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CL. BOTULINUM TYPE C ISOLATED IN THE USSR

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ANTIGENIC STRUCTURE OF THE TOKINS OF
CL. BOTULINUM TYPE C ISOLATED IN THE USSR

/ Following is the translation of an article by T. I. Bulatova and K. I. Matveyev, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Vol 42, No 8, 1965, pages 79--84. It was submitted on 17 Apr 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr. /

We have studied the antigenic structure of type C botulinum toxins in strains which were isolated in 1956 from minks (Matveyev and coworkers), and also strains which were obtained from France (No 573), the USA (No 91), England (No 365) and Yugoslavia (No 2749 - C_a). In order to establish the presence of antigens which are common for the toxins of types C and D, strain No 359 of type D was taken.

Here it was necessary to clear up whether the strains isolated by us belonged to subtype C_a or C_b, and to which subtype strain No 91 (314) belonged, since it was obtained by the State Control Institute for Medical Biological Preparations in 1931 from the USA without any indication to which subtype it belonged.

The establishing of the subtype of strain No 91 is important because it is an industrial strain -- it is used for the preparation of medicinal and diagnostic antitoxin sera, and also in the preparation of type C toxoid for the immunization of people.

It is known that strain C No 573, obtained from France (isolated from a horse), belongs to subtype C_b (Prevot, 1953; Gunnison, 1953). It may have been thought that the Norka and Biryuli strains No 37 and 47, isolated by us from minks, also belong to subtype C_b, since strains of mainly this subtype are usually isolated from minks. This assumption required confirmation.

Already in 1924 Pfenninger showed that the serum against strain C (Bengtson) neutralized the toxins of C_a and C_b, at the same time that the serum against strain C_b (Seddon) neutralized only the homologous toxin. Thus, only strain C_a may be considered full-value in an antigenic respect. It is known that the toxins of types C and D are not neutralized by the sera of A, B, E, and F, however, small doses of these toxins may be cross neutralized by large doses of the above stated types.

Mason and Robinson (1935) showed that type C toxin contains 3 antigens -- C₁, C₂, and D. The latter is found in C toxin in very small quantities.

They also showed that there is a C component in D toxin.

Antigenic community in the toxins of C and D was confirmed by Prevot and Brigot (1953) and Guillaume et al. (1955), who observed a cross reaction between the serum of C_{β} , and the toxins of C_{α} and D.

We undertook the mission to establish not only the affiliation of our strains of type C to subtypes C_{α} or C_{β} , but to determine, as far as possible, the antigenic structure of the toxins of C_{α} and C_{β} and D, especially of the industrial strains (type C No 91 and type D No 359), in order to clear up if there was the possibility of immunization with strains of type C_{α} against the toxins of C_{β} and vice versa, and to what degree strains of type C may cause an immunity against the toxin of type D.

Strictly type specific antitoxin sera were prepared for the projected tests. We obtained the sera to strain C No 91 by means of immunization of both horses and rabbits. The remaining sera were obtained only on rabbits. One series of antitoxin serum type D was obtained from France (from Prevot). Prior to the immunization the sera of the rabbits and horses did not contain natural antitoxins of types A, B, C, D, and E.

In all the sera we determined the titer of antitoxins by the commonly applied method on white mice. Here we used accordingly the dry standard toxins of type C series No 17 (from strain No 91) and type D (from strain No 359).

The titers obtained for the sera (in AU) were as follows: To strain No 91 -- 500 (horse) and 125 (rabbit), to strain No 573 -- 10, No 37--2, to strain No 359 -- 250 and to the strain obtained from Prevot -- 100.

With the sera and toxins of the various strains we set up the cross neutralization reaction on white mice weighing 14--16 grams. Intravenously the animals received a mixture of 2 Dlm of toxin in a volume of 0.3 ml with 0.2 ml of various (twofold) dilutions of sera.

For purposes of control the mice received the toxin with physiological solution in quantities of 2.1 and 0.5 Dlm. The toxins of type C No 91 and type D No 359 were leached with ammonium sulfate, and the toxins of strains Norka, No 37, 47, 365, 573, 2749 were used original in the form of a sterile filtrate of a 5--6 day culture, diluted 1:1 for storage in glycerin. The glycerin toxins remained stable for a period of 1--1½ months of storage at 4°.

As can be seen from the table, for the neutralization of 2 Dlm of toxins of strains No 91 and No 2749 it required 0.2 AU of type C-91 serum (the results with horse and rabbit sera were the same), for the neutralization of toxins of strains Norka, No 37, 47, 365, and 573 it required 2½ times more (0.5 AU), and for the neutralization of the toxins of strain D-359 -- 50 times more (10 AU) than for strain C No 91. Since strain C No 2749 was obtained by

us as C_a , the conclusion could be made that strain C No 91, the toxin of which was neutralized in the same proportions as the toxin of strain No 2749, was also a subtype of C_p .

