A haemagglutination test for staphylococcal anti-leucocidin

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SYNOPSIS A haemagglutination test using tanned erythrocytes sensitized with the fast and slow moving components of the Panton-Valentine staphylococcal leucocidin is described. From the results obtained it is suggested that this test could be useful in the diagnosis of deep-seated staphylococcal lesions.

The properties of one staphylococcal leucocidin called P.V.-leucocidin after Panton and Valentine (1932) have been investigated by Gladstone and van Heyningen (1957). Woodin (1959) has shown that P.V.-leucocidin consists of two main components, fast and slow, which may be separated by using cation-exchange resins. The two components are distinct antigenically and act together synergistically to produce inhibition of respiration and morphological changes in human or rabbit leucocytes.

Leucocidin or the anti-leucocidal property of a serum has been assayed by two methods. The first used by Gladstone and van Heyningen depends on microscopic changes in living human leucocytes when acted on by P.V.-leucocidin. The second method used by Woodin (1959) depends on the ability of leucocidin to prevent the reduction of phenolindophenol 2-6 dichlorophenol by macrophages.

In an effort to find a test applicable for use in a clinical laboratory the use of a haemagglutination method for detecting anti-leucocidal activity of sera has been investigated and is described below.

METHOD

Purified fast and slow components of P.V.-leucocidin were kindly supplied by Dr. A. M. Woodin. The dry powder was dissolved in distilled water and solutions containing 4L+ units (as defined by Woodin, 1959) were used to sensitize tanned sheep erythrocytes.

Saline used in the preparation of cells was 0.85% sodium chloride in unsterilized distilled water. Buffered saline solutions were prepared from M/15 phosphate and sodium chloride solutions. Tannic acid solution was prepared by diluting a 1 : 100 aqueous solution to 1 : 20,000 with buffered saline pH 7.2. Normal rabbit serum saline consisted of 0.5% serum in saline.

Sheep erythrocytes, which had been stored in Alsever solution for at least three days, were washed three times in saline and resuspended in saline to make a 2% suspension. Equal volumes of this suspension and tannic acid solution were incubated at a 37°C water bath for 10 minutes; the treated cells were washed once with buffered saline, pH 7.2, and resuspended in saline to make a 2% suspension.

These tanned cells were then sensitized by mixing equal volumes of cell suspension and a solution containing 4L+ units of fast or slow components in buffered saline pH 6.4. The mixtures were kept at room temperature for 10 minutes, centrifuged at 2,000 r.p.m. for three minutes, washed once with saline and once with normal rabbit serum saline and finally suspended in normal rabbit serum saline to make a 2% suspension.

The sera to be tested were inactivated at 56°C for 30 minutes. Serial doubling dilutions ranging from 1 in 50 to 1 in 51,200 were made in normal rabbit serum saline and 0.2 ml. volumes of each dilution were added to duplicate sets of tubes (5 × 1 cm.). Serum dilutions less than 1 in 50 were not used since it was found that a zone phenomenon sometimes occurred when high titre sera were tested. Such sera may fail to agglutinate sensitized cells when diluted 1 in 50 or, in some cases, 1 in 100. To one row of tubes fast and to the other slow sensitized cells were added in volumes of 0.02 ml. to each tube. Control tubes were set up, containing sensitized cells plus normal rabbit serum saline, tanned cells plus test serum diluted 1 in 50, and positive and negative serum controls. The tubes were shaken and left to settle at room temperature overnight. Results were read by observing the pattern of the cell deposit on the bottom of the tubes.

