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May 16, 2006

To Whom It May Concern,

I would like to submit comments responding to the recent CDC/FDA/NIAID Emerging Clostridia Workshop in Atlanta, which I attended. Please forward these comments to the appropriate individuals developing a research/surveillance agenda on the emerging clostridia.

Before offering comments, it might be worthwhile to briefly summarize my credentials. As a Professor of Microbiology at the University of Pittsburgh School of Medicine, my laboratory has conducted research on *Clostridium perfringens* for nearly 25 years. During that period, we have been continuously funded by NIAID; for the past 10 years, my laboratory has also been supported by the USDA Ensuring Food Safety Research Program. Currently, our work is sponsored by two NIAID grants (one a MERIT award) and by a USDA grant, all of which concern *Clostridium perfringens*. Our research has produced >110 publications in leading journals and >30 book chapters. I have been a member of the *Infection and Immunity* Editorial Board for the past 12 years, have served on a number on NIH study sections, and have been selected as a Fellow of the American Academy of Microbiology. In June, I will deliver the keynote address to the 5th International Conference on the Genetics and Pathogenesis of the Clostridia, which is the preeminent international meeting on the pathogenic clostridia. With that background, some thoughts/suggestions on the CDC meeting follow:

1. The major news at last week's meeting seemed to be that *Clostridium perfringens* is now also being associated with unusual fatal infections of women after abortion/childbirth. These new cases argue that focusing new research efforts/surveillance efforts strictly on *C. sordellii* or *C. difficile* may miss similar disease caused by other important clostridial species.

a) Hopefully CDC or FDA can provide, ASAP, the clostridial research community with information about the two recent *C. perfringens* cases. My laboratory is particularly interested in learning the type of *C. perfringens* isolates involved in these new infections; we have some unpublished findings that might bear upon this situation.

2) Like Dr. Songer at the Workshop, I would urge the CDC/FDA/NIAID to keep an open mind regarding the possibility of foodborne spread of *C. difficile*, particularly for community-acquired cases. There is ample precedent for food-borne transmission of clostridia to humans, i.e., *C.*

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C2

*perfringens* food poisoning is the third most common foodborne disease in the USA, with >250,000 cases/year.

3) Some panelists urged the development of an antitoxin to treat women suffering from *C. sordellii* infections. This is certainly a worthwhile goal, but Dr. Ballard's data presented at CDC suggests that many *C. sordellii* human isolates may not produce large clostridial cytotoxins. If correct, this means it is not yet clear which specific toxins would need to be neutralized by an antitoxin in order to protect a patient. This fundamental lack of knowledge makes it critical to characterize toxin production by *C. sordellii* clinical isolates; that information can then be used to dissect which *C. sordellii* toxins are actually important for virulence (and therefore must be neutralized by antitoxin). Otherwise, antitoxins produced on a "best guess basis" are, at best, a stopgap measure that could prove ineffective.

4) Further comments on panelist suggestions for a research agenda include:

a) Several panelists commented about the lack of isolates from the recent cases of clostridial infections. The availability of recent clinical isolates is key for research, but a shortage of recent clinical isolates is a major problem hindering the entire clostridial research field. The ATCC offers only a very limited collection of pathogenic clostridial isolates, nearly all of which were deposited more than 30 years ago. Some individual researchers (like myself) have large personal collections of a particular clostridial spp. However, providing such isolates to qualified researchers has become impractical due to biosafety/biodefense shipping issues (involving costly permits, containers, etc.).

i) To address this problem, the clostridial research field badly needs a centralized culture repository, as exists for some other bacteria. Given the biodefense concerns for some clostridia, perhaps the botulism laboratory of the CDC might collect and administer such a clostridial culture collection?

b) The panelist's are certainly correct that we need to know much more about clostridial sporulation, germination, toxin regulation, and virulence. Again, this effort should not be too narrowly focused: our limited knowledge about sporulation, etc. holds true for all pathogenic clostridia, not just *C. difficile* and *C. sordellii*.

