoghurt or cheese), the engineered strain could be propagated and administered as a dietary supplement. This would provide an inexpensive, safe, nutritious and easy-to-use preventive measure to block sexual transmission of HIV.

HIV: stopping the spread

Despite years of research and public health campaigns, HIV continues to spread globally. New research, however, holds promise in both preventing and treating the disease

MORE THAN 33 million people are infected with HIV worldwide. Despite decades of research, current antiretroviral drugs are of limited use because of the need for a life-long treatment, long-term side effects, the emergence of drug-resistant strains and drug-associated toxicity. While alternative protein-based drugs are well tolerated and highly effective, their frequent injection is very expensive and impractical.

In 2009, a German research team reported the astonishing news that a stem-cell therapy they were trialling to treat HIV had been successful and had resulted in 20 months of remission for the patient. Transplantation of CCR5 Δ32/Δ32 hematopoietic stem cells into a patient with acute myeloid leukemia and HIV lead to complete removal of HIV infection, without anti-retroviral therapy. This was and is the first and only case of a person being 'cured' of HIV.

Unfortunately, despite the excitement surrounding this result, the lack of matching donors, cost and risks associated with transplantation make this approach impractical and dangerous. But it did offer hope to both patients and researchers that HIV infection can be cured.

In theory, if the patients' own cells were to be modified via gene therapy, many of the problems with the German researchers' strategy could be mitigated. Moreover, the majority of anti-HIV gene therapy strategies developed to date, including those that are being evaluated in clinical trials, are designed to express interfering RNAs/proteins that act intracellularly, and therefore inhibit HIV infection in the gene-modified cells. HIV infection of unmodified target cells could lead to a selection of gene-modified target cells and the reconstitution of an HIV-resistant immune system.

PREVENTATIVE STRATEGIES

Dr Sadhna Joshi from the Department of Molecular Genetics at the University of Toronto is a leading expert in HIV gene therapy. She believes that this technique holds great promise: "Gene therapy has the potential to provide a one-time treatment that would reduce viral load and avoid side effects as well as toxicities associated with antiretroviral therapy. Ultimately, the person would be freed of HIV and his immune system would be reconstituted with unmodified and gene-modified target cells".

In the absence of a vaccine, there is also a greater need for preventive strategies. HIV prevention policies have been implemented globally by NGOs, governments or other organisations, and have been reasonably successful in informing populations and reducing the spread of HIV/AIDS. But no one has yet developed a prevention mechanism administered to individuals which inhibits the transmission of HIV in the body. Such a prevention mechanism could have a significant impact on the spread of the disease.

STRATEGIES FOR HIV PREVENTION AND TREATMENT

Joshi is leading a project entitled 'Genetic therapies for HIV prevention and treatment', aiming to develop two distinct but interrelated strategies to fight HIV infection: microflora defence (prevention) and gene therapy (treatment).

The first step in both strategies is to inhibit the HIV infection. Joshi and collaborators have developed and tested a number of antiviral proteins that inhibit HIV infection. They have specifically targeted genes to engineer cells to secrete highly active bifunctional antiviral proteins. These genes have already been developed in Joshi's laboratory and, encouragingly, have been shown to express proteins that could inhibit HIV infection in culture. Of these, the one demonstrating the most promise is sCD4-17b: "We have shown that unmodified as well as the gene-modified HIV-1 target cells expressing sCD4-17b are highly resistant to infection," Joshi states.

During the project, the antiviral protein genes will be manipulated in two ways: to modify an organism that colonises the vagina and gastrointestinal tract to secrete the antiviral protein into the local environment in order to prevent sexual transmission of HIV (microflora defence) and to modify the patients' own cells to secrete the protein into the blood which will then hopefully inhibit HIV-1 infection of target cells (gene therapy).

MICROFLORA DEFENCE

To prevent the sexual transmission of HIV-1,

GENETIC STRATEGIES FOR HIV PREVENTION AND TREATMENT

OBJECTIVES

This research seeks to develop genetic strategies for HIV prevention and treatment using antiviral proteins that inhibit HIV infection. The genes encoding these proteins may be used to modify the patients' own cells to secrete these proteins into the blood to inhibit HIV-1 infection of target cells (gene therapy). These genes can also serve to modify an organism that colonises the vagina and gastrointestinal tract to secrete them into the local environment to prevent sexual transmission of HIV (microflora defence).

KEY COLLABORATORS

Dr Stanley E Read, MD, PhD, FRCPC,
Hospital for Sick Children; University of Toronto

Dr Juan-Carlos Zúñiga-Pflücker, PhD,
University of Toronto; Sunnybrook Research Institute

