The green light gives the game away: New method for direct identification of antigens

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The immune system is a vital part of our defenses against pathogens, but it can also attack host tissues, resulting in autoimmune disease. The antigens that induce destructive immune reactions can now be identified directly – without any prior knowledge of their possible structure.

Molecules that activate immune responses, generically termed antigens, are recognized by circulating immune cells. In the case of autoimmune reactions, such responses may lead to the destruction of body tissues. A new method that can identify the antigens that initiate such reactions may help to prevent misdirected attacks in the future. Using genetic engineering techniques, researchers at LMU and the Max Planck Institute for Neurobiology have generated cells that emit green fluorescent light when stimulated by the binding of a cognate antigen.

The immunological needle in a haystack

The new method is based on the isolation of T cells present in samples of affected tissues obtained from patients with autoimmune diseases. The research team, led by Dr. Klaus Dornmair (Institute for Clinical Neuroimmunology at LMU and the Department of Neuroimmunology at the MPI for Neurobiology), first recovered the genetic blueprints for the specific antigen-binding T-cell receptors (TCRs) produced by these cells, and transferred them into a cultured cell line that grows well in the laboratory.

This line also contains a version of the gene for the Green Fluorescent Protein (GFP) that is specifically expressed if a TCR is activated. Finally, the cells are incubated with a collection of some 100 million peptides - short amino acid sequences like those normally recognized by TCRs. If even a single peptide represented in the library is recognized by a specific TCR, the corresponding cell synthesizes GFP and can be detected by its green fluorescence, allowing the bound antigen to be identified. The method thus provides a relatively simple way of identifying single autoimmune antigens from huge numbers of possible suspects.

An initial test carried out using cells specific for a known influenza antigen confirmed the efficacy of the method. The researchers were able unequivocally to select out and identify the correct antigen from all the other peptides used in the test. The technique is so rapid and so sensitive that several million antigens can be analyzed in a matter of hours. This opens up a wide range of possible applications – ranging from the analysis of the reactive antigens responsible for autoimmune diseases like multiple sclerosis or psoriasis to the identification of new tumor or viral antigens. Indeed, its practical potential is so significant that the method is the subject of a patent application.(*Nature Medicine*,8.4.2012) göd

More information: Unbiased identification of target antigens of CD8+ T cells with combinatorial libraries coding for short peptides, K. Siewert, J. Malotka, N. Kawakami, H. Wekerle, R. Hohlfeld & K. Dornmair, *Nature Medicine* Advanced Online publication, doi:10.1038/nm.2720

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