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Effects of Panton-Valentine Leukocidin of *Staphylococcus aureus* on Leukocytes from Patients with Leukemia

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Mature granulocytes from six patients with myelocytic leukemia and monocytic cells from two patients with monocytic leukemia were damaged after incubation with leukocidin from *Staphylococcus aureus*. The immature granulocytes were not significantly affected. The lymphocytic cells from one patient with lymphocytic leukemia were not markedly damaged. The numbers of erythrocytes from all nine patients remained practically unchanged with lower concentrations of leukocidin.

Panton-Valentine leukocidin (PVL) of *Staphylococcus aureus* damages rather specifically granulocytes from humans (1, 3, 4). The primary site of leukocidic action appears to be the cell membrane (4–6). Within 30 min at 37 C, the damaged cells lose their mobility and become round. Most of their granules disappear (1, 3). We observed similar initial changes on granulocytes and monocytes from leukemic patients after incubation with partially purified leukocidin (Fig. 1.).

MATERIALS AND METHODS

Partially purified PVL was obtained from *S. aureus* strain V8 by selective adsorptions of alpha hemolysin and some other staphylococcal substances to aluminum oxide cholesterol by chromatography on diethylaminoethyl Sephadex and treatment with aluminum oxide (2). The final product had a PVL activity of 5,700 MLD (3) per mg of protein. It was free from alpha hemolysin, coagulase, egg yolk opacity factor, fibrinolysin, and nuclease.

Leukocytes of nine healthy persons were isolated from native blood on microscope slides (3), and those of patients with leukemia were isolated from citrated blood samples by sedimentation in siliconized glass tubes (internal diameter of 3 mm). The latter method of isolation was necessary, since the "leukemic" leukocytes did not regularly attach themselves to the surface of the microscope slides. The leukocytes obtained by sedimentation were washed three times in Hanks balanced salt solution and finally suspended in a tissue culture medium ("TC 199," Difco). The suspension was adjusted to about 1,000 leukocytes per mm^3. Leukocidic effects were determined by phase-contrast microscopy (3) after incubation with PVL at 37 C for 1 hr. In addition, 0.2 ml of citrated whole blood from each patient was incubated at 37 C for 1 hr with, respectively, 0.2 ml of PVL in twofold dilutions containing from 512 to 1 MLD. The diluent was Hanks solution. Leukocidic effects were evaluated by differential counts of the stained (Pappenheim) blood cells.

RESULTS

Blood cells from nine leukemic patients and nine healthy persons were subjected to various concentrations of PVL. Of the nine patients, four suffered from chronic myelocytic, two from acute myelocytic, two from monocytic, and one from lymphocytic leukemia. The four patients with chronic myelocytic leukemia had been treated previously with 1,4-bis-methanesulfonoxybutane (Myleran, Burroughs Wellcome Laboratories, London). In all six cases of myelocytic leukemia, the mature granulocytes disappeared after incubation with 4 to 512 MLD of PVL. The numbers of immature granulocytes decreased only slightly (Fig. 2, 3). The susceptibility of the leukemic granulocytes to PVL was similar or higher than that of the granulocytes from healthy persons. The numbers of erythrocytes, which varied from 0.96 to 3.06 million with the leukemic patients, were not markedly changed with 1 to 16 MLD of PVL but were reduced by 15% with 32 to 512 MLD of PVL. In the two cases of monocytic leukemia, the monocytic cells disappeared after incubation with 32 to 128 MLD of PVL (Fig. 4). The lymphocytic cells from the patient with lymphocytic leukemia, those from the other leukemic patients, and those from healthy persons were not markedly damaged by PVL.

