THE CLINICAL EVALUATION OF STAPHYLOCOCCAL ANTI-ALPHA HAEMOLYSIN TITRES IN PATIENTS' SERA

BY

HERTA SCHWABACHER AND A. J. SALSBURY
From Group 9 Laboratory, Watford

(RECEIVED FOR PUBLICATION APRIL 24, 1958)

The relationship between the production of α haemolysin and the pathogenicity of Staphylococcus aureus has long been a matter for discussion. Christie, North, and Parkin (1946) stated that the production of α haemolysin is essential for full pathogenicity, and that coagulase-positive strains which produced no α haemolysin might be regarded as non-pathogenic. Marks (1952) found a close correlation between pathogenicity and the production of α haemolysin. On the other hand, Elek and Levy (1950) and Lack and Wailling (1954) found that α haemolysin was not an absolute criterion of pathogenicity. It was suggested by Lack and Wailling that the discrepancy of results might partly be explained by a difference in sources, and on occasions by an over-growth of non-toxigenic strains in necrotic foci. Rogers (1956) has performed experiments which suggest that certain macro-anions, such as chondroitin sulphate, hyaluronic acid, heparin, and the nucleic acids, may, in vivo, inhibit the formation of toxins by the organism. The five cases reviewed in this paper were all of infections by a virulent strain of Staphylococcus aureus, phage type 80. An attempt will be made to show that in such cases the staphylococcal anti-α haemolysin titre of the patient's serum is a valuable guide to progress and an aid to the detection of latent infection by Staphylococcus aureus.

Methods

All the staphylococci isolated from the five cases mentioned were examined for the "clumping factor" by the slide method (Cadness-Graves, Williams, Harper, and Miles, 1943). Hyaluronidase production was determined by the M.C.P. test (McCLean, Rogers, and Williams, 1943). Dr. R. E. O. Williams, of the Central Public Health Laboratory, Colindale, phage-typed the organisms.

The strains were tested for α haemolysin by the method of Gillespie, Devenish, and Cowan (1939). The α haemolysin titres of the patients' sera were estimated using the technique described by Lack and Wailling (1954) as follows:

Serial dilutions of the patient's serum, previously inactivated at 56° C. for 30 minutes, were made in 0.5 ml. volumes in saline (to a dilution of 1 in 32). To each tube was added toxin (S.670 Wellcome Research Laboratories), equivalent to 1 international unit of antitoxin, in 0.5 ml. saline. The tubes were shaken, and allowed to stand at room temperature for 30 minutes, after which 0.1 ml. of a 10% suspension of thrice-washed rabbit cells in saline was added to each tube, and to toxin and serum control tubes. The tubes were incubated at 37° C. for one hour, after which the titre of anti-α haemolysin was read. The titre was taken as the tube exhibiting 50% haemolysis.

Anti-leucocidin titres were kindly obtained for us by Dr. G. P. Gladstone, of Oxford, using the technique described by Gladstone and Heyningen (1957). This is based on the fact that staphyloccocal leucocidin produces characteristic changes in a coverglass preparation of living human polymorphonuclear leucocytes.

The L+ dose of leucocidin is first assayed by making up mixtures of a standard antileucocidin, and logarithmically falling dilutions of leucocidin. The mixtures are placed on slides, and a coverglass preparation of living leucocytes inverted on top of each. After sealing with paraffin wax, and incubating at 35° C. for 30 minutes, the preparations are examined on a warm stage with light phase contrast.

The standardized leucocidin is then taken, diluted to a 0.2 L+ dose, and put up against logarithmically falling dilutions (10% steps) of the patient's serum, starting at a dilution of 1/10. The procedure is then similar to that used in assaying the L+ dose.

In addition, 15 cases of superficial infection by staphylococci producing α haemolysin were followed for periods up to 18 months to see whether any rise in the patients' anti-α haemolysin titre was produced.

