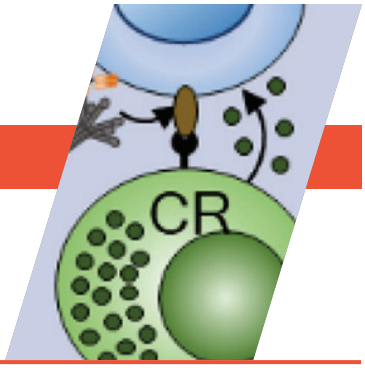
BIOLOGICAL SCIENCES

Modified Antibody Clarifies Tumor-Killing Mechanisms



Scientific Achievement

The structure of an antibody was modified to selectively activate a specific pathway of the immune system, demonstrating its value in killing tumor cells.

Significance and Impact

The work provides a platform for disentangling the effects of different immune-system pathways and could lead to the design of improved immunotherapies.

The immunotherapy approach

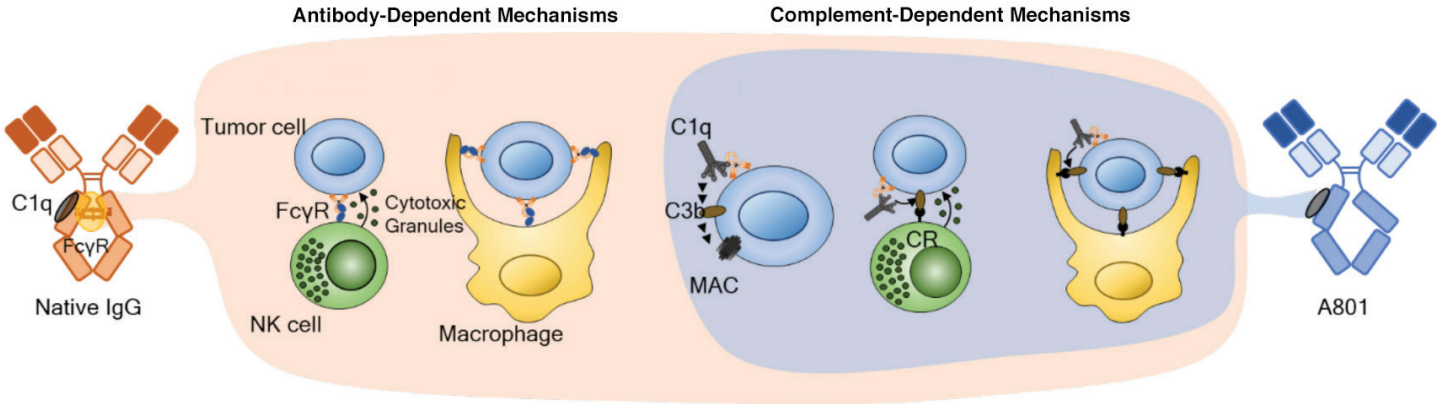
Immunotherapy—the use of the immune system to fight disease—has made tremendous progress in the fight against cancer. Antibodies such as immunoglobulin G (IgG) can identify and attack foreign or abnormal substances, including tumor cells. But to control and amplify this response, scientists need to know more about how elements of the immune system recognize tumor cells and trigger their destruction. There are two main pathways for this: antibody-dependent mechanisms and complement-dependent mechanisms.

The antibody pathway involves coating the surfaces of tumor cells with antibodies that recruit "natural killer" (NK) cells and macrophages (a type of white blood cell) to destroy the tumor cells. The complement pathway (so named because it complements the antibody pathway) also engages NK cells and macrophages and includes a third mechanism—a cascade of events culminating in tumor-cell destruction via a membrane attack complex (MAC).

Antibodies engineered for one pathway

Because both the antibody and complement pathways are triggered by the same IgG antibodies, disentangling their effects is difficult. The lower arm of the Y-shaped antibody (the "Fc tail") contains binding sites for molecules that initiate the antibody pathway (FcγR) as well as for molecules that trigger the complement pathway (C1q).

