Memory is skin deep

By Tracey Baas, Senior Editor

A **Harvard Medical School** team has identified a T cell-based immune response in the skin that does a better job of protecting mice from some viral infections than circulating T cells.¹ **Trem Rx Inc.** is using associated proprietary technology as the basis of a vaccine platform against infectious diseases and cancer.

“Conventional vaccines typically focus on the B cell arm of the immune system to create disease-fighting antibodies. The Trem Rx platform works to engage the T cell arm of the immune system through the generation of a newly discovered subpopulation of immune cells—the T_{RM} cells—that reside in the skin, lung, gut and other epithelial tissues,” said Thomas Kupper, professor of dermatology at Harvard Medical School. Kupper is also chair of dermatology at **Brigham and Women’s Hospital** and the **Dana-Farber Cancer Institute**, and he is the scientific founder of Trem Rx.

Memory T cells reside in either blood or lymphoid tissue, where they are called T central memory (T_{CM}) cells, or in epithelial tissues outside the blood and/or lymph compartment, where they are called T resident effector memory (T_{RM}) cells. An open question had been the relative contributions of T_{CM} and T_{RM} cells to protecting the host from invading pathogens.

A team led by Kupper thus set out to determine whether T_{CM} cells or T_{RM} cells provided superior protection against infection.

In mice, a vaccinia virus (VACV) infection delivered by scarification led to the production of both $CD8^+ T_{CM}$ and $CD8^+ T_{RM}$ cells that produced proinflammatory cytokines and persisted throughout the skin for at least six months postinfection.

Mice with both $CD8^+$ skin T_{RM} and $CD8^+ T_{CM}$ cells cleared a subsequent VACV reinfection 10,000-fold more effectively than naïve mice. Similar protection was seen in mice with $CD8^+$ skin T_{RM} and $CD8^+ T_{CM}$ cells treated with a compound that blocks movement of T_{CM} cells from lymph nodes to blood, indicating the T_{RM} cells were the primary mode of defense.

Finally, the team used a parabiotic mouse model, consisting of a previously VACV-infected mouse surgically connected to a never-infected naïve mouse, to create animals that had only $CD8^+ T_{CM}$ cells. While the mice were connected, $CD8^+ T_{CM}$ cells from the infected mouse were transferred through the shared bloodstream to the naïve mouse.

Following separation, the naïve mouse now containing $CD8^+ T_{CM}$ cells but not $CD8^+$ skin T_{RM} cells cleared the virus only 30-fold more effectively than completely naïve mice.

Together, the findings suggest $CD8^+ T_{RM}$ cells provide superior protection over circulatory $CD8^+ T_{CM}$ cells, and a vaccine that boosts production of $CD8^+$ skin T_{RM} cells could lead to long-lasting protective immunity against skin infections.

Results were published in *Nature*.

The new findings support work reported in *Nature Medicine* by Kupper’s laboratory in 2010 that showed VACV immunization by skin scarification was superior to other injection routes—subcutaneous, intradermal, intramuscular and intraperitoneal—and provided better protection from subsequent VACV skin or respiratory challenge.² The skin scarification with VACV was shown to produce T_{CM} cells in the blood and T_{RM} cells in both the skin and lung epithelia.

Because the team did not yet have the parabiotic mouse model, they used antibody-deficient mice and mice treated with the compound that blocks movement of T_{CM} cells from lymph nodes to blood to show the animals were protected against respiratory challenge. These results suggested that lung T_{RM} cells alone were sufficient for protection; however, the protection was better when both T_{RM} and T_{CM} cells were present.

Kupper’s laboratory plans to use the parabiotic mouse model to confirm which specific $CD8^+$ T cells contribute to the antiviral effects in the lung.

The skin immunization strategy used in both studies also protected mice from melanoma challenge. All mice receiving immunization by skin scarification with VACV engineered to express the melanoma antigen ovalbumin (OVA) survived a challenge with melanoma cells. In contrast, the survival rate was less than 50% for animals receiving subcutaneous, intradermal, intramuscular or intraperitoneal immunization.

“The insights gained from the studies definitely open ways for future vaccine development, but to proceed we first need more basic knowledge on these protective cell populations—what does it take to induce these T_{RM} cells, how long they will be around and how far away do they distribute,” said Anke Huckriede, professor of vaccinology at the **University Medical Center Groningen**.

Thus, she said, “it has to be investigated to what extent T_{RM} cells in the skin can reduce overall virus load.” Huckriede also wanted to see the studies “extended to other animal models with better predictive value for the human situation than mice.”

Peter Openshaw, director of the Centre for Respiratory Infection at the National Heart and Lung Institute at **Imperial College London**, thinks demonstrating the effectiveness of skin-delivered vaccines is going to be a challenge because the skin-specific vaccine platform does not generate neutralizing antibodies.

“Currently, new batches of flu vaccines are evaluated for generating a strong immune response by using surrogate markers such as

“The insights gained from the studies definitely open ways for future vaccine development, but to proceed we first need more basic knowledge on these protective cell populations—what does it take to induce these T_{RM} cells, how long they will be around and how far away do they distribute.”

**—Anke Huckriede,
University Medical Center
Groningen**

serum neutralizing antibody inhibition or hemagglutination inhibition,” he said. “Although these markers may show that the vaccine satisfies the regulatory criteria, it may not actually be effective against the targeted disease. To actually test effectiveness, the evaluation process is tough, a real challenge.”

