IL-21 goes antiviral

By Lev Osherovich, Senior Writer

Two papers in *Science* make a case for harnessing IL-21 to treat chronic viral infections like HIV and HCV.^{1,2} The studies may open a new therapeutic space for recombinant IL-21, a compound **ZymoGenetics Inc.** is developing as an immunity-boosting cancer therapeutic.

"IL-21 is the most recently identified member of a family of cytokines with broad effects on a variety of immune cells, including antibody responses and helper T cell responses—it does a bit of everything," said Allan Zajac, associate professor of microbiology at the **University of Alabama at Birmingham** and senior author of one of the papers.

The new studies add to a mounting body of evidence supporting the cytokine's role as a potent booster of virus-fighting cytotoxic T cells (CTLs), which become exhausted during long bouts of viral infection.³ In exhausted CTLs, inhibitory signaling pathways block normal responses to stimulatory cytokines and antigens, allowing viral infections to persist indefinitely.

"The punch line is that IL-21 is a factor involved in sustaining a virus-fighting response during chronic infection," said David Brooks, assistant professor of microbiology, immunology and genetics at the **University of California, Los Angeles** and senior author on the other paper.

In a therapeutic setting, he said, this could mean that IL-21 treatment could amplify a natural antiviral mechanism.

A new face for IL-21

Drawing on earlier hints that IL-21 could broadly activate the immune system, both research teams used mice infected with lymphocytic choriomeningitis virus (LCMV) as a model for chronic viral infection. The teams measured IL-21 levels after infection and found that the virus stimulated IL-21 production in CD4⁺ T helper cells, which coordinate the antiviral immune response.

Both groups then disrupted IL-21 or its receptor on CTLs and examined the effect on chronic viral infection. Zajac's team found that IL-21 knockouts had lower active CTL levels than healthy controls. Similarly, both teams found that deletion of the IL-21 receptor specifically in CTLs rendered the cells unable to proliferate during the chronic phase of viral infection, in contrast to what was seen in wild-type controls.

In both cases, in the absence of IL-21, the mice were unable to sustain a CTL response and clear the infection.

To further show that IL-21 could have a therapeutic effect, Zajac's team injected IL-21 into mice that lacked helper T cells and were thus unable to launch an antiviral response. The researchers found that the cytokine boosted CTL proliferation and allowed the mice to survive infections that were lethal to untreated controls.

Finally, both teams found that IL-21 did not protect mice from the initial or acute effects of infection, but rather allowed the mice to survive once chronic infection set in. This situation may be relevant to HIV and HCV, which typically present in the clinic as deep-seated chronic infections.

Indeed, Brooks noted that in HIV and HCV, there is an initial burst of CTL activity that gets downgraded over time. He suspects IL-21 may help sustain a low level of CTL activity, which could help clear infections in the long term.

"In chronic infection, you have a homeostasis in which viral response leads to adaptation. There's something that says 'We've lost, let's turn the CTL response down,'" said Brooks. "At the same time, it doesn't fully go away."

In contrast, he added, in the absence of IL-21, the CTL response goes back to zero after the acute phase of infection is over.

Additionally, research published today in *Science* by a team at the **Swiss Federal Institute of Technology Zurich** describes results from experiments with IL-21 receptor knockout mice,⁴ and the data are comparable to those from Zajac's and Brooks' teams. The Zurich team was led by Manfred Kopf, professor of molecular biomedicine at the institute.

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—Allan Zajac,
University of Alabama at Birmingham

Hitting the chronic phase

"The interesting part for me is the specific role of IL-21 in the chronic phase, not in the acute phase," said Pallavur Sivakumar, principal scientist at ZymoGenetics.

The company, which was not involved with either of the two *Science* studies, is developing recombinant IL-21 as an immunity-boosting adjuvant to chemotherapy in renal cell carcinoma (RCC) and as a single agent in metastatic melanoma. In cancer, IL-21 is thought to work by enhancing CTL and NK cell activity to help the immune system fight off tumors.

Earlier this month, ZymoGenetics reported positive results for IL-21 in two Phase II cancer trials.

Stacey Dillon, also a principal scientist at ZymoGenetics, suspects that IL-21 may have a similar mechanism of action in cancer and viral infection.

"We have seen activation markers for NK cells and CD8⁺ T cells go up in cancer patients after IL-21 treatment," said Dillon. "Activating the immune system with IL-21 may provide similar responses in cancer and viral therapy due to these common mechanisms."

Zajac and Brooks suggested that IL-21 may combat the tendency of CTLs to become exhausted during the long battle against chronic infection.

Recent studies have identified proteins that influence CTL exhaustion, including IL-10, cytotoxic T lymphocyte-associated protein 4 (CTLA4; CD152) and programmed cell death 1 (PDCD1; PD-1; CD279), but it is unclear how IL-21 relates to these mechanisms.⁵

Sivakumar said that the two academic groups should now be able to test how IL-21 influences the production of other exhaustion-related cytokines and surface markers.

In HIV, he noted, the helper T cells that produce IL-21 are killed by the virus, resulting in CTL exhaustion. In HCV, he added, “there’s evidence of long-term exhaustion, and drugs that have a direct antiviral effect like interferon- α end up activating [exhausted] CTLs.”

Clinical prospects

As a proof of principle for therapeutics, the two teams now must demonstrate that recombinant IL-21 can help clear viral infection in mice with fully intact immune systems, not just in mice lacking helper T cells.

“Therapeutically, it remains to be determined whether adding IL-21 will have a beneficial effect in the chronic phase,” said Brooks.

Zajac further cautioned that IL-21 infusion could lead to undesirable autoimmune or inflammatory effects.

“We must be really cautious about immunopathology upon IL-21 treatment,” said Zajac. “Given IL-21’s broad range of immunological targets, there could be many side effects.”

However, Dillon and Sivakumar noted that ZymoGenetics’ clinical experience with IL-21 suggests that immunological side effects will be minimal. In the melanoma trial, the most common side effects were mild or moderate fatigue and rash.

Zajac is already planning to try IL-21 therapy in animal models of HIV. He noted that there is evidence of IL-21 deficiency in HIV infection, and thus he would like to test the effect of restoring IL-21 to normal levels.⁶

In the case of HCV, there are no small animal models, so monkey

studies may be the only avenue.

“The next step will be to take IL-21 into nonhuman primates,” said Brooks. “LCMV is a nice exploratory system, but we need to do this in the big fish.”

ZymoGenetics acquired worldwide rights to recombinant IL-21 from **Novo Nordisk A/S** in February. After restructuring in April, the company is now seeking partners to take the cytokine into Phase III cancer trials.

According to company spokesperson Susan Specht, ZymoGenetics does not currently plan to test IL-21 in virology indications and is instead developing pegylated interferon- λ , which is in Phase I for HCV.

The company has received patents for the use of IL-21 to improve immunity. Neither Brooks nor Zajac filed patents on their discoveries.

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