For the neutralization of 2 Dlm of the toxins of all the strains of type C, isolated from minks (Biryuli, No 37 and 47, Norka and strain No 365), it required as much of the serum of C-91 as for the neutralization of 2 Dlm of the toxin of strain C_p No 573. Besides this, the sera of strain No 573 neutralized the toxins of strains No 37, 47, 365 and Norka in the same proportions as homologous toxin. From this it was very apparent that strains Biryuli, No 37, 47, Norka and 365, just as strain No 573, belonged to subtype C_a .

Thus, this demonstrated the feasibility of a cross neutralization with sera of C_a and C_p of the toxins of the corresponding subtypes.

We also observed the cross neutralization of the toxin D with sera of C_a and C_p and the toxins of C_a and C_p with the sera of type D.

As a result of setting up numerous neutralization reaction experiments, we determined the amount of AU for each serum which is necessary for the neutralization of 2 Dlm of toxins of various strains, and then we calculated the index of the multiplicity factor, that is, the relative number, which expressed the ratio of the amount of AU, necessary for the neutralization of toxin of a heterologous type, to the amount of AU, necessary for the neutralization of the same amount of toxin of a homologous type. In other words this number showed how many times more serum it was necessary to take for the neutralization of a heterologous toxin than for the neutralization of a homologous toxin. It also made it possible to judge the ratio of various toxic components (C_a , C_p , and D) in the toxins of various strains. For example, for the neutralization of 2 Dlm of toxin D of No 359 it was necessary to have 0.1 AU of serum of D 359, for the neutralization of the same amount of toxin C_a , 10 AU of this serum was necessary, and of toxin C_p -- 1 AU. For the toxin C_a the index of the multiplicity factor during its neutralization by the serum of D-359 equaled 100 (10:0.1), and for the toxins of C_p -- 10 (1:0.1). This index indicated that in the toxin of D No 359 there was 100 times less of component C_a than of component D, and of component C_p -- 10 less than of component D.

It is apparent from the drawing that in the toxin of strain C-91 there is $2\frac{1}{2}$ times less of component C_p than of C_a , and of component D -- 50 times less than of component C_a .

A similar amount of the component C_a was contained in the toxin of strains No 37 and 573, but a various amount of component D. Strain No 37 contained 10 times less of it than of component C_p , and in strain No 573 it was found in the form of traces (undiluted serum of C-573 did not neutralize 2 Dlm of toxin D).

Strain No 359 contained very little of component C_{β} , there was more of it in the strain which we conditionally named Prevot. Component C_{α} was found in still smaller quantities in these strains, especially in strain No 359.

Thus, the toxins of strains C_{α} , C_{β} , and D were made up of 3 toxic components. Apparently the quantitative ratio of these components could change, depending on the strain and also on the conditions under which they were cultivated (medium, pH, various growth factors, etc.).

There is no doubt in the fact that in strains of type C_{α} the α -component prevailed, in strains of type C_{β} -- the β -component, and in strains of type D -- the D component. In each toxin the two other components were found in significantly lesser quantities.

Such a complex mosaic in the antigenic structure of the toxins of the indicated types may also explain the errors which were allowed by Prevot (1953) during the identification of strains No 468, 571, and 573, which were isolated by him from horses and cats. These strains belong to subtype C_{β} (Prevot, 1953; Gunnison, 1953), but since their toxins were neutralized by type D serum, then they were initially regarded by Prevot et al. (1950) to type D.

From the results of our tests it follows that the opinion of Pfenninger (1924) that the serum to strain C_{α} neutralizes the toxins of C_{α} and C_{β} , but the serum of C_{β} neutralized only the homologous toxin, has a weak foundation. The results of our investigations show that the sera of types C_{α} , and C_{β} cross neutralized the toxins of C_{α} , and C_{β} , the homologous toxin neutralized the equivalent amount of serum completely, and for the neutralization of the heterologous toxin approximately $2\frac{1}{2}$ times more of serum was necessary. Pfenninger set up the neutralization reaction on guinea pigs. Apparently his serum to the Seddon strain (C_{β}) was of a low titer, therefore in a volume of 0.5 ml it did not protect the animals from a lethal dose of the toxin C_{α} , though their death set in later (after 40--59 $\frac{1}{2}$ hours following administration of the toxin) than in a control pig (after 24 $\frac{1}{2}$ hours) and in pigs which had received, together with the toxin of C_{α} , the antitoxin sera of types A (death after 24 $\frac{1}{2}$ hours) and B (death after 28 $\frac{1}{2}$ hours). In his tests the serum of C_{α} in a volume of 0.5 ml protected all the animals from a lethal dose of the toxins of C_{α} , and C_{β} . There is no doubt that based on titer this serum was stronger than the serum to the Sedden strain.

