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# Scientists boost potency, reduce side effects of IL-2 protein used to treat cancer

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The utility of a naturally occurring protein given, sometimes to great effect, as a drug to treat advanced cancers is limited by the severe side effects it sometimes causes. But a Stanford University School of Medicine scientist has generated a mutant version of the protein whose modified shape renders it substantially more potent than the natural protein while reducing its toxicity.

The findings will appear online March 18 in *Nature*.

The protein, known as interleukin-2 or IL-2, is a master regulator of the immune system. It acts as a growth factor for many different kinds of immune cells, including an all-important class called T cells. These cells can both recognize and organize attacks against pathogens or tumors.

IL-2 stimulates T cells' proliferation in response to these threats. That makes it a potent anti-cancer drug. When injected into a patient, it spurs fierce anti-tumor activity.

"In a substantial subset — about 7 percent — of patients with advanced metastatic melanomas or kidney cancers, IL-2 treatment actually cures the disease," said Christopher Garcia, professor of molecular and cellular physiology and of structural biology and the study's senior author. That's an impressive result, considering the failure of most treatments at such a late stage of cancer.

IL-2 is also used off-label for various other cancers and a wide range of

other indications including HIV. But its use is restricted because it can cause severe toxic side effects such as difficulty in breathing due to pulmonary edema, or swelling of the lung, caused by the buildup of fluid in that organ. This in turn is the result of leakage from the copious capillaries that permeate lung tissue, the better to carry away oxygenated blood to distant tissues.

"The cells that cause these toxic effects appear to express different levels and types of IL-2 receptors than do the cells that produce the therapeutic effects," Garcia said. The various classes of immune cells activated by IL-2 have their own characteristic receptor complexes for the protein. Accordingly, each different cell type requires a different concentration of IL-2 for its activation, and each responds in its own way.

In 2005, Garcia and his colleagues determined the structure of IL-2, making it possible to visualize its internal features. "We thought we might be able to tilt the balance of therapeutic-to-toxic effects by modifying this protein in a way that preferentially trips off activation of a desired immune-cell type, while minimizing the activation of an unwanted cell type," he said.

For this study, Garcia's group produced a vast variety of mutated versions of the protein, and then, in a test-tube competition, compared the strength of these mutant proteins' binding to a particular cell-surface receptor, a process that is crucial to the T-cell activation needed to treat cancer. The researchers eventually obtained a mutant that Garcia dubbed "Super-2," which had more than 300 times the receptor-binding strength of natural IL-2. In subsequent tests designed to assess Super-2's ability to impede tumor growth, the new molecule outperformed natural IL-2 by a significant margin.

The researchers also tested Super-2 to determine the extent of the side

effects it would cause. To do this, they collaborated with a co-author of the paper, Onur Boyman, MD, of University Hospital Zurich in Switzerland, who had previously found that the type of cells in the lung that are responsible for capillary leakage have receptors for IL-2. Boyman developed an assay for IL-2's most dose-limiting side effect, pulmonary edema. This assay compares the weight of lungs from mice treated with a test compound versus lungs that are not thusly treated. The greater the weight difference, the more fluid buildup has occurred.

Boyman and a member of his group, Carsten Krieg, PhD, who is one of four investigators sharing first authorship of the *Nature* paper, carried out all the animal research used for the study. By this assay, pulmonary edema caused by Super-2 was significantly and substantially less than by natural IL-2.

Others sharing first authorship of the study were Aron Levin, PhD, (now at Technion University in Israel) and Darren Bates, PhD, (now a scientist at Amgen) who were formerly in Garcia's lab, and his MD/PhD student Aaron Ring.

What makes Super-2 so effective, said Garcia, is its altered shape. A T-cell's IL-2 receptor complex consists of three separate protein components sitting on the cell's surface. These receptors, sometimes referred to as alpha, beta and gamma, act in concert: IL-2 must first touch bases with alpha before it can assume the right shape to bind to beta. Typically, T cells that have never been activated in the past have vanishingly small amounts of alpha on their surfaces, and so require high concentrations of IL-2 to get the process started.

But the mutations Garcia's team induced lock Super-2 into a configuration whose optimized shape lets it bind directly to beta, bypassing alpha. The three-dimensional structure of Super-2, together with computer simulations from the laboratory of associate professor of

chemistry Vijay Pande, PhD, suggested this was because the mutant form of IL-2 was less "floppy" than the natural form, so that it presented a "tighter" binding surface to the beta receptor. This souped-up form of the protein was several times as potent as the naturally occurring form of IL-2 at slowing tumor growth, as measured by assays employing three different tumor types in culture.

But Super-2 is no more proficient than natural IL-2 at activating the immune cell type responsible for causing capillary leakage and the ensuing pulmonary edema. So, its ratio of activation is skewed much more favorably toward T-cell activation. Because of that, it's possible to give amounts of Super-2 that jump-start T-cell activation without setting off the type of cells that cause pulmonary edema.

Major pharmaceutical companies have expressed an interest in Super-2, according to Garcia, who said he suspects that a licensing agreement from one of them may be in the offing. Stanford has applied for a patent on Super-2.

However, he said, a group at the National Institutes of Health, including some of the heavyweight scientific experts who originally put IL-2 through its clinical paces some years ago, is now testing Super-2 from Garcia's lab in a large number of tumor models, in the hope of fast-tracking its development as a new therapy for additional cancer indications. "I hope it goes this route, because that would mean human trials would get started more quickly," Garcia said.

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