

Learning from the 2001 Anthrax Attacks: Immunological Characteristics

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(See the article by Doolan et al., on pages 174–84.)

The article by Doolan et al. in this issue of the *Journal of Infectious Diseases* [1] describes the immune responses to anthrax among persons exposed and possibly exposed when a letter containing spores that had been sent to Senator Daschle was opened in the Hart Senate Office Building (HSOB) in October 2001. The authors took advantage of this unfortunate but unique opportunity to conduct an observational study to determine whether high levels of inhalational exposure to anthrax spores resulted in clinical disease, as well as in humoral immune or cell-mediated immune (CMI) responses.

This is one of the few studies of the immune response to high-level, naturally occurring anthrax exposure in humans, and it may be the first to describe CMI responses to this pathogen. The immune responses of persons with various levels of exposure, including an unexposed group and an unexposed but recently vaccinated group, are described, with data collected at 3 points in time: ~6, 8, and 12 weeks

after exposure. The degree of exposure was determined by proximity in the HSOB and Capitol complex to the letter at the time when it was opened. The proximity measure of exposure correlated directly with the percentage in each group who had positive nasopharyngeal cultures within 24 h of the incident. In addition, the article describes the immune response to vaccination against anthrax among the subset of these groups who were vaccinated as part of the public health response. The key findings are that, although all highly exposed persons had immediately received antibiotics and there was no evidence that exposure resulted in clinical illness, sizeable percentages of persons highly exposed but not vaccinated had evidence of either humoral immune (40% to protective antigen [PA] and 14% to lethal factor [LF]) or CMI (80% to PA and ~60% to LF) responses. In addition, immune responses occurred mainly in persons with higher levels of exposure, and, for all measures of immunity, there was a notable dose-response gradient. Vaccination of highly exposed persons also provoked detectable anti-LF antibodies in up to 40% of vaccinees. No one in the recently vaccinated, unexposed control group had detectable anti-LF antibodies; the anthrax vaccine used (anthrax vaccine adsorbed [AVA]) is geared toward the reliable production of PA antibodies only and not of LF [2]. Finally, a small percentage of persons min-

imally exposed (outside the HSOB but in the Capitol complex where positive environmental cultures were obtained) also had evidence of CMI responses.

In addition to adding to the library of information about the immune response to anthrax spore exposure, this study may shed additional light on the pathogenesis of anthrax. The data obtained support the concept that exposure to high doses of potentially virulent anthrax spores in the setting of antibiotic prophylaxis to abort infection is sufficient to provoke an immune response. Furthermore, the variety of responses—particularly the humoral, cell-mediated, and postvaccination responses to LF—suggest that some people may have had spore germination with toxin secretion and exposure that was limited in extent and duration in the setting of antibiotics. The current anthrax vaccine used in the United States, AVA, consists principally of PA adsorbed onto aluminum hydroxide. As demonstrated in the study by Doolan et al., vaccination reliably produces responses to PA but only rarely to LF.

There are several possible practical applications of the findings of this study. First, during the 2001 attacks, there was considerable uncertainty among both possibly exposed postal workers and public health officials as to how many persons were really exposed and at risk of inhalational anthrax. In particular, there was

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interest among possibly exposed groups of postal workers in Connecticut and New York who did not develop any disease as to whether there had been early disease that may have gone undetected and been aborted if antibiotics were received without antecedent blood cultures. Several workers had been ill shortly after exposure and received antibiotics. Except for the HSOB exposure, antibiotic prophylaxis for exposed workers was started well after the shortest expected incubation periods for inhalational anthrax, and the absence of diagnosed disease may have contributed to poor adherence to recommended prophylactic antibiotic therapy [3, 4]. Serological studies were done at the US Centers for Disease Control and Prevention (CDC) to examine this possibility in exposed and symptomatic individuals and in selected postal workers who were treated with antibiotics [5, 6]. These serological studies were done using a CDC-developed serological assay to detect antibodies to PA in persons with clinical anthrax [7]. None were positive, except in clinical cases of anthrax confirmed by other means [8]. Although this was reassuring to persons involved in responding at the time, it is possible that an examination of CMI responses would have been more disquieting. On the basis of the work of Doolan et al., whose anti-PA antibody assay appears to be more sensitive than the assay developed by the CDC, it appears that measurements of CMI are considerably more sensitive than humoral measures. More study is needed to determine the meaning of cell-mediated responses and whether any are unique to germinated, compared with nonviable, spore exposure.

A second possible practical implication regards vaccination. As outlined by Doolan et al., the goals of vaccination against anthrax are to produce circulating hu-

moral antibodies to neutralize the effects of toxins such as LF, block PA binding to cells, enhance phagocytic opsonization, and inhibit spore germination. The current vaccine is designed to block PA binding to cells. A more-recent, promising experimental vaccine targets the suppression of cell germination by including capsular poly- γ -D-glutamic acid (PGA), a polypeptide that makes up the capsule, in addition to PA [9]. The vaccine is effective in producing anti-PGA antibodies. Its effect on CMI was not reported. Thus, the potential involvement of CMI remains unclear, although it could be important for the inhibition of spore germination and bacterial growth. Further research should include an examination of the potential role played by CMI with regard to surface antigens and toxins and means to stimulate this type of immunity.

Large-scale point-in-time anthrax exposures have been rare. Although the authors are to be commended for taking advantage of what we all hope to be a unique event and for studying all highly exposed persons, their work is only a starting point for further study of the human immune response to anthrax. The study was limited by having only 4 individuals who were clearly exposed but who did not receive vaccination in whom to monitor the full natural evolution of immune responses and only 2 of the surviving persons with inhalational anthrax from the same attack agreeing to provide samples for the study. In addition, the target antigens for the study of CMI were toxins, not bacterial surface structures. Thus, the full range of immune responses, particularly cell-mediated responses in persons surviving clinical anthrax, remains to be characterized and compared with those with exposure to viable and to nonviable spores. Optimally, plans should be made to obtain

specimens from both highly exposed persons and any clinical cases that may occur if there is another anthrax attack. If the opportunity presents itself, such observational studies should examine the full evolution of not just humoral immune but also CMI responses in persons developing clinical anthrax, in those with viable spore exposure and in those exposed to inert spores.

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