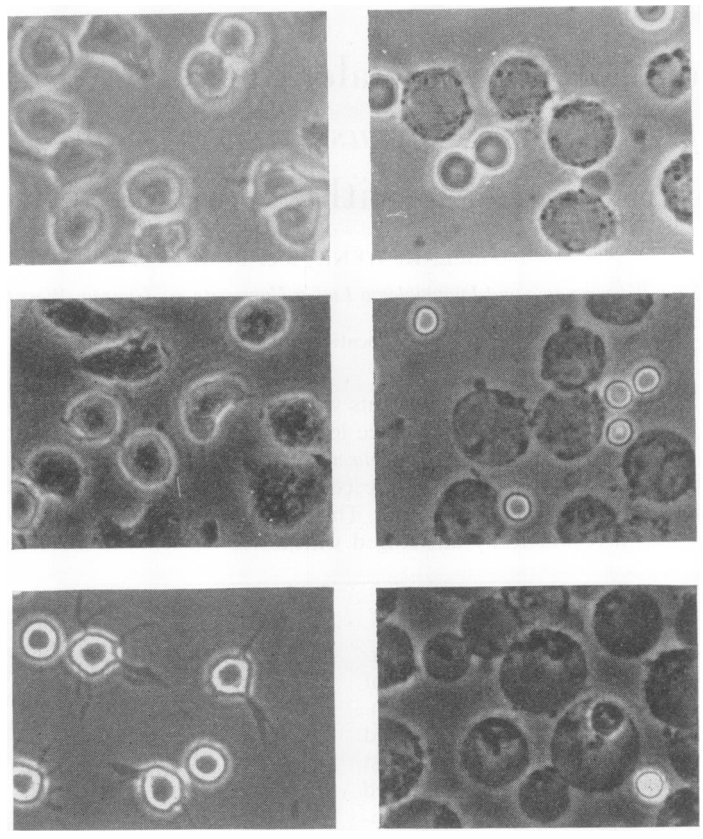

FIG. 1. *Cellular changes observed by phase-contrast microscopy after incubation with leukocidin of Staphylococcus aureus. On the left are cells before incubation, and on the right are cells after incubation. Granulocytes from healthy persons are shown in the top row, and those from patients with myelocytic leukemia are shown in the middle row. Monocytic cells from patients with monocytic leukemia are shown in the bottom row.* ×400.

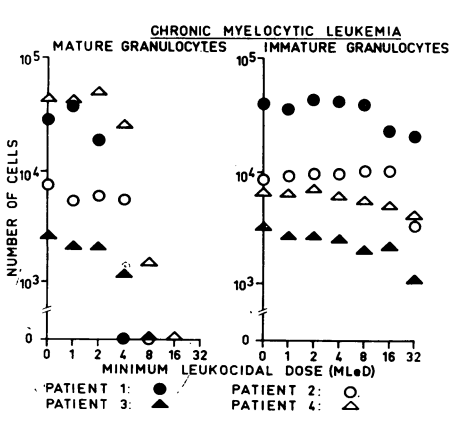

FIG. 2. *Effects of leukocidin on granulocytes from patients with chronic myelocytic leukemia.*

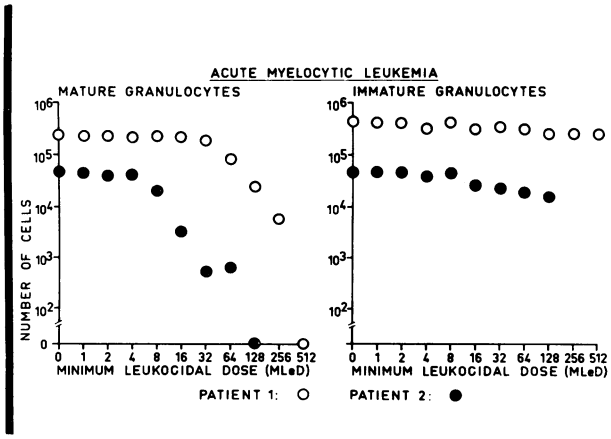

FIG. 3. *Effects of leukocidin on granulocytes from patients with acute myelocytic leukemia.*

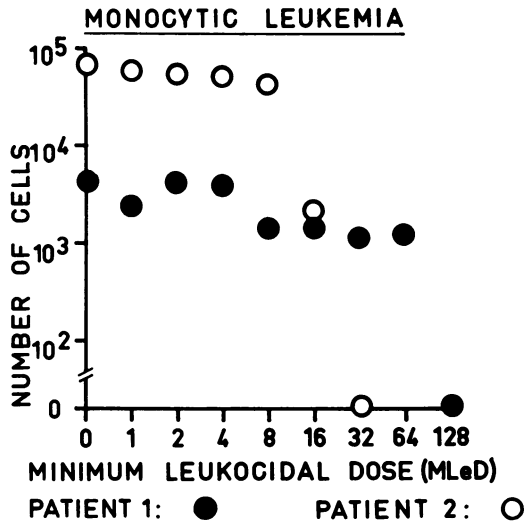

FIG. 4. *Effects of leukocidin on monocytes from two patients with monocytic leukemia.*

DISCUSSION

The rather selective effects of PVL on the more mature granulocytes were of particular interest. If the primary site of leukocidic action is the cell membrane (4–6), the observation that the membranes of mature cells were more susceptible to PVL than those of immature cells cannot be readily explained. Perhaps a co-factor for the PVL was involved, which was fully developed or accessible only in the more mature cells. At any rate, further investigations on the mechanism of PVL activity at the cellular level appear to be indicated.

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LITERATURE CITED

1. Gladstone, G. P., and W. E. Van Heyningen. 1957. Staphylococcal leucocidins. Brit. J. Exp. Pathol. 38:123–137.
2. Kanoe, M., H. Blobel, and W. Schaeg. 1969. Selektive Anreicherung von Staphylokokken-Panton-Valentine-Leukozidin mit Aluminiumoxid—Cholesterin. Z. Naturforsch. 24b:1431–1435.
3. Wenk, K., and H. Blobel. 1970. Untersuchungen an "Leukozidinen" von Staphylokokken verschiedener Herkunft. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Abt. I 213:479–487.
4. Woodin, A. M. 1965. Staphylococcal leucocidin. Ann. N.Y. Acad. Sci. 128:152–164.
5. Woodin, A. M., and A. A. WIENEKE. 1964. The cytotoxic effect of leucocidin. Ciba Found. Symp. Cell. Inj., 1963, p. 30–52.
6. Woodin, A. M., and A. A. Wieneke. 1966. The interaction of leucocidin with the cell membrane of the polymorphonuclear leucocyte. Biochem. J. 99:479–492.