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Researchers Make Progress in Understanding Anthrax Resistance

Researchers have discovered that certain strains of mice are resistant to anthrax toxin because they have slightly different versions of a molecular motor protein that is involved in transporting molecules within immune system cells called macrophages. Presumably, the different forms of the protein are either more effective at protecting macrophages or less effective at facilitating the damaging effects of the toxin, say the scientists.

Their finding may help researchers understand how anthrax wreaks damage in macrophages. The research may also aid efforts to protect humans against the deadly anthrax spores, which have the potential to be used in biological weapons.

In an article published in the October 2001 issue of *Current Biology*, Howard Hughes Medical Institute investigator William F. Dietrich and his colleagues report that the protein produced by the gene *Kif1C* exists in slightly different forms, or polymorphisms, in mice. Dietrich and colleagues at Harvard Medical School collaborated with researchers at the Whitehead Institute for Biomedical Research.

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"While the effects of anthrax lethal toxin on cells are quite complicated, the primary impact of the toxin is on the body's macrophages," said Dietrich. "The rest of the physiological impact of the disease on humans and animals

seems to be secondary to those effects. The bacterium has evolved this mode of attack since, as a key weapon in the body's innate immune system, macrophages would gobble up the bacteria. Wiping out this first line of defense allows the bacteria to proliferate unchecked.

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According to Dietrich, previous studies by other researchers had shown that the differences in anthrax resistance among mouse strains were not due to differences in the uptake of the toxin by macrophages. In previous experiments, Dietrich and his colleagues used genetic techniques to map the resistance gene to an area, or locus, of mouse chromosome 11, but they had not pinpointed the specific gene.

"In this latest work -- with considerable help from our colleagues at the Whitehead Institute -- we used DNA sequencing and both the mouse and human genome databases to pick out the genes in that region," said Dietrich. "Once we identified those genes, we searched through each gene, looking for sequence differences between susceptible and resistant strains that could account for the differences in anthrax toxin resistance."

The researchers found that the differences between susceptible and resistant strains were caused by missense polymorphisms in the gene *Kif1C*. Missense polymorphisms represent single nucleotide changes in the gene that can subtly affect the functioning of the protein for which it codes, said Dietrich. Different forms of the same gene are known as alleles. Thus, the scientists set out to show that the resistant alleles of the *Kif1C* gene did, indeed, increase resistance to the toxin in macrophages.

"The most convincing of these experiments were those in which we inserted a resistant allele of *Kif1C* into susceptible macrophages and made them become more resistant to the anthrax lethal toxin," said Dietrich.

The researchers also treated resistant macrophages with the drug, brefeldin A, which is known to prevent the *Kif1C* protein from reaching its usual destination in the cell. This treatment induced susceptibility to the toxin in resistant macrophages. And in other experiments, the scientists showed that uptake of the toxin was normal in resistant cells, demonstrating that the toxin cleaved one of its normal protein targets -- called map kinase kinase -- in the macrophages.

Kif1C codes for a transport protein related to kinesins, which are known to carry molecular cargo along cellular highways, called microtubules. Dietrich speculates that the polymorphisms in the *Kif1C* gene alter the protein's ability

to carry cargo involved in the macrophage's response to the anthrax toxin.

"Research by other groups has indicated that anthrax toxin kills macrophages by inducing a runaway reaction called an oxidative burst," said Dietrich. "We speculate that this kinesin protein might be transporting either the elements that are part of this oxidative burst, or the actual oxidative compounds themselves. Or, the protein may be ensuring the prompt and appropriate delivery of compounds that protect the macrophage against its own oxidative bursts.

"So, while the subtle mutations that we see in *Kif1C* probably don't affect the macrophages in their normal functioning, the decrease in efficiency during the stress of intoxication by the anthrax toxin can mean the difference between death and survival of the macrophage." Further studies should clarify the effects of the polymorphisms and yield even more insight into the effects of the toxin, he said.

"This work represents the first concrete connection between a molecule inside the cell and a direct effect of lethal factor on whether the cells live or die," said Dietrich. "And understanding such effects are important to know if we are to learn how to fight susceptibility to anthrax." While Dietrich does not see immediate clinical benefits from the new findings, he does see the beginning of an important research pathway.

"We intend to look for these variations in human populations; to figure out what cargo this protein is carrying, and to learn why *Kif1C* is activated under anthrax intoxication," he said. "Once we understand such phenomena, we might be able to work toward clinical applications in terms of better diagnostics and treatments."