Results

All the staphylococci from the five cases mentioned belonged to phage type 80, and produced a positive clumping test. They all produced...
hyaluronidase, although it has been shown that virulent strains may not produce hyaluronidase (Schwabacher, Cunliffe, Williams, and Harper, 1945). Lack (1956) has suggested that hyaluronidase may not act as a "spreading factor" but, by depolymerizing hyaluronic acid in connective tissue, it is removing a potential inhibitor.

All the strains produced α haemolysin. This provoked a rise in anti-α haemolysin titre in four of the cases, but in Case 2 there was no antibody response.

Case 2, a woman aged 68, had chronic osteomyelitis of the right femur from August, 1955, which was still persisting in March, 1958. Although the infecting organism produced α haemolysin, the anti-α haemolysin titre had always been less than 0.5 u./ml. The anti-leucocidin titre was 10 u./ml. This may be regarded as slightly raised, as Dr. Gladstone (personal communication) states that an anti-leucocidin titre of 10 u./ml. or over can be taken as abnormal. However, the anti-leucocidin titre was raised to a much greater extent in the other cases.

In the other four cases, the anti-α haemolysin titre gave a good indication of clinical progress, rising when the infection was most marked and falling on clinical improvement. Fig. 1 shows the variation in anti-α haemolysin titre in Case 1. The maximum anti-α haemolysin titre in this case was 6 u./ml. (An anti-α haemolysin titre of 2 u./ml. or over was regarded as being raised.) The raised anti-α haemolysin titre in Case 1 was of particular value, as the patient presented with several cystic swellings on the scalp, which showed as punched-out defects on radiographs, and a destructive lesion of the left hip. A provisional diagnosis was made of myelomatosis, and it was not until pus was aspirated from the cysts, coupled with the raised anti-α haemolysin titres, that the true diagnosis of pyaemia was made. During an active stage of the patient's disease, when the anti-α haemolysin titre was 4 u./ml., the anti-leucocidin titre was 64 u./ml.

Fig. 2 shows the anti-α haemolysin titre variations in Case 3. This patient had a diffuse, hard swelling of the left thigh, with enlarged inguinal glands. A diagnosis was made of sarcoma, but the anti-α haemolysin titre was 32 u./ml., and the anti-leucocidin titre 100 u./ml. The thigh was therefore incised, and 20 oz. of pus removed. After clinical improvement, the anti-α haemolysin titre had fallen to less than 2 u./ml. and the anti-leucocidin titre to 60 u./ml.

It will be noticed from both graphs that there is a time lag between the appearance of active infection and the elevation of the anti-α haemolysin titre. There is also a delay after clinical improvement before the anti-α haemolysin titre falls to normal, and this may vary between a few weeks and several months.

As can be seen from Figs. 1 and 2, the rise in the total leucocyte count precedes the rise in anti-α haemolysin titre, and the leucocytes fall before the titre. The white cell count may be regarded as a more sensitive index to the momentary condition of the patient. On the other hand, the continued elevation of the anti-α haemolysin titre may indicate a persistence of the infection which is not
revealed by the white cell count. For instance, it will be seen in Fig. 2 that the leucocyte count was falling towards normal while active infection was still present.

The anti-leucocidin titre bears a close relation to the anti-\( \alpha \) haemolysin titre. Fig. 3 shows the anti-leucocidin readings in the four cases, and classifies them according to anti-\( \alpha \) haemolysin titres taken at the same time. Case 2 was omitted, as the anti-\( \alpha \) haemolysin titre was never raised, and the anti-leucocidin titre only just above normal (Dr. Gladstone has found anti-leucocidin titres of up to 6 u./ml in normal sera). It appears that an anti-\( \alpha \) haemolysin titre of 2 will yield an anti-leucocidin titre of 60 to 70; a titre of 4, an anti-leucocidin titre of 60 to 100; a titre of 16, one of 80 to 100; and a titre of 32, an anti-leucocidin titre of 80 to 125.

With clinical improvement, the anti-\( \alpha \) haemolysin titre may fall to less than 2 u./ml., but the anti-leucocidin titre takes much longer to fall to normal. In Case 5, however, both titres have remained elevated, despite complete clinical improvement. This patient, after having fractured the left femur, developed a large gluteal abscess, staphylococcal pneumonia, and septicaemia.

