ACTION OF STAPHYLOCOCCAL TOXINS ON BLOOD AND TISSUE CULTURE CELLS

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The principal role in the pathogenesis of staphylococcal infections is played by staphylococcal toxin. The injuries which it causes are considered to be mainly due to the presence of an α-hemolysin in this toxin. Subsequently other components of staphylococcal toxin were found, possessing experimental activity on erythrocytes and leukocytes of various animals and also on the cells of various tissues and organs, including kidney and liver epithelium.

Johanowsky [2], and Swejcar and Vancuric [4] have recently shown that the severity of staphylococcal lesions in man is correlated with the production of Panton–Valentine leucocidin by the microorganisms, a substance with a lethal action on human leukocytes. The study of this toxin is extremely urgent, but several technical difficulties are encountered. Titration of leucocidin by the bioscopic method does not allow differentiation between its effect and that of α-hemolysin. The use of the microscopic method, on the other hand, means that freshly obtained human blood must be constantly available.

The object of the present investigation was to study the possibility of replacing human leukocytes during titration of leucocidin by a culture of human epithelian HeLa tissue which is available in most laboratories. For this purpose a parallel study of the action of staphylococcal toxins on HeLa tissue cultures and on various blood cells was necessary. The blood cells used included rabbit's and sheep's erythrocytes and also human and rabbit's leukocytes.

At the same time, these various cells were used as indicators for determination of the components of staphylococcal toxins.

EXPERIMENTAL METHOD

Staphylococcal toxin was obtained by cultivating a toxigenic strain on 0.3% meat-peptone agar at 37° for 4-5 days in an atmosphere containing 30% carbon dioxide, followed by filtration through a candle. The titer of α- and β-hemolysins was determined by standard methods [3]. The presence of Neisser–Wechsberg and Panton–Valentine leucocidins was determined by treating rabbit's and human leukocytes with various dilutions of the filtrate at 37° for 1 h. For each drop of these mixtures applied to a slide, one drop of 1% Congo red was added, followed after 3 min by 1 drop of 0.2% methylene blue. A cover slip was applied, and under the high power of the microscope the number of dead and living leukocytes was counted. The former stained redish-brown, the latter blue.

Staphylococcal toxin was titrated in suspensions of HeLa tissue culture cells by a microscopic method similar to that suggested for titration of leucocidin. Salk's color test [4], based on the ability of living cells to change the pH of a medium to the acid side, was used as a parallel method. In the presence of doses of toxin causing death of the cells no changes took place in the pH of the medium or in the color of the indicator.

EXPERIMENTAL RESULTS

The results of parallel titration experiments using several batches of toxins revealed no definite relationship between the content of α- and β-hemolysins and of leucocidins. The α-hemolysin titers were always higher than the leucocidin titers and the ratio between them varied from case to case. The β-hemolysin titers were sometimes higher than the leucocidin titer, but in some cases they were equal or

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even lower. Comparison of the leucocidin titers with the intensity of the cytopathogenic action of staphylococcal toxin on the HeLa tissue culture showed extremely interesting relationships. In the 17 experiments in which leucocidin was titrated on rabbit's leukocytes, agreement between the titers of this toxin and the titers of cytopathogenic effect on HeLa cells was observed 13 times, and disagreement 4 times. When the action of staphylococcal toxin on human leukocytes and HeLa cells was compared, in all the experiments complete agreement was found between the leucocidin titers and the cytopathogenic effect. It follows that the sensitivity of HeLa cells and human leukocytes to Panton–Valentine "human" leucocidin is comparable, and that staphylococcal human leucocidin can be titrated on a culture of HeLa cells. It is more convenient to use the fast and simple technique of microscopic titration for this purpose; on the other hand, in investigations demanding greater accuracy it is preferable to use the color test, the results of which are read on the 4th day of the experiment. Comparison of these two methods of titration of staphylococcal toxin showed that deviations of the leucocidin titers determined by the microscopic method from those determined by the color test are insignificant. Of 25 parallel experiments, only in 4 did the titers differ by one dilution.

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