IMMUNOLOGY

Protein Proves to Be a Key Link in Innate Immunity

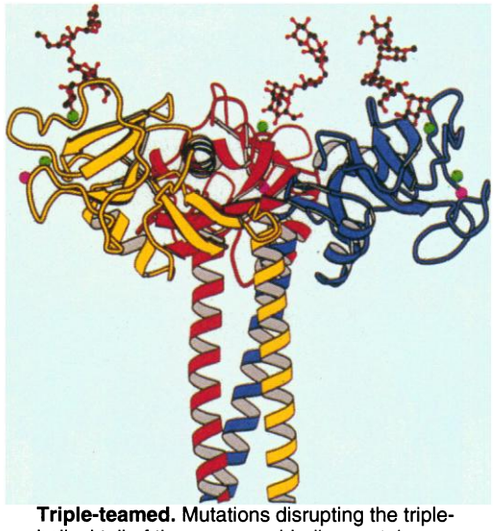
A pediatrician confronted with a puzzling case of a child who keeps coming down with serious infections is likely to start running down a list of suspects, starting with the AIDS virus and continuing through a host of rare inherited immunodeficiency diseases, such as the one suffered by the well-known "boy in the bubble." Until recently, few physicians would have thought of pinning the problem on an obscure protein known as the mannose-binding protein (MBP). But that may be changing.

Within the past few years, researchers have learned that the protein is a linchpin of the innate immune system—a nonspecific type of immunity that helps people ward off infections until they develop the more specific immune responses carried out by antibodies and T cells. "Mannose-binding protein has made an important contribution to immunological research," says immunologist and infectious diseases expert Michael Frank of Duke University in Durham, North Carolina. "It's a piece of the puzzle as to how nonspecific immunity causes protection against infection."

What's more, inherited defects in the gene encoding MBP may be a relatively common cause of immunodeficiency in children. Firm estimates of the numbers affected are hard to come by, but up to 25% of cases in which children have frequent unexplained infections may be due to mutations in the MBP gene. And even though the conventional wisdom has been that such children outgrow the problem as their immune systems mature and the more specific responses kick in, evidence obtained just this spring indicates that MBP gene mutations may also cause immunodeficiencies in adults.

This development of a clearer picture of MBP's role in immunity, coupled with identification of the mutations causing the immunodeficiency, has opened the way to better diagnosis and treatment of individuals with MBP defects. It might be possible, for example, to treat individuals who turn out to have the gene defects with antibiotics—or perhaps with MBP itself—to prevent infections before they develop. Indeed, predicts immunologist John Pound of Birmingham University in the United Kingdom, "I see a time when people will routinely test for this MBP immunodeficiency."

Although immunologists only began to understand MBP's importance about 5 years ago, the story began in 1968 with a report by Michael Miller, then an immunologist at the State University of New York Downstate Medical Center in New York City, of a 3-month-old child with symptoms suggesting that her immune system was impaired. She suffered from recurrent ailments ranging from minor skin infections to the serious blood infection septicemia, had persistent diarrhea, and in general failed to thrive. While her physicians performed a battery of tests on her immune system, they could detect only one problem: in a lab assay, there was an inability to prepare yeast cells for attack by the phagocytes, immune cells that help rid the body of infectious bacteria by engulfing and digesting them.



WILLIAM WEIS/STANFORD UNIVERSITY AND ARTHUR PALMER/COLUMBIA UNIVERSITY

Triple-teamed. Mutations disrupting the triple-helical tail of the mannose-binding protein may lead to immunodeficiency.

The cause of the defect was unclear, but a rash of similar cases over the next few years prompted researchers to search for the culprit. By 1976, for example, immunologist John Soothill of the Institute of Child Health in London had found the same immunodeficiency in 11 of 43 children with recurrent infections. Soothill "found there was clearly something absent from the blood that was needed to phagocytose the bugs [causing the infections]," says molecular biologist John Summerfield of St. Mary's Hospital, London.

By 1989, the team at the Institute of Child Health, now headed by Malcolm Turner, had shown that the substance, whatever it was, normally binds to mannan, a sugar residue present on the outer yeast cell wall. To try to pin down its identity, Michael Super, a graduate student in Turner's group who is now at Fuji Immunopharmaceuticals in Boston, searched through a database looking for molecules that show specific binding to mannose. He found one that seemed to fit the bill: a mannose-binding protein that is now recognized as one of several proteins produced in inflammatory reactions.

