Journal of Bacteriology and Mycology

Open Access

Austin Publishing Group

Special Article - Botulism

Clostridium botulinum Subtype Ba Neurotoxins and Antitoxins: An Immunological Enigma

Giménez DF*

Cátedra de Microbiología, Facultad de Ciencias Médicas, National University of Cuyo, Mendoza, Argentina

***Corresponding author:** Giménez DF, Galera 16, 5 B, 28042 Madrid, Spain, Email: domingobesso@gmail.com

Received: October 12, 2016; **Accepted:** October 14, 2016; **Published:** October 18, 2016

In 1976 a toxigenic strain of *Clostridium botulinum* was isolated from feces of a clinical case of Infant Botulism (IB). This strain, identified as type B 657, was extensively studied at the CDC due to its erratic serological behavior: low titer of this neurotoxin could be neutralized with B antitoxin while higher titers (100LD_{50} and up) were not. Also, it was noted that lesser amount of antitoxin B 657 was required to neutralize the toxin B control than its own toxin [1]. Later on, I showed that this strange serological behavior was due to the presence of a second toxin, type A; hence, I defined this strain as serological subtype Ba [2].

Besides its dual antigenicity, results of neutralization tests run at different concentrations of Ba 657 toxin (from 10 to 20,000LD_{50}) showed an apparent incongruous behavior in the consumption of antitoxins B and A when compared with what we know on toxin-antitoxin reaction of botulinal neurotoxins (BoNTs) A and B [3-5]. Even more important, comparative lower titers of antitoxins B and A (from Ba 657 antitoxin) were required for the neutralization of BoNTs A and B control (Table 1).

These serological results had little or no impact when they were reported. However, i) the increased interest in BoNTs research recorded in the last two decades driven by its application in several scientific fields, ii) the development of genomic, proteomic and molecular biochemistry methods applied to the study of BoNTs, iii) the emphasis of genetical research on the structure of complex genetical strains as the serological subtypes Af, Ba and Baf [6-8], and iv) the production of the genetical variant A4 (fraction A from Ba toxin) as holotoxin in a nontoxigenic *C. botulinum* expression system [9], those serological results summarized in Table 1 attain a particular relevance. In this context, the following comments may be pertinent:

1. Concentrations of toxins B and A of strain Ba 657 were roughly estimated to be 95% B and 5% A. Same concentrations were arbitrarily estimated for their respective antitoxins, by analogy with concentrations of toxins and antitoxins recorded on strain Af 84 [10].

2. If it is generally accepted that the specificity of an antibody is unique to its own antigen, then the *specificity index* of a homologous toxin-antitoxin system correspond to the unity, i.e. is equal to 1. It is necessary to note, however, that in this work the serological tests were performed with crude toxins and polyclonal antitoxins. So, the term *specificity* intends to define the effectiveness of antitoxins in terms of amounts of their consumption in the neutralization tests, rather than the respective specificities *sensu stricto*.

Table 1: Cross-neutralization tests of botulinal neurotoxins Ba 657, A 110, B CN5009 and antitoxins Ba 657, A 110 and B, at 2,000 LD_{50} test level [2].

Strains	Toxins (LD_{50})	Antitoxins		Specificity Index[1]	Results[2]
		Type	Dose (anti-LD_{50})		
Ba 657[3]	2,000	Ba 657	2,000	1	0/12
A 110[3]	2,000	A 110	2,000	1	0/12
B CN5009[3]	2,000	B	2,000	1	0/12
A 110	2,000	Ba 657	(700) 35[4]	57	0/12
B CN5009	2,000	Ba 657	(100) 95[4]	21	0/12
Ba 657[4]	B 1,900	B	130,000	0.0146 (-68)	0/12
	A4 100	A 110	67,000	0.0015 (-670)	0/12

[1]Specificity Index: Ratio of consumption of the homologous antitoxins to the consumption of the heterologous antitoxins.
[2]Mice dying in 72 h/number injected.
[3]Controls of titer and specificity.
[4]Estimated 95% and 5% respectively of neurotoxins and antitoxins B and A of the strain Ba 657.

3. First paradox: Antitoxin A from Ba 657 toxin is 57 times more specific for a heterologous toxin (A110) than for its own toxin (A4). That is, only 35 anti-LD_{50} of antitoxin A (5% of the 700a-LD_{50}) neutralize 2,000LD_{50} of A 110 toxin.