By making a few amino-acid substitutions, researchers were able to modify a native ("wild type") IgG antibody so that it would bind exclusively to C1q. The resulting monoclonal antibody (mAb), denoted A801, thus decouples the complement pathway from the antibody pathway. Such mAbs with absolute C1q-binding selectivity provide a much-needed platform for studying complement-dependent cell-killing mechanisms, a topic that has suffered from a lack of experimental tools with sufficient specificity.



Unaltered IgG antibodies (far left) trigger both antibody-dependent and complement-dependent mechanisms for the destruction of tumor cells. Binding of FcγR molecules triggers the antibody-dependent mechanisms, and binding of C1q molecules triggers the complement-dependent mechanisms. Researchers have now engineered a version of the IgG antibody (A801, far right) that binds exclusively to C1q molecules, providing a much-needed platform for exploring just the complement-dependent mechanisms.

Crystallography reveals a twisted tail

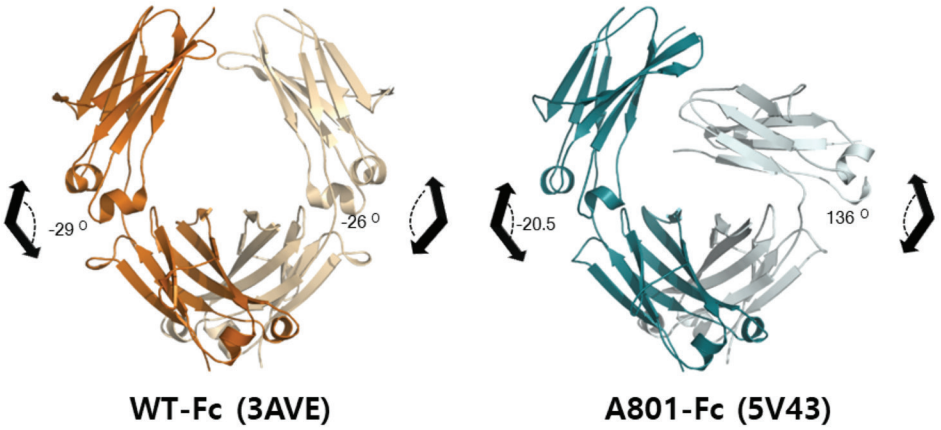
To understand the molecular basis of the unique selectivity of the A801 variant, the researchers solved the crystal structure of the Fc tail and compared it to the unmodified wild-type version. The diffraction data were collected at ALS Beamline 5.0.3 (part of the Berkeley Center for Structural Biology) and Beamline 23-IDD at the Advanced Photon Source.

Although the IgG Fc tail is a homodimer (i.e., it's composed of two identical subunits), the A801 variant displayed an extreme twist in the angle between the planes of the second subunit. The details of this asymmetry in the structure—which include changes in chain flexibility, variations in electrostatic interactions, and loss of access to binding sites—provided a structural explanation for enhanced C1q binding and lack of FcγR recognition.

Evaluation of anti-tumor effects

The researchers applied the engineered Fc tails to well-documented therapeutic antibodies (rituximab) to evaluate their ability to kill tumor cells in vitro as well as in mouse models. The results showed that complement-dependent mechanisms very effectively cleared tumor cells with speed and efficacy comparable to those of antibody-dependent mechanisms, while circumventing some of the adverse reactions of the latter.

Collectively, the data highlight the importance of complement-dependent mechanisms in mAb function and provide an experimental approach for delineating the effects of the complement pathway in immunotherapeutic treatments and studies.



Solved structures of the Fc tail domain of the IgG antibody, wild type (WT) and engineered (A801). The homodimers that make up each domain are indicated by darker and lighter coloring. An extreme twist in the second homodimer of A801 accounts for its selectivity for C1q binding.

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