That was an intriguing result, because the Turner group had evidence indicating that the protein missing from the children's blood activates the classical complement pathway, a cascade of several proteins whose concerted action helps protect against infection by causing the rupture of invading bacteria, and there was already circumstantial evidence suggesting that MBP plays such a role. Kurt Drickamer's team at Columbia University in New York City had cloned and sequenced the rat MBP gene in 1988. The sequence revealed that MBP is structurally similar to a key component of the complement pathway, a protein called C1q whose job is to interact with antibodies that have bound to bacteria and then draw in the other complement proteins that rupture the bacterial cells. The structural similarity between C1q and MBP suggests that MBP essentially mimics the role of C1q in activating the complement pathway.

If so, then loss of MBP could make people more susceptible to infection by reducing complement pathway activity. Loss of the protein might also explain the specific phagocytosis defect seen in the children. Bacteria, as well as some viruses, also carry the mannose sugars recognized by MBP. Consequently, the protein could act as a universal antibody by binding to bacterial cells and attracting phagocytic cells to destroy the pathogens. Indeed, when Super and his colleagues measured the levels of the protein in children known to have the phagocytosis defect, they found that MBP was either absent or present in very low concentrations—the expected result.

Also in 1989, Maureen Taylor from the St. Mary's group and Kedernath Sastry, working with Alan Ezekowitz at Harvard University, cloned the human MBP gene. And at this point, Turner's and Summerfield's groups joined forces to pin down the genetic cause of the low MBP levels. "They had the families [with the defect] and the protein expertise, and we were working on the MBP gene and could do the molecular genetics," says Summerfield.

The London team scanned the human gene in the family members, looking for mutations that might cause the patients' immune problems. They found two similar mutations: In Eurasians, amino acid 54, normally a glycine, was replaced by aspartic acid, and in sub-Saharan Africans, another glycine at position 57 was replaced by glutamic acid. The frequency of these mutations turned out to be surprisingly high: About 17% of Caucasians and 29% of Gambians carry a mutant MBP gene. In addition, Peter Garred's group

at National University Hospital in Copenhagen, Denmark, found a third, much less common mutation in which an arginine (at position 52) is replaced with cysteine.

What apparently happens, Turner says, is that the mutations disrupt MBP's structure, thus preventing it from performing its functions. The protein has two parts: a head that binds sugars plus a tail consisting of the structural protein collagen in which three chains wind together in a triple helix. And as Turner notes, the mutations should prevent formation of the helical structure, which is needed for complement activation, because glycine is the only amino acid that can fit in the turn of the helix. "If you introduce a dicarboxylic acid, such as aspartic acid or glutamic acid, which is what we see with the major mutations, the protein can't polymerize," says Turner. In work in press in *Immunology*, his team has evidence in support of that hypothesis. The researchers found that in patients with the defective MBP gene the protein is not polymerized.

What's more, MBP problems may not be limited to children as previously thought. In the 8 April issue of *The Lancet*, Summerfield and his colleagues report that they've found mutations in one or both copies of the MBP gene in five adult patients with severe bacterial infections, and in three of these individuals extensive testing had ruled out HIV infections and other immunodeficiencies as the cause of the problem. How extensive a problem MBP deficiency is in adults remains to be established, however.

Also unclear, given the apparent importance of MBP in immune defenses, at least in children, is the question of why so many people seem to carry mutations in the gene. One possibility is that the mutated gene might confer a selective advantage that outweighs its deleterious effects. Ezekowitz suggests, for example, that people with reduced levels of MBP might be protected against the complement-mediated damage often seen in inflammatory conditions, such as rheumatoid arthritis or the septicemia caused by meningococcal bacteria.

Despite these uncertainties, researchers are looking to explore the clinical implications of the MBP work. At the very least, identification of the gene defects should help clinicians diagnose children with unexplained immunodeficiencies. And eventually, it might be possible to produce recombinant MBP and use it to treat patients with active infections. As Super points out, however, because the protein is very large and complex, "it's going to be a major headache to manufacture." Even so, during the past few years MBP has risen from obscurity to prime suspect as a cause of immunodeficiency disease.

