

APRIL 22, 2007

Crab Shells and Fungi May Hold a Key to Asthma

Chitin is the stuff of crab shells and the carapaces of dust mites, the cell walls of lichens, and even the rigid innards of parasitic worms. It may also be the stuff that sets off asthma.

Despite being the most common biopolymer on earth after cellulose, chitin never occurs naturally in humans and other vertebrates. However, according to a new study led by Howard Hughes Medical Institute (HHMI) investigator Richard M. Locksley, our immune systems possess an innate ability to recognize and eliminate chitin from our bodies. The researchers found that when this potent response to the ubiquitous particles goes awry, it can cause an allergic response that could potentially lead to asthma. The new findings, published in the April 22, 2007, online version of the journal *Nature*, may help elucidate the biological mechanisms underlying asthma and provide new ways of preventing and possibly treating it.

Pronounced kī-tin, the polysaccharide cement lends many organisms their rigidity. It can be found in the cell walls of fungi and the exoskeletons of shellfish, plankton and insects, along with insect egg cases and the powerful grinders and rigid walls in the pharynx of worms. Each year, molting or dead crustaceans, fungi, and insects shed billions of metric tons of chitin. But chitin deposits never build up on the sea or forest floor. Instead, bacteria possessing specialized chitinolytic enzymes cause the chitin to decay, helping recycle it swiftly back into the environment.

"Are persons at risk for developing asthma less efficient at breaking down chitin?"

- Richard M. Locksley

While humans and other vertebrates do not produce chitin, our bodies are equipped to recognize and eliminate it. When exposed to chitin, our innate immune system—the system with which we are born—can muster a response that generates an enzyme called acidic mammalian chitinase (AMCase), which breaks chitin down. The new study indicates that a less active form of AMCase might allow our adaptive, or learned, immune system to recruit too

many cells in its attempt to rid the body of chitin, eventually inflaming airways and setting off asthma.

A team led by Locksley, an immunologist at the University of California San Francisco, had previously traced the pathways involved in the body's immune response to bites from helminths, a common parasitic worm. For these studies, they developed laboratory mice engineered with fluorescent probes in their immune system that light up when mucosal barriers, such as the lining of the intestines or lung, come under attack. Locksley used this mouse model to show that the same immune process that occurs in response to a worm bite in the intestines also takes place following exposure to an allergen in the lungs.

Like most worms, helminths synthesize chitin for their pharynx. Curious to learn how the immune system responds to such an environmentally common allergen, Locksley's team aerosolized purified chitin and sprayed it into the lungs of laboratory mice. This caused a rapid and intense immune response. To rid themselves of the chitin, the mice used the same strategy they use to respond to other allergens, in which interleukin (IL)-4 and IL-13 cells including eosinophils and basophils enter lung tissue to attack the invader. Given that the mice had not been previously exposed to chitin, Locksley wondered, "What actually sensed the chitin in the mice?"

His team, which included scientists from Harvard Medical School and the Free University in Amsterdam in the Netherlands, then used his mouse model to trace the pathway necessary for recognizing chitin. They tested mice with and without a type of white cell in the lining of the lung known as alternatively activated macrophages, a part of the innate immune system. They showed that mice without the cells did not launch an immune response, indicating that the macrophages signaled the presence of the chitins.

The scientists observed an important difference from the standard process for eliminating a foreign particle, however, when they observed activation of AMCase. The lungs produce AMCase in response to the presence of IL-4 and IL-13 following exposure to chitin. The researchers found that when mice have more AMCase than normal, the immune response to chitin is greatly reduced. They also observed a dampened immune response when they exposed chitin to AMCase before the animals inhaled it. Locksley believes that AMCase, comparable to the chitinolytic enzyme found in marine and soil bacteria—and likely conserved in human evolution—attenuates the chitin-induced innate immune response by degrading the chitin. This removes the stimulus for further eosinophil and basophil recruitment more rapidly and halts the allergic response.

Locksley believes his study points to the importance of chitin exposure and a plausible explanation for the role of AMCase in the development of asthma. "Are persons at risk for developing asthma less efficient at breaking down chitin?" he asks. His findings about chitin and AMCase may help explain the extremely high rates of asthma—as high as 25 percent—found in previously

asymptomatic workers in shellfish processing plants. He has begun to study *in vivo* human lung cells' reaction to chitin exposure and to search for variants of AMCase found in asthma patients.

His findings could promote further study of the role in asthma of environmental chitin from dust mites and cockroaches and other sources, about which little is presently known. It is also possible that identification of the human AMCase variants responsible for asthma could lead to tools for predicting who is likely to develop asthma and possibly new treatments to prevent asthma attacks.