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Ozone produced by antibodies during bacterial killing and in inflammation

Scientists at The Scripps Research Institute report

Professor Richard A. Lerner, M.D., Associate Professor Paul Wentworth, Jr., Ph.D., and a team of investigators at The Scripps Research Institute (TSRI) is reporting that antibodies can destroy bacteria, playing a hitherto unknown role in immune protection. Furthermore, the team found that when antibodies do this, they appear to produce the reactive gas ozone.

"[Ozone] has never been considered a part of biology before," says Lerner, who is Lita Annenberg Hazen Professor of Immunochemistry and holds the Cecil H. and Ida M. Green Chair in Chemistry at TSRI. The report will appear in an upcoming issue of the journal Science.

The ozone may be part of a previously unrecognized killing mechanism that would enhance the defensive role of antibodies by allowing them to subject pathogens to hydrogen peroxide and participate directly in their killing. Previously, antibodies were believed only to signal an immune response.

This ability of antibodies to generate toxic compounds may also link them to a number of inflammatory diseases, such as atherosclerosis, lupus, multiple sclerosis, and rheumatoid arthritis. Furthermore, this research opens up exciting possibilities for new antibody-mediated therapies for conditions ranging from bacterial and viral infection to cancer.

Recognition and Killing in the Same Molecule

Also called immunoglobulins, antibodies are secreted proteins produced by immune cells that are designed to recognize a wide range of foreign pathogens. After a bacterium, virus, or other pathogen enters the bloodstream, antibodies target antigens--proteins, fat molecules, and other pieces of the pathogen--specific to that foreign invader. These antibodies then alert the immune system to the presence of the invaders and attract lethal "effector" immune cells to the site of infection.

For the last hundred years, immunologists have firmly held that the role of antibodies was solely to recognize pathogens and signal the immune system to make an immune response. The conventional wisdom was that the dirty work of killing the pathogens was to be left to other parts of the immune system.

Now, Lerner, Wentworth and their colleagues have demonstrated that antibodies also have the ability to kill bacteria. This suggests that rather than simply recognizing foreign antigens and then activating other parts of the immune system to the site of infection, the antibodies may further enhance the immune response by directly killing some of the bacteria themselves.

Antibodies do this by producing the chemical oxidant hydrogen peroxide--best known as the foamy formulation used for first-aid. Hydrogen peroxide is lethal to bacterial cells because it pokes holes in their cell walls, bursting the cells and killing them.

In the Science paper, the TSRI team reports the effective killing of E. coli bacteria through hydrogen peroxide production by antibodies specific for that bacteria.

The Ozone Hole in Each One of Us

Certainly the most surprising result that Lerner, Wentworth, and their colleagues found was that antibodies appear to make ozone, which they detected through its chemical signature. They have not yet demonstrated conclusively that what they found is ozone, but they are highly confident that ozone is what the antibodies are producing because no other known molecule has the same chemical signature.

Ozone is a particularly reactive form of oxygen that exists naturally as a trace gas in the atmosphere, constituting on the average fewer than one part per million air molecules. But it is noted mainly where its presence or absence poses a threat to public health.

The gas is perhaps better known for its crucial role absorbing ultraviolet radiation in the upper reaches of Earth's stratosphere--about 25 km above the surface--where it is concentrated in a so-called ozone layer, protecting life on earth from damaging solar radiation.

Ozone is also a familiar component of air in industrial and urban settings where the highly reactive gas is a hazardous component of smog in the summer months. Never before has ozone been detected in biology.

"All our analytical data point to this oxidant possessing the chemical signature of ozone," says Wentworth, "in which case, this is a new molecule in biology and therefore may have tremendous ramifications for signaling and inflammation."

Proof for a Proposed Reaction Pathway

All antibodies have the ability to produce hydrogen peroxide, the report adds, but they need to first have available a molecule known as "singlet" oxygen--another highly reactive oxygen species--to use as a substrate.

Singlet oxygen is an electronically excited form of oxygen that forms spontaneously during normal metabolic processes or when oxygen is subjected to visible or ultraviolet light in the presence of a sensitizer. "Phagocytic" innate immune cells, like neutrophils, also produce singlet oxygen and are the most likely source of the substrate for antibodies, since during an immune response, antibodies will recruit neutrophils and other immune cells to the site of an infection.

Once there, the neutrophils will engulf and destroy bacteria and other pathogens by blasting them with singlet oxygen and other oxidative molecules. The antibodies reduce singlet oxygen by combining it with water to produce hydrogen peroxide, producing ozone as a side product.

Interestingly, all antibodies have the ability to do this, which leads the TSRI team to speculate that the removal of singlet oxygen may have been the original role of antibodies. In a previous report, the same team speculated an ancient form of antibodies may have existed--molecules whose role was to catalyze singlet oxygen destruction, since singlet oxygen can potentially destroy any cell, making it dangerous to have around. Prior to the evolution of the modern antibody-mediated humoral immune response in vertebrates hundreds of millions of years ago, ancient antibodies may have been responsible for controlling the release of highly reactive and potentially dangerous singlet oxygen. Later, when antibodies developed as part of the adaptive arm of the immune system, they kept their original function because it provided a bit of extra lethality.

Another interesting finding is that the antibodies carry the reaction through an unusual intermediate. Lerner, Wentworth, and their colleagues postulate that the antibodies carry the reaction through an intermediate chemical species of dihydrogen trioxide, a reduced form of ozone.

Dihydrogen trioxide has also never before been observed in biological systems, and its presence as an intermediate has been the source of considerable speculation in the scientific community.

The team's reported detection of ozone is strong support of this proposed dihydrogen trioxide intermediate, and now the team is tackling the larger question of what it means.

"This is a novel set of observations and very interesting ones--there are a million questions [we could ask]," says TSRI Professor Bernard Babior, M.D., Ph.D. "What does the ozone do to the body's proteins, nucleic acids? Are there lethal concentrations of ozone? Does it have anything to do with other reactive species in the body?"

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The research article, "Evidence for Antibody-Catalyzed Ozone Formation in Bacterial Killing and Inflammation" is authored by Paul Wentworth, Jr., Jonathan E. McDunn, Anita D. Wentworth, Cindy Takeuchi, Jorge Nieva, Teresa Jones, Cristina Bautista, Julie M. Ruedi, Abel Gutierrez, Kim D. Janda, Bernard M. Babior, Albert Eschenmoser, and Richard A. Lerner, and appears in the November 18, 2002 "Science Express," the advanced publication edition of the journal Science. The article will appear in Science later this year.

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