c) As emphasized repeatedly by Drs. Gerding, Ballard and Sonenshein at the workshop, studying the subjects listed in 4b requires the ability to perform genetics with the pathogenic clostridia; this is currently difficult (*C. perfringens*) or nearly impossible (other clostridial spp.). The clear consensus of the entire clostridial research field is that developing clostridial genetic systems must be a major, immediate research focus upon which most/all other efforts depend.

i) In response to this pressing need for clostridial genetics, I will be submitting an RO1 application for June 1 that will evaluate (and further develop) promising tools for clostridial genetics. This system, which is independent of the low frequency of recombination in clostridia mentioned by Dr. Sonenshein at the Workshop, has already produced 4 out of 5 attempted toxin mutants in *C.*

*perfringens*. Notably, those mutants were produced in 10-20 fold less time than would be required by conventional, recombination-based techniques. Given these promising results in *C. perfringens*, our RO1 proposal will test whether this revolutionary system can also be used for mutagenesis of *C. difficile*, *C. sordellii*, *C. septicum* and *C. botulinum*. Hopefully, NIH study sections, which normally favor hypothesis-driven work, will recognize the importance, timeliness and urgent need for these genetic tools.

5) It was somewhat surprising that the panelists failed to propose, in addition to studies of the sporulation process, studies of clostridial spores themselves. Clostridial spores have received little study despite being a/the transmissible form (and reservoir for infection) for *C. difficile* and *C. sordellii* infections, as well for clostridial myonecrosis (gas gangrene), *C. perfringens* type A food poisoning, botulism, tetanus and nearly all other clostridial infections. Specific suggestion for study of these spores would include:

i) proteomic identification and analyses of clostridial spore proteins.

ii) characterization of spores of the new *C. diff* isolates: for example, are these spores more resistant to disinfectants than are the spores of traditional *C. difficile* strains? How resistant are these emerging *C. difficile* spores to heating and low temperature storage (this would become important information if *C. difficile* is shown to be transmissible via the foodborne route). We're doing similar studies for *C. perfringens* spores for a USDA grant and have found that spores of food poisoning strains are impressively more resistant to heating, freezing etc. than previous literature reports for spores of "typical" *C. perfringens* isolates.

The issue of spore resistance properties is relevant for prevention, which received little attention at the CDC meeting.

6) From Linc Sonenshein's presentation, there is growing knowledge about the PaLoc element in *C. difficile*; however, it remains unclear whether the large clostridial cytotoxins of *C. sordellii* are associated with a similar genetic element. This information could provide insights into evolution of the new clinical strains and suggest whether their virulence elements are transferable among clostridia. One way to approach this would be a *C. sordellii* genome project, as suggested by Dr. Ballard.

a) In general, there is a need to understand the potential contribution of mobile genetic elements to the pathogenesis of *C. difficile* and *C. sordellii*. We have unpublished data indicating that iota toxin genes of *C. perfringens*, which are closely related to the binary toxin genes of the newly emerging *C. difficile* isolates, are present on a conjugative plasmid- might the emerging *C. difficile* strains have recently acquired the binary toxin genes from *C. perfringens*? If virulence genes are exchanged across clostridial spp., this process might eventually produce a "super strain"(assuming the new *C. difficile* strains do not already fit this description). These are important issues that should be addressed.

7) Knowledge of the microecology of the GI tract and female genital tract is unacceptably

limited. Such information is critical for understanding, for example, why some people are now acquiring *C. difficile* infections in the community, apparently without taking any antibiotics.

a) It would be important to understand which microflora normally suppress *C. difficile* growth and/or colonization in healthy people. Since antibiotics and chemotherapeutics are not involved in the new community-acquired *C. diff* cases, what factors are perturbing the normal GI flora in these seemingly healthy people? Similarly, does *C. sordellii* colonize the reproductive or GI tract in some women? Why? All of this is a mystery now.

8) Given the immense gaps in knowledge evident from the above points, I would urge NIAID (and/or other federal agencies) to consider an RFA on clostridial disease.

Hopefully, NIAID, CDC and FDA will find some of these comments/suggestions of value.

Respectfully submitted by,

  
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