Antibodies

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An **antibody** (**Ab**), also known as an **immunoglobulin** (**Ig**), is a large, Y-shaped protein used by the immune system to identify and neutralize foreign objects such as pathogenic bacteria and viruses. The antibody recognizes a unique molecule of the pathogen, called an antigen. Each tip of the "Y" of an antibody contains a paratope (analogous to a lock) that is specific for one particular epitope (analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can *tag* a microbe or an infected cell for attack by other parts of the immune system, or can neutralize it directly (for example, by blocking a part of a virus that is essential for its invasion).

To allow the immune system to recognize millions of different antigens, the antigen-binding sites at both tips of the antibody come in an equally wide variety. In contrast, the remainder of the antibody is relatively constant.

What do antibodies look like?

The two arms at the top of the antibody's Y shape bind to what's known as the antigen. The antigen can be a molecule, or a molecular fragment — often some part of a virus or bacteria. (For instance, the new coronavirus SARS-CoV-2 has unique "spikes" on its outer coat, and some antibodies bind to and recognize these spike proteins.) The bottom of the Y, or the stalk, binds to several other immune-system compounds that can help kill the antigen or mobilize the immune system in other ways. One set of these, for instance, triggers the complement cascade. Antibodies have the same basic Y-shape, but there are five variations on this theme — called IgG, IgM, IgA, IgD and IgE. Each variation looks slightly different and plays slightly different roles in the immune system. For instance, immunoglobulin G, or IgG, is just one Y, whereas IgM looks a bit like it has 10 arms, with five Ys stacked together, and each prong can bind one antigen.

The constant region at the trunk of the antibody includes sites involved in interactions with other components of the immune system. The class hence determines the function triggered by an antibody after binding to an antigen, in addition to some structural features. Antibodies from different classes also differ in where they are released in the body and at what stage of an immune response.

Together with B and T cells, antibodies are the most important part of the adaptive immune system. They occur in two forms: attached to a B cell or in soluble form in extracellular fluids such as blood plasma. Initially, antibodies are attached to the surface of a B cell – they are then referred to as B-cell receptors (BCR). After an antigen binds to a BCR, the B cell activates to proliferate and differentiate into either plasma cells, which secrete soluble antibodies with the same paratope, or memory B cells, which survive in the body to enable long-lasting immunity to the antigen. Soluble antibodies are released into the blood and tissue fluids, as well as many secretions. Because these fluids were traditionally known as humors, antibody-mediated immunity is sometimes known as, or considered a part of, humoral immunity. The soluble Y-shaped units can occur individually as monomers, or in complexes of two to five units. Antibodies are glycoproteins belonging to the immunoglobulin superfamily. The terms antibody and immunoglobulin are often used interchangeably, though the term 'antibody' is sometimes reserved for the secreted, soluble form, i.e., excluding B-cell receptors. Antibodies are specialized, Y-shaped proteins that bind like a lock-and-key to the body's foreign invaders — whether they are viruses, bacteria, fungi or parasites. They are the "search" battalion of the immune system's search-and-destroy system, tasked with finding an enemy and marking it for destruction. When antibodies find their target, they bind to it, which then triggers a cascade of actions that vanquish the invader. Antibodies are part of the so-called "adaptive" immune system, the arm of the immune system that learns to recognize and eliminate specific pathogens.

Most antibodies have the similar structure except the hypervariable region which is called the antigen binding site. This region is constituted by the combination of various amino acids. When the antigen is a kind of carbohydrate (Polysaccharide), the binding could be regarded as a protein-carbohydrate interaction.

Biological function

Protein–carbohydrate interactions play an important role in biological function.

- Cell adhesion
- Signal Transduction
- Host-Pathogen Recognition
- Inflammation

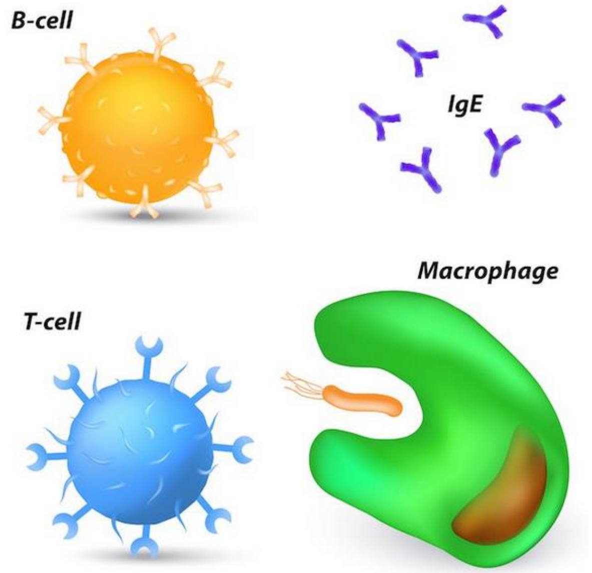
Where do antibodies form?