Kupper said his team has strategies to evaluate the vaccine using blood and very small skin biopsies.

Choosing the right diseases

Trem Rx's vaccines incorporate replication-deficient viruses. The company declined to disclose its lead indications, but President Eric Stromquist said potential areas of interest include HPV, influenza and HIV.

In HPV, Gardasil from **Merck & Co. Inc.** and **Sanofi** is marketed to prevent infection. The vaccine is based on virus-like particles made up of recombinant capsid proteins of HPV types 6, 11, 16 and 18, all of which are HPV subtypes associated with cervical cancer.

Rachael Clark, assistant professor at Harvard Medical School and one of Kupper's collaborators, was the first to carefully study T_{RM} cells in human skin, demonstrating that there were twice as many T cells in skin as in blood. She has been collaborating with groups studying other human tissues and has identified T_{RM} cells in cervical epithelial tissue,³ leading these investigators to hypothesize that boosting this population of protective T cells could help eradicate HPV-infected epithelial cells, thus reducing the risk of malignant transformation.

In the case of influenza, Stromquist said, the conventional trivalent vaccine “needs to be decided upon every year, and sometimes it is a hit or miss, depending on if the prediction of emerging influenza strains was accurate. A number of researchers are trying to create a universal influenza vaccine by producing a universal hemagglutinin antigen. Because our vaccine's protection is independent of antibody production, you don't have to worry about predicting the right antigen to use, and our platform could be used to either replace or to complement any antibody-inducing vaccine platform.”

In HIV, Kupper said the key would be creating a first line of defense at the epithelial layer of the reproductive mucosa. “I think that a vaccine platform that induces protective T_{RM} cells in relevant epithelial tissues, such as the reproductive mucosa, might have a better chance of preventing HIV infection than vaccine platforms optimized to induce T_{CM} cells or produce antibodies in the blood,” he said. “By the time HIV infection takes hold in the epithelial tissues, it is very difficult to eradicate.”

Trem Rx's other areas of interest include respiratory syncytial virus (RSV), polio, tuberculosis and intracellular bacterial infections.

Although the company's vaccines are in preclinical development, there is clinical evidence supporting the hypothesis that T_{RM} cells provide superior protection over circulatory T_{CM} cells in humans.

In January 2012, Clark and Kupper published results from a study on the immunological effects of Sanofi's Campath alemtuzumab in 18 patients with leukemic cutaneous T cell lymphoma (CTCL), a malignancy of skin-homing TCM cells.³ Patient skin and blood samples showed the

“I think we are beginning to reappraise skin scarification for delivery of vaccines. It should not be considered an archaic method from the history of medicine. Most vaccines today are injected into muscle, bypassing the innate and adaptive immune effectors of both the epidermis and dermis.”

— Thomas Kupper,
Harvard Medical School

drug depleted malignant T_{CM} cells and B cells circulating in the blood but spared normal T_{RM} cells in the skin. Despite the complete absence of T and B cells in the blood, 17 patients did not experience any infections.

These data give further support to the idea that T_{RM} cells alone can provide immunologic protection against infection.

Leukemic CTCL is often refractory to multiple therapies. Median survival is three years, and most patients die from uncontrolled infections due to dysfunction of the immune system.

Campath is a humanized mAb against CD52 that is marketed to treat chronic lymphocytic leukemia (CLL).

Back to the future

Vaccination through skin scarification is hardly a new idea, as it is how the smallpox vaccine is delivered. The current data provide a mechanistic explanation for the clinical success of the approach.

“I think we are beginning to reappraise skin scarification for delivery of vaccines. It should not be considered an archaic method from the history of medicine,” Kupper said. “Most vaccines today are injected into muscle, bypassing the innate and adaptive immune effectors of both the epidermis and dermis.”

He added, “While scarification works, there are a number of approaches being worked on outside of our laboratory and outside of Trem Rx to deliver vaccines to skin.”

Huckriede noted that although scarification has a long track record of human use, “now there are alternative techniques to penetrate the stratum corneum. These include intradermal injection, microneedle-based delivery, tattooing, jet injectors or patches. It needs to be evaluated which of these techniques would work best for the induction of skin-resident T cells, as this might depend on the exact type of antigen-presenting cells targeted.”

The results in the *Nature* and *Nature Medicine* papers are patented by Kupper and colleagues, and Brigham and Women's Hospital. The IP has been assigned to Trem Rx.

Baas, T. *SciBX* 5(13); doi:10.1038/scibx.2012.322

Published online March 29, 2012

REFERENCES

1. Jiang, X. *et al. Nature*; published online Feb. 29, 2012; doi:10.1038/nature10851
Contact: Thomas S. Kupper, Brigham and Women's Hospital, Boston, Mass.
e-mail: tkupper@partners.org
2. Liu, L. *et al. Nat. Med.* **16**, 224–227 (2010)
3. Clark, R.A. *et al. Sci. Transl. Med.* **4**, 117ra7 (2012)

COMPANIES AND INSTITUTIONS MENTIONED

Brigham and Women's Hospital, Boston, Mass.
Dana-Farber Cancer Institute, Boston, Mass.
Harvard Medical School, Boston, Mass.
Imperial College London, London, U.K.
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
Trem Rx Inc., Boston, Mass.
University Medical Center Groningen, Groningen, the Netherlands