4. Second paradox: Antitoxin B from Ba 657 is 21 times more specific for a heterologous toxin (B CN5009) than for its own toxin (B).

5. Taking into account these results, both toxins of Ba 657 strain, but specially toxin A (A4), appear as powerful antigens. So, they could liberally be called "hyperantigens".

6. A great amount of antitoxin A 110 (67,000 anti-LD_{50}) is required to neutralize 100LD_{50} of toxin A4, another indication of the strange antigenic structures of this toxin. To add more complexity to this behavior, it has been reported that A4 toxin is, by weight, around 1,000-fold less toxic than other A toxins [11].

7. From the serological standpoint, these results would permit to infer:

a) Antigens B and A4 (both from Ba 657 toxin) appear as highly effective for the preparation of anti-AB vaccine.

b) Likewise, their antitoxins for the treatment of A or B botulism.

c) Even considering the comparative lower toxicity of A4 toxin, clinically could be expected a more serious botulism when produced by serological subtype Ba than that produced by serotypes A or B due to their resistance to the current A and B antitoxins treatment.

d) The risk of the use of these toxins as a biological weapon for the reasons stated above.

Citation: Giménez DF. *Clostridium botulinum* Subtype Ba Neurotoxins and Antitoxins: An Immunological Enigma. J Bacteriol Mycol. 2016; 3(4): 1036.

References

1. Hatheway CL, McCroskey M, Lombard GL, Dowell, Jr. VR. Atypical toxin variant of *Clostridium botulinum* type B associated with infant botulism. J Clin Microbiol. 1981; 14: 607-611.

2. Giménez DF. *Clostridium botulinum* subtype Ba. Zbl. Bakt. Hyg. A. 1984; 257: 68-72.

3. Ciccarelli AS, Giménez DF. Estudio serológico de toxinas de *Clostridium botulinum* tipo A y cepa 84. Rev. lat-amer. Microbiol. 1971; 13: 67-74.

4. Shimizu T, Kondo H. Immunological difference between the toxin of a proteolytic strain and that of a non-proteolytic strain of *Clostridium botulinum* type B. Jpn. J Med Sci Biol. 1973; 26: 269-271.

5. Rymkiewicz D, Sawicki J, Bruhl A. Study on the immunological heterogeneity of *Clostridium botulinum* type B toxin. Arch Immunol Ther Exp. 1979; 27: 709-714.

6. Dover N, Barash JR, Hill KK, Davenport KW, Teshima H, Xie G, et al. *Clostridium botulinum* Strain Af84 Contains Three Neurotoxin Gene Clusters: Bont/A2, bont/F4 and bont/F5. PLoS ONE. 2013; 8(11): 10.1371/annotation/b482f80f-c5b6-4b9c-8e9b-8b7139dc37f1.

7. Arndt JW, Jacobson MJ, Abola EE, Forsyth CM, Tepp WH, Marks JD, et al. A structural perspective of the sequence variability within botulinum neurotoxin subtypes A1-A4. J Mol Biol. 2006; 362: 733-742.

8. Pellet S, Tepp WH, Bradshaw M, Kalb SR, Dykes JK, Guangyun L, et al. Purification and characterization of botulinum neurotoxin FA from a genetically modified *Clostridium botulinum* strain. mSphere. 2016; (1): e00100-15.

9. Bradshaw M, Tepp WH, Whitemarsh RCM, Pellet S, Johnson EA. Holotoxin activity of botulinum neurotoxin subtype A4 originating from a nontoxigenic *Clostridium botulinum* expression system. Appl. Environ. Microbiol. 2014; 80: 7415-7422.

10. Giménez DF, Ciccarelli, AS. Studies on strain 84 of *Clostridium botulinum*. Zbl. Bakt. I Abt Orig. 1970; 215: 212-220.

11. Whitemarsh RCM, Tepp WH, Bradshaw M, Lin G, Pier CL, Scherf JM, et al. Characterization of botulinum neurotoxin A subtypes 1 through 5 by investigation of activities in mice, in neuronal cell cultures, and *in vitro*. Infect Immun. 2013; 81: 3894-3902.

J Bacteriol Mycol - Volume 3 Issue 4 - 2016
ISSN : 2471-0172 | www.austinpublishinggroup.com

Citation: Giménez DF. *Clostridium botulinum* Subtype Ba Neurotoxins and Antitoxins: An Immunological Enigma. J Bacteriol Mycol. 2016; 3(4): 1036.