To understand antibodies, you first need to know about B-cells, which are a type of white blood cell that forms in the bone marrow. There are about a trillion B-cells in the body, and each one has a unique IgM antibody that sits on the B-cell surface and each binds, to one antigen. This staggering level of variation allows the body to recognize almost any substance that could enter. Here’s how it achieves that diversity: In each B-cell, the genes that code for the antibody's binding site are shuffled like playing cards in a deck. These B-cells then patrol the body, often lingering longer in areas like the lymph nodes or the tonsils.

Most of the time, these B-cells don't bind anything. But if, by a one- in-a-million chance, a B-cell does bind some foreign substance, that triggers the B-cell to get activated. The B-cell grows in size and starts to divide in what's called the clonal expansion. It's an identical copy of the parent, just like the mother. After a week or so, there may be hundreds of thousands to a million of these copies. Eventually, these clonally expanded B-cells differentiate into plasma cells, which are antibody factories. They secrete 10,000 antibodies per cell per second and they can do that for weeks or years for some.

But not all B-cells divide the same amount.

If you consider the B-cell to be a lock, and you consider all of these different things to be floating around to be different keys, then some of the keys will fit better, some will fit worse, and some won't fit at all. And depending on how well the key fits into the lock on the surface of a particular B-cell, that cell will be triggered to divide more. Then, the more prolific B-cells produce more plasma cells and churn out more of a specific type of antibody. The body doesn't just produce one type of antibody either; it produces a messy, chaotic zoo of them. Each locks onto different parts of an invader.



Drawings of a B-cell, T-cell, antibodies and a macrophage. (Image credit: Shutterstock)

And antibodies don't all do the same thing once they've bound to a target. Some will nip infection in the bud by directly neutralizing a threat, preventing a pathogen from entering a cell. Others tag invaders, so that the immune system's killer cells (which aren’t antibodies) can remove it. Still others may wrap viruses or bacteria in a gooey coating. And other antibodies send ‘instructions’ to immune cells called macrophages to come gobble up the invader. (That strategy can sometimes backfire with viruses, which may co-opt this response to invade new cells.) The first type of antibody to form after you are exposed to a virus is IgM, which emerges within 7 to 10 days after exposure. IgM can bind to an invader, but each "Y" in this 10-armed protein does so fairly weakly. But, just as five weak people working together can tackle a large, strong adversary, IgM's five Y's (10 arms) working together can bind tightly to an antigen. At about 10 to 14 days, the body begins making IgG, which is the immune system's "major workhorse". IgG can cross the placenta in a pregnant woman, giving a new born passive protection against disease until their own immune system can ramp up.

Normally, the immune system is stunningly good at recognizing the enemy and ignoring, or tolerating, our own cells. Sometimes, however, this process goes awry. That’s when T-cells (another type of white blood cells) come in. The body uses these T-cells to cross-check targets — only if both a B-cell and a T-cell recognize something as a foreign invader will an immune response be triggered. The body is supposed to remove B-cells that make so-called auto-antibodies, which react to the body's own cells. But when that doesn't happen, the body may mark its own cells for destruction and then relentlessly eliminate them. Autoimmune diseases such as lupus, rheumatoid arthritis, or type 1 diabetes can result. There are more than 100 autoimmune disorders.

What are monoclonal antibodies?

Antibodies have become the basis for some of the most useful medicines, as well as some of the most powerful lab techniques in biology. One of these clinical and therapeutic milestones is what's known as a monoclonal antibody. To create a monoclonal antibody, researchers vaccinate an animal (or possibly a human) to stimulate the production of antibodies against a particular substance. The body will gradually make antibodies that are more and more effective against that antigen. These antibody-producing cells are then filtered out of white blood cells and put into a dish to see which cells bind the antigen best. The cell that binds the best is then isolated — it is an antibody-producing factory, specifically honed to churn out one super-selective antibody. From there, that cell is fused to a blood cancer cell, producing something called a hybridoma. This hybridoma, or monoclone, is an inexhaustible generator of exactly the same antibody, over and over and over. (Researchers bind the monoclonal cell to a cancer cell because cancer just continues to reproduce.)