The sera of 129 persons were tested by this method. The subjects included 48 without a history of recent staphylococcal infection and 81 with active infection at the time of examination.
A haemagglutination test for staphylococcal anti-leucocidin

TABLE

<table>
<thead>
<tr>
<th>Source of Sera</th>
<th>Anti-fast Or Anti-slow Leucocidin Titres of Various Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Sera Tested</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients without recent history of staphylococcal infection</td>
<td>48</td>
</tr>
<tr>
<td>Patients with staphylococcal infection</td>
<td></td>
</tr>
<tr>
<td>1 Superficial skin infections</td>
<td>41</td>
</tr>
<tr>
<td>2 Boils and breast abscesses</td>
<td>24</td>
</tr>
<tr>
<td>3 Bone and joint disease</td>
<td>16</td>
</tr>
<tr>
<td>All staphylococcal infections</td>
<td>81</td>
</tr>
</tbody>
</table>

RESULTS

Slight activity to fast or slow sensitized cells was obtained in the majority of the sera of patients without a recent history of staphylococcal infection but in only one instance was the haemagglutination titre over 1 in 200. In this study therefore the arbitrary titre of 1 in 200 has been taken as the upper limit of normal and values above this as indicating the presence of staphylococcal infection.

The results of the haemagglutination tests are shown in the Table in which the highest readings obtained with fast or slow sensitized cells are recorded. Positive tests were obtained in a high proportion of patients who had staphylococcal abscesses and especially in those with bone and joint disease. In the latter titres of up to 1 in 25,600 were sometimes observed. Only a third of patients with superficial staphylococcal lesions gave a positive test.

The sera of nine patients with breast abscesses were examined and six gave positive tests. In the remaining three, which were negative, the lesions were subsiding as a result of treatment when the sera were collected.

Most positive sera showed greater haemagglutinating activity with slow than with fast sensitized cells. Half were positive with both fast and slow and in six of the 44 positive sera fast activity alone was in the positive range.

DISCUSSION

Towers and Gladstone (1958) tested 156 sera for anti-P.V.-leucocidial activity using the human leucocyte technique and a crude P.V.-leucocidin preparation. It was found that 82% of 83 cases of staphylococcal osteomyelitis gave a positive test. The value of a test such as this has been questioned by Woodin (1961) on the grounds that the test would be influenced not only by the ratio of antibodies to fast and slow components but also by the composition of the test toxin used. While this is a valid criticism it is of interest that the results obtained in cases of acute osteomyelitis by the haemagglutination test using purified fast and slow components are similar to those obtained by Towers and Gladstone.

The disadvantage of the haemagglutination test is that it requires a relatively large amount of pure fast and slow components for the sensitization of the tanned cells and these substances are not easy to prepare. This objection is now rather less cogent since a modified method for their preparation has been worked out by Woodin (1961).

During the course of this work it was found that tanned cells could be sensitized simultaneously with fast and slow components if equal amounts of each component were used in a mixed solution for tanned cell sensitization. When cells sensitized in this way were used the haemagglutination titre of a serum was the same as the highest titre observed using fast or slow sensitized cells separately.

In spite of some difficulties inherent in the estimation of anti-P.V.-leucocidal activity of sera it seems possible that the haemagglutination test may be useful in the diagnosis of deep-seated staphylococcal lesions and especially in bone and joint disease.

I am most grateful to Professor R. E. O. Williams in whose former department, at the Central Public Health Laboratories, Colindale, the initial part of this work was done. At that time I was in receipt of a travel grant from the Wellcome Trust which I gratefully acknowledge. I am also very grateful to Dr. A. M. Woodin for making available to me the purified P.V.-leucocidin components and to Dr. G. P. Gladstone for his interest and advice. I am indebted to Dr. A. G. Towers who supplied me with some of the samples of sera tested.
ADDENDUM

Since the preparation of this paper was completed an article by A. G. Towers (J. clin. Path. 1961, 14, 161) has reached New Zealand. A haemagglutination test for anti-P.V.-leucocidin in serum is described in which the cells were sensitized with crude P.V.-leucocidin. The results obtained show that 71% of sera from patients with staphylococcal osteomyelitis gave high haemagglutination titres when tested with cells sensitized by this method. It is notable, however, that sera from patients without staphylococcal infection also had very considerable activity to factors in the crude P.V.-leucocidin.

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