ACTION OF STAPHYLOCOCCAL LEUKOCIDINS ON HUMAN GRANULOCYTES

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The action of staphylococcal α- and δ-hemolysins and of P. V. leukocidin on human granulocytes was studied. To detect structural changes in the cells, blood films were examined under the phase-contrast microscope. P. V. leukocidin produced structural changes in the cell nuclei, making them look like "bags of granules." The action of δ-toxin was directed simultaneously to both nucleus and membrane and produced rapid and complete lysis of both. The α-toxin caused no marked destructive changes in the blood cells. Phase-contrast microscopy can be used as an additional method for differentiation of staphylococcal leukocidins. The time at which morphological changes developed in the granulocytes depended on the strength of the preparations and varied from 1-2 min to 1.5-2 h.

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One of the principal factors reflecting pathogenicity of the staphylococci is their ability to form toxins. The presence of various staphylococcal toxins has been established, including α-, β-, γ-, and δ-hemolysins, P. V. leukocidin, enterotoxin, and so on.

The object of the present investigation was to study P. V. leukocidin in relation to the hypothesis of the possible essential role of leukocidins in the pathogenesis of staphylococcal infections. The existence of at least three leukocidins has now been demonstrated: 1) P. V. leukocidin [3], known as "true" hemolysin, because it is not identical to any other known staphylococcal hemolysin; 2) N. W. leukocidin [2], identical with α-hemolysin; and 3) G. H. leukocidin [1], identical with δ-hemolysin.

The use of insufficiently standardized and unified methods of determination of leukocidin activity makes the identification and differentiation of leukocidins difficult. In methods used previously, determination of leukocidin activity was based on quantitative changes in the relative proportions of living and dead leukocytes under the influence of staphylococcal toxins (the methods of Loganovskii, Valentine, and of Shveitsar and Vanchurzhik). In 1957 Gladstone and van Heyningen, examining blood films (of leukocytes) under the phase-contrast microscope, discovered a leukocidin which differed from the other two known leukocidins P. V. and N. W. in the types of morphological changes produced in the structure of the leukocytes under the action of staphylococcal toxins.

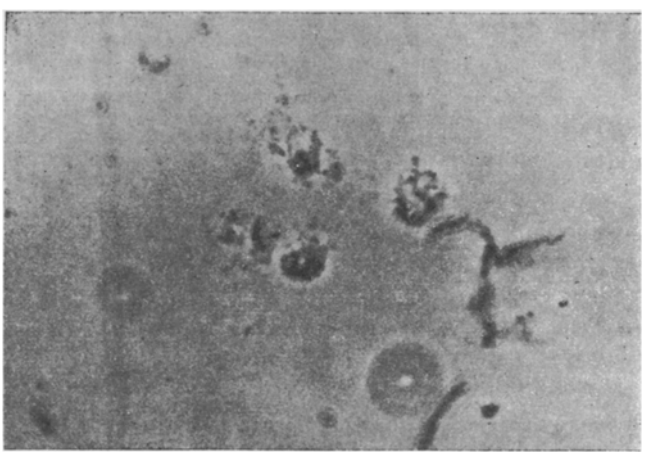

Fig. 1. Action of P. V. leukocidin. Formation of "bags with granules."

The object of this investigation was to achieve complete differentiation of staphylococcal toxins (α- and δ-hemolysins and P. V. leukocidin) by the study of their action on human granulocytes.

EXPERIMENTAL METHOD

The method of Gladstone and van Heyningen mentioned above was used to study the action of

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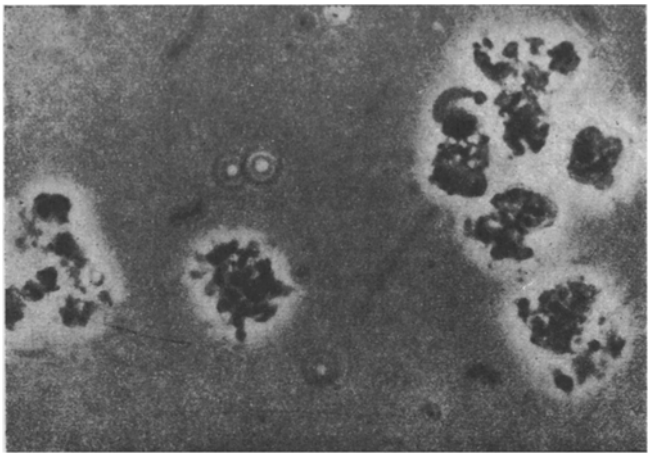

Fig. 2. Action of δ-toxin. Deformation of neutrophils.

