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J. B. Gunnison

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COMPLEMENT FIXATION REACTIONS OF THE HEAT LABILE AND THE HEAT STABLE ANTIGENS OF *CLOSTRIDIUM TETANI*

J. B. GUNNISON

From the Division of Bacteriology, University of California Medical Center, San Francisco, California

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The antigenic structure of *Clostridium tetani* has been studied intensively by means of agglutination reactions. The most recent report on the subject is that of MacLennan (1). The species is divided into at least ten serologic types based on agglutination of the heat labile "H" or "flagellar" antigens which are type specific (1, 2, 3). All types possess a common heat stable "O" or "somatic" antigen. In addition, types II, IV, V, and IX fall into a group which possesses another heat stable O antigen lacking in the remaining types (1, 3). In this study, preparations containing both H and O antigens of *Cl. tetani* were tested by complement fixation.

METHODS

The strains of *Cl. tetani* studied were the same ones used in a previous investigation of agglutination reactions (3). The organisms were cultivated and then suspended in salt solution as described in that report. The suspensions used as antigens in complement fixation tests were heated at 56 C for one hour and were shown by agglutination tests to possess both H and O factors. A few suspensions were prepared by heating at 100 C so that they no longer gave H agglutination.

The antisera were the same as those employed in the studies on agglutination. Adsorption tests were made as in the previous report.

Complement fixation tests were performed by two methods. In one series, the standard quantitative Kolmer test was used with overnight fixation in the refrigerator and a dose of ten units of bacterial antigen. In another series, the technic employed in a similar study of *Clostridium parabotulinum* was followed using a fixation period of one hour at 37 C. and a dose of two and a half units of antigen (4).

RESULTS

Complement fixation tests were carried out with antisera for each type of *Clostridium tetani*. These antisera were known to contain agglutinating antibodies for both the heat labile H and the heat stable O antigens. The suspensions of *Cl. tetani* used as antigens contained both H and O antigens and were representative of the various types.

Types specific reactions were obtained with types I, III, VII, and VIII. For example, antiserum for type I reacted in a dilution of 1:2,000 with suspensions

of type I strains but only in dilutions of 1:20 to 1:200 with other types. The cross-reactions between the types were more evident than in agglutination tests, but there was no difficulty in differentiating types I, III, VII, and VIII by complement fixation. Hence, both type specific H antigens and the common O antigen seemed to be involved.

Suspensions of types I, III, VII, and VIII heated at 100 C. did not give type specific reactions, but reacted to low titer with all antisera. Thus the type specific antigen was shown to be thermolabile and so altered by heat that it no longer combined with antibody.

The reactions of types II, IV, V, and IX were not type specific and antisera for each of these types reacted strongly with suspensions of other members of this group. For example, antiserum for type II reacted in a dilution of 1:1,000 with types II, IV, V, and IX; but only in dilutions of 1:20 to 1:100 with other types. Both the common O antigen and the additional O antigen characteristic of this group of types seemed to be concerned. The latter antigen seemed to play the predominant part and to mask fixation by the type specific H antigens if it occurred.

Type VI strains are not flagellated and lack the heat labile H antigen. As MacLennan (1) has stated, this is not a valid type but a "dumping ground" for non-flagellated strains. Antiserum for type VI reacted in dilutions of 1:100 to 1:200 with organisms of all types regardless of whether they were heated at 56 C. or 100 C. Only the common O antigen was concerned here.

Cross reactions among the types due to the common somatic O antigen were more marked with the Kolmer technic than with the method using a shorter fixation time and a smaller dose of antigen.

Antisera prepared by injection of either a non-flagellated type VI strain or of a type I strain heated at 100 C. reacted equally with suspensions of all types. This confirms the findings based on agglutination tests that the type specific antigens of *Cl. tetani* are found only in the flagellate strains and are altered by heating at 100 C. so that they do not stimulate antibody formation.

An antiserum for type VIII containing antibodies for both H and O was adsorbed with a suspension of the same type heated at 100 C. After adsorption, this antiserum no longer reacted with organisms of other types nor with a suspension of type VIII heated at 100 C. The same results were obtained by adsorption with cells of either type I or type VI. This showed that all antibodies for the common O antigen had been removed. Complement fixation still occurred in only slightly reduced titer with a suspension of type VIII containing the type specific heat labile antigen. This seems conclusive evidence that the thermolabile flagellar or H antigen of *Cl. tetani* type VIII reacts with its specific antibody in such a way that complement is fixed.

DISCUSSION

It has never been clearly established whether the heat labile flagellar or H antigens of bacteria are significantly involved in complement fixation reactions. According to Felix and Robertson (5), the H antigens of *Cl. tetani* and other

organisms do not take part in complement fixation nor stimulate the formation of complement-fixing antibodies. The evidence for and against the participation of H antigens is discussed fully by Craigie (6) and by Henderson (7). As the latter has pointed out, the conflicting opinions may be due to differences either in the technic used or in the antigenic structure of the organisms studied. In particular, some bacteria possess heat labile antigens, such as the Vi antigen of *Salmonella typhi*, which are not situated in the flagella.

In the case of *Cl. tetani*, it has been demonstrated that heat labile components are confined to the flagella and hence are properly designated as H antigens. It has been shown further that type specificity depends solely upon reactions of the H antigen and antibody. Therefore, the type specific complement fixation given by types I, III, VII, and VIII must be due to fixation of complement by union of H antigen with its specific antibody.

The failure of types II, IV, V, and IX to give type specific complement fixation may be due to the additional somatic antigen found in all these types. Inasmuch as both H and O antigens and antibodies are involved in fixation, the specificity of the reaction must depend upon the relative proportions of each. In the presence of an excess of the non-specific somatic antigens the effect of the specific flagellar antigens may not be observable.

Additional evidence that flagellar antigens take part in complement fixation is afforded by studies of *Cl. parabotulinum*. This species has been divided into a number of serologic groups by agglutination reactions (8). It was later shown that this group specificity depended upon the flagellar or H antigens and that all strains had identical somatic or O antigens (9). Complement fixation tests were specific for the serologic groups, indicating that the flagellar antigens must have been involved (4).

SUMMARY

Both the heat labile flagellar or H antigens and the heat stable somatic or O antigens of *Clostridium tetani* take part in complement fixation reactions. Type specific reactions due to the flagellar antigens are given in high titer by types I, III, VII, and VIII. Type VI strains are non-flagellated and devoid of type specific H antigen. Types II, IV, V, and IX, which possess an additional somatic antigen lacking in the other types, did not show type specific fixation but reacted strongly with each other. Cross-reactions in low titer due to the common somatic antigen occurred between all types.

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