Validity of Botulinum Neurotoxin Serotype H

To The Editor—I write in reference to the publication of the articles and commentaries regarding the purported new botulinum neurotoxin serotype H [1–4]. For the past 30 years, the genetics and physiology of Clostridium botulinum, particularly the properties of serotypes and subtypes of botulinum neurotoxins, have been studied in my tier 1 select agent laboratory. I also have a dual use research of concern issues (DURC) program in place, including a risk-mitigation plan, whereby my laboratory’s work and publications, including those on recombinant botulinum neurotoxins, must be rigorously approved by the University of Wisconsin–Madison Office of Biological Safety, the Centers for Disease Control and Prevention, and the National Institute of Allergy and Infectious Diseases. Although developing a program to satisfy DURC requirements has required much effort, time, and extensive communications, we feel it represents the opinion of the majority of researchers in the field of C. botulinum and its neurotoxins that the presentation and sharing of new information and results of important findings is integral to advances in this important field.

I was dismayed by the publication in the Journal of a purported new serotype of botulinum neurotoxin (“serotype H”), wherein critical data were intentionally omitted because of presumed “DURC issues.” In addition, there are significant scientific concerns in the studies, in that certain strategies and experiments did not yield decisive and definitive findings for defining a new serotype of botulinum neurotoxin. Specifically, in dual toxin–producing strains, it is extremely difficult to distinguish the lethal effects in mice between the high-titer toxin (BoNT/B) and the low-titer toxin (BoNT/H). It is unclear whether the antitoxins used by the authors were validated for efficacy and whether death was due to nonneurotoxin mouse lethal compounds that can be observed in C. botulinum cultures grown in complex medium. In the “serotype H” study, protection in the appropriate mouse lethality assay was observed by combinations of antitoxins A, B, and F, supporting the conclusion that the new serotype is primarily a hybrid between A and F serotypes. These results resemble the mosaic botulinum toxins observed between serotypes C and D, for which antitoxins to C or D alone do not neutralize “by the conventional serotyping method” [5, 6]. It has been discussed that such C/D-hybrid toxins constitute a new serotype, but molecular understanding of the hybrid nature has not provided support for designating a new serotype. Furthermore, the percentage homology of the “new serotype H” is actually higher to A and F than among the serotypes A–G, providing support that “H” is primarily a hybrid.

Most importantly, for definitive designation of a new serotype of botulinum neurotoxin in these advanced times of molecular biology, it is essential to isolate and purify the putative new toxin so that its specific toxicity, catalytic activity, and immune response can be definitively determined. The principles for proposing new serotypes since the discovery of type G >40 years ago have advanced to more-rigorous determinations. Purification and characterization of botulinum neurotoxins is the specialty of my laboratory, and we purify all 7 confirmed serotypes and many subtypes [7]. However, our oral and written requests for the culture or genomic DNA for type H have been denied or gone unanswered by the corresponding author. This “H” strain has been recognized for >3 years. Furthermore, the sequestering of the DNA sequence of the putative novel toxin prevents capable laboratories with large culture collections to perform analyses to determine whether they also have strains that produce this botulinum neurotoxin.

In conclusion, it is impossible to critically evaluate the novelty of a declared new serotype or potentially significant discovery when other laboratories do not have access to the materials and information. It was and still is feasible to develop a DURC program in which these data can be openly shared with colleagues. I sincerely hope that the practices orchestrated by the authors and the Journal do not carry forth to other discoveries in the botulinum field, as this will severely impede advances in this important discipline of science.

Notes

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References


This letter was sent to the authors at the California Department of Public Health. The corresponding author has informed the Journal of Infectious Diseases that the California Department of Public Health elected not to submit a reply.

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