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Clue to Smallpox Immunity

A team of scientists led by a Howard Hughes Medical Institute (HHMI) international research scholar has discovered the immune system mechanism that causes some mice to be more susceptible to mousepox than others. The discovery could pave the way to better protection for humans against the threat of smallpox, a related virus, as a weapon of bioterrorism.

HHMI international research scholar Gunasegaran Karupiah, a scientist at the John Curtin School of Medical Research at the Australian National University, and colleagues have identified proteins that determine which mice succumb to mousepox and which do not. The new insights into the immune response of mice to the mousepox virus could enable scientists to combine antivirals and cytokines such as gamma interferon to increase the efficacy of the treatment of poxvirus infections, including smallpox, said Karupiah.

The researchers found that strains of mice that are resistant to mousepox infection generate three types of the regulatory proteins called cytokines that are released by immune system cells to produce an immune response: interferon gamma (IFN- γ), interleukin-2 (IL-2), and tumor necrosis factor (TNF). Collectively, this is known as a type-1 cytokine response. Strains of mice susceptible to infection produce little or none of these cytokines, but they do produce IL-4a type 2 cytokine. The findings were published online in the June 7 edition of the *Proceedings of the National Academy of Sciences*.

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- Gunasegaran Karupiah

A potentially important application could be treating or protecting the increasing numbers of persons being vaccinated against smallpox with the vaccinia virus primarily health professionals in the United States and

elsewhere who are on the front lines of a potential bioterrorist attack.

We are interested in not only overcoming an acute infection to save the individual, but at the same time helping to induce long-term immunity which will provide protection from a secondary infection, Karupiah said. The cytokine effect may apply not only to poxviruses, according to Karupiah, but to other generalized viral infections.

Previous studies have shown that poxviruses such as smallpox, monkeypox, and vaccinia, the virus used to vaccinate against smallpox, all make proteins that bind to IFN- γ , to interfere with its signaling pathway. Karupiah's findings bolster suspicions that IFN- γ , and other cytokines play key roles in pathogenesis of these infections. He and his colleagues are the first to rigorously characterize the distinct cytokine responses in susceptible mice and compare them to those observed in resistant mice.

Many in the field had believed IL-4 to be a hallmark for a type-2 cytokine response, and IFN- γ , to be characteristic of the type-1 response. Karupiah's group demonstrated that simply taking away IL-4 in susceptible animals is not by itself enough to reverse susceptibility, meaning that it's a lot more complicated than people think, said Karupiah. But the prerequisite for efficient virus clearance seems to be high levels of gamma interferon production.

Scientists know relatively little about the immune response to smallpox, primarily because the virus has been all but eradicated for years. Researchers and public health officials therefore had little motivation to understand the immune responses generated. Also, although Karupiah's group has been working with mousepox for 15 years, investigators worldwide have been generally wary of working with the pathogen for fear it might spread to other mouse colonies. Smallpox was one of the biggest human scourges, said Karupiah, noting that in some populations there was a 30% mortality rate. And yet, because it was successfully eradicated, no one was interested in understanding how individuals recovered. But now, of course, the interest is back because of the threat of bioterrorism.

Karupiah's group used immunohistochemistry to look for cytokine proteins in vivo, an approach that insures the detected cytokines actually are being produced by the host during the course of infection. They found that while the messenger RNA for most of the cytokines is expressed following infection, the protein is not always produced an important observation, since the cytokine only has a biological effect when the protein is produced. Investigators, therefore, have been misled at times by looking only at gene expression.

Interestingly, the pattern of protein production also differs between organs. Unexpectedly, in animals that made IFN- γ , investigators found the protein in the spleen and not the lymph node, though both organs are associated with

the generation of an immune response. Conversely, they found IL-2 in the lymph node but not in the spleen. The reasons why this happens are still unclear, said Karupiah, but understanding them may help researchers figure out what constitutes an effective immune response.

The mousepox model has proven a useful tool for studying smallpox biology because of the ample mouse gene knockouts available. Karupiah also emphasizes the importance of the mousepox virus having co-evolved with the mouse much as smallpox has co-evolved with humans. For millions of years, the mouse immune system has adapted to the pathogen, and the pathogen has likewise adapted to new adaptive responses generated by the host. Scientists do not have the opportunity to study the outcome of such a longstanding immunological arms race in a mouse model of, for example, influenza.

Karupiah and colleagues continue to probe the mousepox-triggered immune response. They're investigating not only interferon response, but also the killer T cell and antibody responses.