The data obtained by us concerning the presence of common antigens in botulinum toxins of strains C_{α} , C_{β} , and D must be taken into consideration during the laboratory diagnosis of botulism and when investigating the soil and other objects of the external medium for the presence of Cl. botulinum.

The presence of just the serum of C_{α} (to strain No 91) in the series of diagnostic antitoxin sera produced in the Soviet Union may lead to a mistaken conclusion.

Among the strains which are neutralized by this serum there may be not only C_a, but also C_β, and D, since for setting up the neutralization reaction they usually take 0.2 ml of undiluted diagnostic antitoxin serum C_a, which contains around 1000 AU in 1 ml. Usually 0.2 ml of this serum contains around 200 AU, which may completely neutralize not only 1--2 Dlm of the toxins C_β, and D, but also a significantly greater amount of lethal doses of these toxins.

For a correct and timely identification of strains of Cl. botulinum of types C and D it is necessary to develop a method for the preparation of diagnostic type specific antitoxic sera of types C_β, and D.

On the basis of our investigations of the antigenic structure of toxins of strains C_a, C_β, and D, we consider that toxoids prepared from toxins of the strain C_a may produce a reliable active immunity, which will protect from the toxin C_a and partially from the toxin C_β, which was demonstrated by us in the immunization of minks with toxoid of strain C-91 (Matveyev et al., 1958).

Toxoids of type D may guarantee a reliable immunity against the toxin D, but to a much lesser degree against the toxin C_β and vice versa.

Boroff and Reilly (1959) showed that pheasants immunized with toxoid of type C endured 1000 and more LD₅₀ of C_β toxin, at the same time that they were resistant to only 20 LD₅₀ of D toxin.

Complex preparations which are recommended in the Soviet Union at the present time for the active immunization of persons (Matveyev et al., 1956, 1960; Bygodchikov et al., 1961--1963), including types C_a, and D toxoids, will apparently also protect against the C_β toxin, however, the problem of the intensity of immunity against the C_β toxin requires experimental verification on animals.

Conclusions

1. Strains of Cl. botulinum type C, which were isolated from minks in the USSR, belong to subtype C_β.
2. The strain of Cl. botulinum type C No 91, which is used in the production of medicinal and diagnostic antitoxin sera, and also toxoids, belongs to subtype C_a.
3. Botulinum toxins of types C_a, C_β, and D consist of 3 toxin components -- C_a, C_β, and D, but in each of them the main component prevails in a quantitative respect. The remaining two are found in considerably lesser quantities. Due to the presence of common antigens in

the toxins, a cross neutralization reaction is observed between the botulinum sera and the toxins of types C_α, C_β, and D.

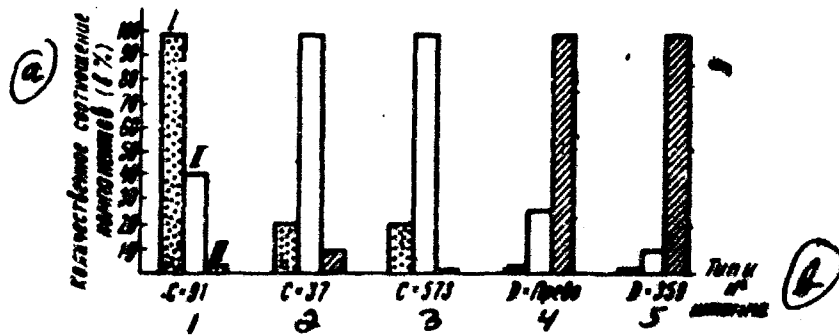
4. For the correct identification of botulinum causative agents, isolated from various objects (soil, patients, corpses, etc.) it is necessary that the series of diagnostic antitoxic antibotulinum type specific sera include sera of types C_β, and D on a level with sera of types A, B, C, and E.

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Cross neutralization reaction of antitoxic antitoxin sera of types C_a , C_p and D with botulinum toxins of the same types.

Type of toxin	Number of strain	Amount of AU, necessary for neutralization of 2 Dlm of toxins by sera of types				
		C_a -91	C_p -37	C_p -573	D-Prevot	D-359
C_a	91 2749	0.2	0.2	0.2	4	10
C_p	Norka 37 47 365 573	0.5	0.04	0.04	0.4	1
D	359	10	0.4	>2	0.1	0.1



Antigenic structure of botulinum toxins C_a , C_p and D on the basis of the results of the neutralization reaction. I - C_a ; II - C_p ; III - D.

a - quantitative ratio of components (in %); b - type and No of strain;

1 - C-91; 2 - C-37; 3 - C-573; 4 - D-Prevot; 5 - D-359.