–Clare Thompson

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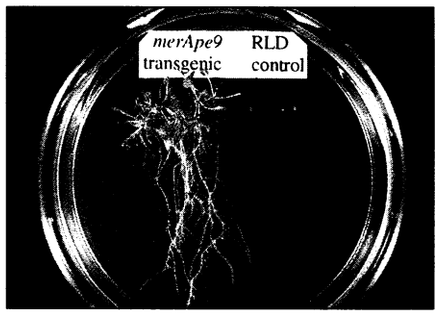
ENVIRONMENTAL BIOLOGY

Plants Proving Their Worth in Toxic Metal Cleanup

Delbert Hershbach, a grower of ornamental plants in California's San Joaquin Valley, probably doesn't think of himself as a scientific pioneer. But he is. About 5 years ago, his farming operation ran into trouble. The irrigation water he was using was leaching toxic selenium salts from the soil, allowing them to escape into the drainage water, polluting local evaporation ponds and endangering aquatic wildlife. To solve the problem, Hershbach began growing plants, such as mustard, that remove the toxic compounds, concentrating them in their own tissues and keeping the toxic compounds from entering drainage ponds. Soon, he expects to return to growing his regular, profitable crops. "This strategy of cleansing soils makes a lot of sense," says Hershbach. "Without it, I would have to abandon the field or grow something I'm not happy about."



RICHARD MEAGHER

Mercury lover. The *Arabidopsis* plants *(left)* carry a bacterial gene that enables them to grow on a mercuric chloride solution.

Indeed, the strategy Hershbach used, known as phytoremediation, is making sense to many other people. Faced with a growing list of sites contaminated with toxic materials, researchers are increasingly turning to plants as a possible means of cleansing the soils. "Interest in phytoremediation has exploded in the last few years," says Norman Terry, a plant biologist at the University of California, Berkeley. One indication of the growing enthusiasm: The first international conference devoted to the subject, held in April at the University of Missouri, Columbia, was oversubscribed, attracting a multidisciplinary crowd of 250 biochemists, plant physiologists, ecologists, and soil scientists, among others.

Currently most of the interest of this growing crowd is focused on removing metals, mainly because much less is known about how plants handle organic contaminants such as oils and cleaning fluids. But Terry says "lots of sites" need phytoremediation to remove metals, so business is booming, even for this single application. Among the poisoned sites: abandoned mines laced with zinc and lead; military sites fouled with lead and cadmium; municipal waste heaps where copper, mercury, and lead can be hazards; and dump sites for sewage sludge where all these metals are problems.

Although phytoremediation research is in its early stages, the hope is that plants will prove easier and cheaper to use than the current technique for dealing with metal-tainted soils—excavation and reburial. Such cleanup is practical only for small areas, often a half hectare or less, and cleaning one hectare to a depth of one meter costs between $600,000 and $3,000,000, depending on the type and intensity of pollution.

Metal-scavenging plants may be able to improve on this record, because they can be planted over large areas and they are cheap to grow. An added economic benefit may come from the fact that the harvested plants can be burned and metals, such as nickel, recovered. "Contamination of soils is a big-time social issue," says Scott Cunningham of DuPont in Wilmington, Delaware. "Phytoremediation is going to be much better and cost-effective than conventional cleanup strategies," he predicts.

Both amateur and professional botanists have long known that certain plants can concentrate metals to levels that would kill off most species. For example, for hundreds of years, prospectors looking for copper used this knowledge to hunt for precious ores lurking near the surface. "Hyperaccumulating plants are an evolutionary response to soils of a particular geologic origin," says Alan Baker, a plant ecologist at the University of Sheffield, U.K. "They grow on a range of geologic substrates, including nickel-rich serpentine soils, soils containing calamine and other zinc and lead minerals, and those rich in copper and cobalt."

The plants are especially common in the tropics or subtropics, apparently because metal accumulation is a defense against plant-eating insects and microbial pathogens. Because these organisms are most numerous in areas that escaped glaciation, the hyperaccumulating plants are, too. In an August 1994 trip to Cuba, for example, Baker turned up more than 80 species of nickel-accumulating plants in just two families, the Buxaceae, which includes boxwood, and Euphorbiaceae, which includes many