The anti-\( \alpha \) haemolysin titre was 32 u./ml. and anti-leucocidin titre 125 u./ml. After a three-month course of erythromycin, the patient was perfectly well, but the anti-\( \alpha \) haemolysin titre had fallen only to 16 u./ml. and the anti-leucocidin titre to 37.5 u./ml. Six months later, the anti-\( \alpha \) haemolysin titre was <2 u./ml. and anti-leucocidin 60 u./ml.

The amplitude of anti-leucocidin titre variation is greater than that of anti-\( \alpha \) haemolysin, and produces a more dramatic picture. For example, during the convalescence of Case 5, the anti-\( \alpha \) haemolysin titre fell from 32 to 16 u./ml whereas the anti-leucocidin titre fell from 125 to 37.5 u./ml. In Case 4, a diabetic woman who died of staphylococcal enteritis, the anti-\( \alpha \) haemolysin titre rose only to 4 u./ml., but the anti-leucocidin titre rose to 60 u./ml.

A raised anti-\( \alpha \) haemolysin titre has only been obtained when the staphylococci have been situated in muscle, bone, or blood stream. None of the 15 cases with superficial infections by \( \alpha \)-haemolysin-producing organisms ever showed a rise in anti-\( \alpha \) haemolysin titre in the serum.

**Discussion**

To-day, when the community is becoming infected with increasing numbers of penicillin-resistant staphylococci, the sooner a diagnosis of staphylococcal infection is made, the better. This is particularly the case with Staphylococcus aureus, phage type 80.
It is in the deep-seated infections—in muscle, bone, or blood stream—where isolation of the organism may be impossible or delayed until surgical procedures are taken, that a diagnostic test for active staphylococcal multiplication is of its greatest value. Case 1 was provisionally given the diagnosis of myelomatosiis, and the raised anti-\( \alpha \) haemolysin titres in Case 3 made it possible to decide on a diagnosis of infection as opposed to one of sarcoma. In latent staphylococcal infections, it must be remembered that the white cell count may be within normal limits. A raised anti-\( \alpha \) haemolysin titre, even in the absence of signs of infection, is indicative of the continued presence of the pathogen.

The fact that \( \alpha \)-haemolysin antibodies may not be detected in a patient's serum, despite a deep-seated staphylococcal focus, must also be considered. When present at a titre of 2 u./ml and higher, they are of significance. Leucocidin antibodies are more readily stimulated, and, as exemplified in Case 2, may suggest staphylococcal infection in the absence of a raised anti-\( \alpha \) haemolysin titre.

The value of the anti-\( \alpha \) haemolysin and anti-leucocidin titres as a diagnostic procedure has been reported by Towers and Gladstone (1958).

The importance of avoiding all delay in the eradication of resistant \textit{Staphylococcus aureus}, phage type 80, from the host is seen by the protracted nature of the illnesses in the cases reviewed. The average duration of infection was two years. Any diagnostic test which can speed the onset of effective treatment for the elimination of the staphylococcus is of value, and the estimation of anti-\( \alpha \) haemolysin titres has a definite place in the control of staphylococcal infections.

**Summary**

Five cases of infection with \textit{Staphylococcus aureus}, phage type 80, have been recorded.

It has been shown that the anti-\( \alpha \) haemolysin titre in four of these cases was a good index to the condition of the patient, though less sensitive to the momentary changes of the patient's condition than the total white cell count.

The anti-\( \alpha \) haemolysin titre corresponds well with the anti-leucocidin titre.

It is suggested that the anti-\( \alpha \) haemolysin titre is of its greatest value in deep-seated infections, when diagnosis may be difficult, or treatment delayed until surgical procedures reveal the causative organism.

We would like to record our thanks to Mr. K. I. Nissen, Mr. Staveley Gough, and Dr. J. S. Richardson for their kind permission to use their cases as a basis for this paper.

**REFERENCES**


