Enzyme-activating antibodies revealed as marker for most severe form of rheumatoid arthritis

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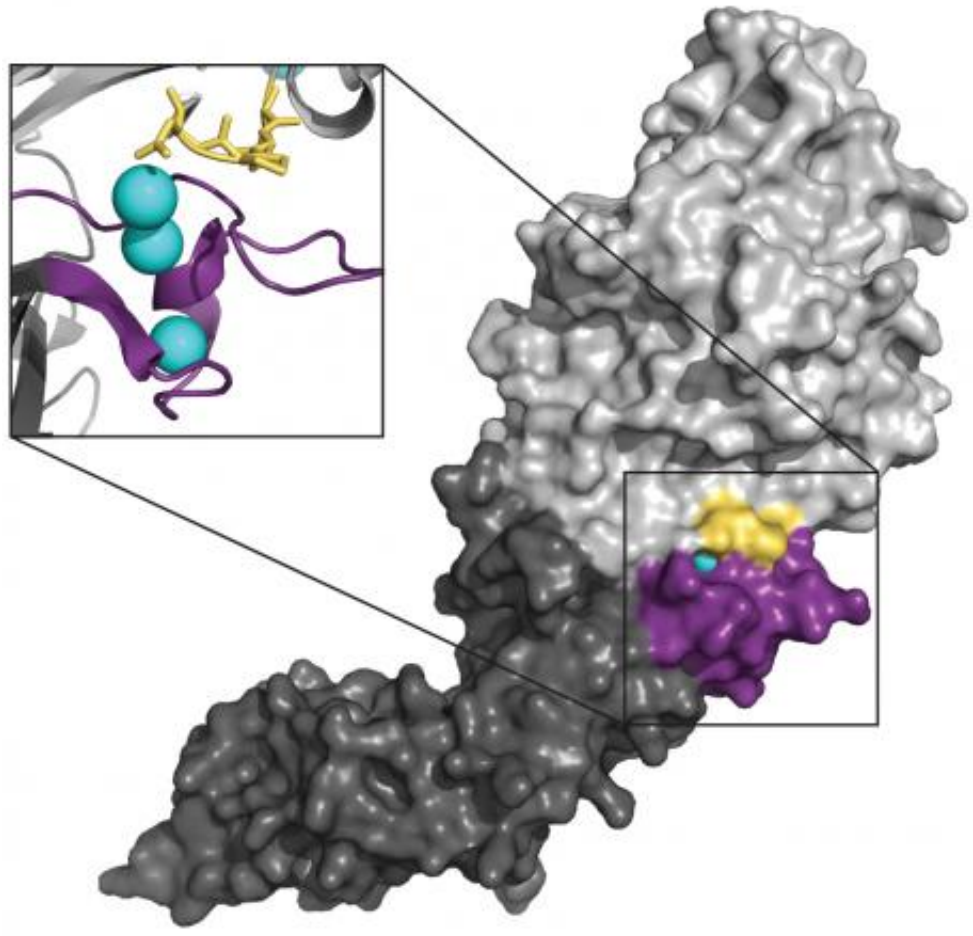

The proposed binding site of the PAD4-activating antibody. The PAD4 enzyme is composed of two pieces (light gray and dark gray) that are connected in the middle. This connection point between the two halves is important for binding to calcium (blue spheres) and interacting with proteins that will be modified by the

PAD4 enzyme. The newly discovered PAD4-activating antibody seems to bind in this region (shown in yellow and purple) and substitutes for some of the calcium that is usually required to modify proteins. This allows the enzyme to work with the small amounts of calcium normally present in the body. Credit: Science Translational Medicine/AAAS

In a series of lab experiments designed to unravel the workings of a key enzyme widely considered a possible trigger of rheumatoid arthritis, researchers at Johns Hopkins have found that in the most severe cases of the disease, the immune system makes a unique subset of antibodies that have a disease-promoting role.

Reporting in the journal *Science Translational Medicine* online May 22, the Johns Hopkins team describes how it found the novel antibodies to peptidylarginine deiminase 4, or PAD4, in blood samples from people with aggressive inflammation and connective tissue damage.

Researchers say the presence of so-called PAD3/PAD4 cross-reactive autoantibodies could serve as the basis for the first antibody-specific diagnostic test to distinguish those with severe rheumatoid arthritis from those with less aggressive forms of the disease.

"Identifying early on a subset of patients with severe rheumatoid arthritis could benefit their health, as these patients could start aggressive drug therapy immediately and find the most effective treatment option," says senior study investigator Antony Rosen, M.D. Rosen, director of rheumatology and the Mary Betty Stevens Professor at the Johns Hopkins University School of Medicine, says that a third, or 1 million of the more than 3 million Americans - mostly women - estimated to have rheumatoid arthritis have an aggressive form of the disease.

In the study, the antibodies were present - in 18 percent of 44 fluid samples from one research collection and in 12 percent of another collection of 194 - but only in people with severe cases of rheumatoid arthritis. Past research shows that those with the most aggressive disease are less likely to respond to anti-inflammatory treatments with steroids and other drugs.

An examination of patients' medical records revealed that 80 percent of patients with the antibody saw their disease worsen over the previous year, while only 53 percent without the antibody showed disease progression. In comparing average scores of disease-damaged joints, researchers found that those with the antibody had an average deterioration in joints and bones by a score of 49. Those without the antibody had an average degradation in their score of 7.5, indicating much milder disease.

In a related finding, the Johns Hopkins team also uncovered how the PAD3/PAD4 cross-reactive auto-antibodies might contribute to more severe, erosive disease in rheumatoid arthritis. The team performed a series of experiments to gauge the antibodies' effects on PAD4 in response to varying cell levels of calcium, on which PAD enzymes depend.

Lab experiments showed that the antibodies greatly increase PAD4 enzyme function at the low levels of calcium normally present in human cells. Results showed that PAD4 activity was 500 times greater in the presence of antibodies than when they were absent. Tests of the antibody and enzymes' chemical structures later showed that the antibodies bind to PAD4 in the same region as calcium, suggesting to researchers that the antibodies might be substituting for calcium in activating the enzyme.

According to Rosen, the series of experiments, which took two years to

complete, represents the first evidence of an antibody having a direct role in generating the targets of the immune response, or auto-antigens, in rheumatoid arthritis.

"Our results suggest that drugs inhibiting the PAD4 enzyme may have real benefit in patients with severe rheumatoid arthritis and represent an important field of study for investigating new and alternative treatments," says lead study investigator and biologist Erika Darrah, Ph.D.

Darrah says the team next plans long-term monitoring of arthritis sufferers to find out when the antibody first appears in the blood, and when intervention may have maximum impact in preventing or stalling disease progression. The team also plans further experiments to see if the antibody is taking control of the chemical pathways normally used by other cell proteins to control PAD4 sensitivity to calcium.

More information: "Erosive Rheumatoid Arthritis Is Associated with Antibodies That Activate PAD4 by Increasing Calcium Sensitivity," by E. Darrah, *Science Translational Medicine*, 2013.

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