Dr John J Rossi, PhD,
Beckman Research Institute of the City of Hope

FUNDING

Canadian Institutes of Health Research; funding requested from other sources

CONTACT

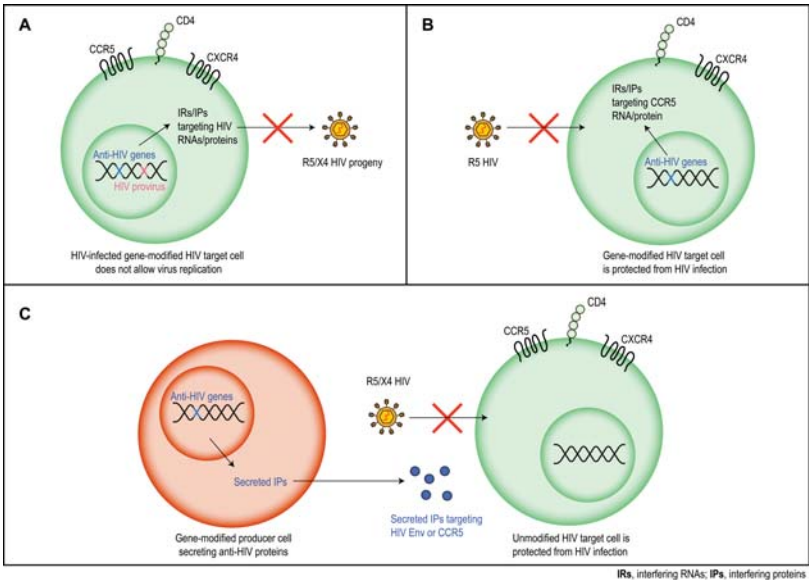
Sadhna Joshi, PhD, DSc
Associate Professor

Department of Molecular Genetics
Department of Laboratory Medicine and Pathobiology
Faculty of Medicine
University of Toronto
150 College Street, Room 210
Toronto, Ontario
Canada, M5S 3E2

T +1 416 978 2499
E sadhna.joshi.sukhwal@utoronto.ca

DR SADHNA JOSHI completed her PhD and DSc from University Paris VII, France. She joined Allelix Biopharmaceuticals, Missisauga, Ontario, in 1983 where she worked as a Senior Research Scientist and Principle Investigator on AIDS and Immune Regulation. In 1989 she became Associate Professor in the Department of Molecular Genetics and the Department of Laboratory Medicine and Pathobiology at the University of Toronto.

GENE THERAPY STRATEGY (C) DIFFERS FROM TRADITIONAL METHODS (A AND B)



the virus infection in the vagina or rectum has to be inhibited. As Joshi explains, the microflora defence strategy uses the location of the HIV-1 infection to its advantage: "Commensal bacteria naturally inhabit the vagina and gastrointestinal tract and these bacteria may be best suited for expression and secretion of proteins that would inhibit HIV infection at these sites".

Using a genetically engineered strain of *Lactobacillus* as a live substance (known as a microbicide) which can attack the HIV infection in the body, the group is developing a microflora defence strategy. *Lactobacillus* is a bacterium commonly present in the vagina and gastrointestinal tract of healthy individuals, and the strategy aims to modify *Lactobacillus* to secrete the bifunctional protein.

The modified *Lactobacillus* must simultaneously strengthen the natural barrier and boost the existing defence mechanisms, and also provide the necessary antiviral properties. This is no easy task, notes Alexander Falkenhagen, a graduate student working on this project: "In the human genital tract, the success of a microbicide can be affected by many variables including local pH, the presence of biological substances, such as semen or cervicovaginal fluid, composition of the natural bacterial microbiota and stimulation of immunomodulatory molecules. In fact, microbicides that disrupt the natural barrier of the genital tract by causing inflammation or disturbing the microbiota may inadvertently facilitate infection".

PREVENTING TRANSMISSION

The microbicide will be applied to the vagina of mice which will then be infected with HIV to test if transmission is inhibited. If promising results are obtained, clinical trials will be designed and, if successful, the genetically engineered strain of *Lactobacillus* could be harnessed to produce treatment in capsule or cream form.

GENE THERAPY

Prevention is not the only strategy necessary to stem the spread of HIV. Those who have already contracted the virus still desperately need an effective treatment regimen. Joshi notes that the gene therapy approach holds great promise in this regard: "Clinical trials have proven that gene-modified cells give rise to multilineage progeny cells expressing therapeutic genes, and these trials have shown both the safety and the feasibility of the gene therapy approach".

Current HIV gene therapy strategies including those tested in clinical trials protect gene-modified HIV target cells, but not unmodified target cells. Secretion of a bifunctional antiviral protein that inhibits HIV entry from gene-modified blood cells would protect the entire HIV target cell population.

Even with low numbers of gene-modified cells, sCD4-17b secretion from various gene-modified progeny cells, especially from cells that are not susceptible to HIV-1 infection, could lead to a high systemic and local concentration of this protein at sites of HIV infection, resulting in a therapeutic benefit.

THE NEXT STEP

The safety and feasibility of Joshi's gene therapy strategy will be investigated by introducing modified hematopoietic stem cells into humanised mice. The development of a functional human immune system, the presence of the antiviral protein in the blood and the reduction of HIV following infection will be tested in these mice and compared to mice that received unmodified stem cells.

If Joshi and her colleagues obtain the results they hope for, clinical trials will be designed to evaluate the approach to treat HIV-infected individuals. To further decrease the cost of HIV gene therapy and broaden accessibility to this treatment, alternative *in vivo* gene delivery into more accessible producer cells, such as muscle cells, may also be considered in the future.