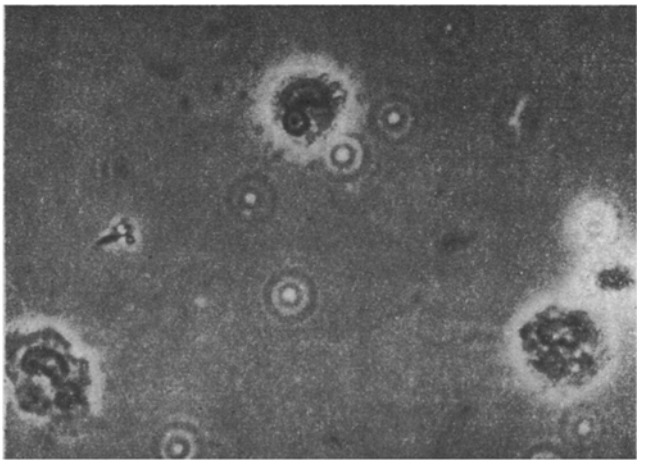

Fig. 3. Action of α-toxin.

staphylococcal leukocidins on blood cells (granulocytes). A drop of blood taken by a sterile needle from the finger of a healthy person was placed on a defatted cover slip; blood films thus made were incubated for 10-15 min at 37°. Blood clots (erythrocytes) formed on the cover slips were washed off with 0.5% gelatin solution (pH 6.8), the procedure being done very carefully so as not to injure the leukocytes adherent to the glass, to which the test toxin was subsequently added. A cover slip was placed over the films obtained in this manner and they were ringed with petroleum jelly and examined under the microscope.

The following preparations of staphylococal toxins were studied: 1) P. V. leukocidin prepared from leukocidin-producing strain V-8. Concentration and purification were carried out by Woodin's method [4]. The leukocidin activity determined by the method of Shveitsar and Vanchurzhik was 1:80-1:160; 2) δ-toxin, prepared from producer strain No. 88 at the Gamaleya Institute of Epidemiology and Microbiology. It was used in a concentrated and purified form. The hemolytic activity was 1:80-1:160; 3) a native preparation of staphylococcal α-toxin obtained from strain 0-15, producing the α-toxin. The hemolytic activity was 1:160-1:320.

EXPERIMENTAL RESULTS

Morphological changes detected in the human blood cells (granulocytes) as a result of the action of the various staphylococcal toxins differed in character.

Under the influence of P. V. leukocidin, the cell nuclei were principally affected. The nuclei of the neutrophils 5-10 min after addition of the preparation had lost their well marked lobular appearance and were breaking up into separate lobules. The time from the beginning of exposure to appearance of the morphological changes depending on the strength of the preparation used had varied from 20 min to 1.5 h. During this time the nuclei almost completely disappeared although the granules remained, so that the cells resembled "bags of granules" (Fig. 1).

The character of structural changes in the cells produced by the action of staphylococcal δ-toxin was rather different. A well marked deformation of most neutrophils began 1-2 min after its application, the clearly defined outlines of nuclei and membranes disappearing simultaneously (Fig. 2). In the course of 10-15 min these cells underwent lysis and only separate granules could be seen under the microscope. The solitary intact cells which remained also underwent destruction and lysis during the next 10-15 min. The time from the beginning of exposure to the appearance of morphological changes depended, as when P. V. leukocidin was used, on the strength of the toxin applied, but in this case it was shorter – from 1 to 40 min.

Test of the α-toxin preparation revealed no destructive changes in the granulocytes during the first 50-60 min. The cells simply lost their mobility (Fig. 3). Changes in the morphology of the neutrophils discovered after 1.5-2 h varied in character and were evidently due to the presence of other toxic components in the preparation and, in addition, to natural death of the cells.

By means of phase-contrast microscopy it is thus possible to demonstrate the specific action of staphylococcal leukocidins (P. V., α-, and δ-toxins) on leukocytes. The varied character of the changes produced

by the action of these toxins on human leukocytes confirms that different leukocidins are in fact produced by pathogenic staphylococci. To determine the activity of these leukocidins precisely, their strength must be measured by titration.

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