What it produces is a monoclonal antibody. Such cell lines have an incredibly diverse range of uses. There are millions of commercial monoclonal antibodies, which are used in labs to tag the tiniest, most specific cellular targets for study. Monoclonal antibodies also form the basis for many blockbuster drugs. For instance, the drug adalimumab (brand name Humira), is a monoclonal antibody that treats rheumatoid arthritis by inhibiting an inflammatory protein known as a cytokine. Another, called bevacizumab (Avastin), targets a molecule that fuels blood vessel growth; by blocking this molecule, bevacizumab can slow the growth of lung, colon, kidney and some brain cancers.

And in the SARS-CoV-2 pandemic, doctors around the world are racing to create monoclonal antibodies that will hopefully neutralize the new coronavirus. These antibodies are filtered from the plasma of people who have recovered from COVID-19 (also called convalescent serum). The hope is that by isolating the most effective antibodies, and then producing them en-masse, doctors can create a treatment that provides a temporary, "passive" immunity until the body can catch up and mount an effective, more long-lasting response on its own. By contrast, polyclonal antibodies are derived from multiple B-cells. Polyclonal antibodies are a library of antibodies that all bind to slightly different parts of the antigen, or target. Polyclonal antibodies are typically produced by injecting an animal with the antigen, stimulating an immune response, and then extracting the animals' plasma to produce antibodies en masse. Unlike monoclonal antibodies, which can take up to 6 months to produce, polyclonal antibodies can be made in 4 to 8 weeks, and require less technical expertise. In addition, for certain types of tests where you are trying to detect the antigen, polyclonal antibodies might have a better chance of binding to the target antigen, making them potentially more sensitive. The downside of polyclonal antibodies is that, because each individual animal might produce a different array of antibodies, making polyclonal antibodies that are consistent from batch to batch can be more challenging, and it isn't as easy to have a large supply.

How do antibody tests work?

Antibody tests detect whether the body has produced detectable quantities of antibodies to a certain molecule, and can therefore reveal whether someone has been infected by a specific virus or bacteria in the past. Usually, these tests are detecting IgM or IgG. For instance, SARS-CoV-2 antibody tests typically detect either part or all of the coronavirus' spike protein and can reveal whether someone has had COVID-19 in the past. Because the body takes time to ramp up its production of antibodies, people usually only test positive about two weeks after they were first exposed to the pathogen. There are two common types of antibody tests — lateral flow assays and enzyme-linked immunosorbent assay (ELISA) tests. Both involve fixing an antigen to a surface and then detecting whether an antibody binds to that antigen. Usually, a chemical reaction, such as fluorescence or a color-change, is triggered when the antibody binds to the antigen. Lateral flow assays are similar to pee-on-a-stick pregnancy tests; rather than pee, for antibody tests, blood or serum is washed over the flat surface, which is usually paper. ELISA tests work on a similar principle, only the tests are conducted in microplates and require a lab technician, and the results may not read out instantly. A good antibody test is one that produces few false positives and few false negatives.

Methods of study

- X-ray crystallography - Just like other organic molecule study, X-ray crystallography is a very useful tool to know the detail information on the interaction between carbohydrate and protein.

- NMR Study - By using titration, NOESY(Nuclear Overhauser Effect SpectroscopY), CIDNP experiments, the specificity and affinity of binding, association constants and equilibrium thermodynamic parameters of carbohydrate–protein binding can be studied.
- Molecular Modelling - In many cases, the conformation information is required, however, sometimes it is not able to get directly from the experiments. So the knowledge-based model building approach is used.
- Fluorescence Spectrometry - Fluorescence spectrometry is a useful tool and has its advantages: no procedure for separation and plenty of ways to get fluorophore source: there are some of amino acids and ligands that have fluorophore after they are activated.
- Dual polarisation interferometry - Dual polarisation interferometry is a label free analytical technique for measuring interactions and associated conformational changes.

Advances in the study of protein–carbohydrate binding

- Microarray-Based Study by Metal Nanoparticle Probes - Recently, studies by using metal nanoparticle probes to detect the carbohydrate–protein interactions were reported. Use of gold and silver nanoparticle probes in resonant light scattering (RLS) gives particular high sensitivity.
- Carbohydrate biosensor - As Lectin can strongly bind to specific carbohydrate, scientists develop several lectin-based carbohydrate biosensors. Designed lectin contains specific groups can be detected by analytical method.
- Isothermal Titration Calorimetry

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