CHARACTERISTICS OF HUMAN NEUTROPHILS OBTAINED BY THE "SKIN WINDOW" CHAMBER METHOD

A. N. Mayanskii and M. E. Viksman

UDC 612.112.91-085.23

The viability, morphological composition, and functional state of human cells migrating into a "skin window" chamber were studied. After 18-20 h the chamber contained $(42.0 \pm 5.3) \cdot 10^6$ viable cells/cm^2 with a high proportion of mature neutrophils (98.6 ± 0.6%). Normal reactivity of the neutrophils of the cell exudate was established by the nitro-BT reduction test. The chamber variant of the "skin window" method is recommended as a technically simple and physiological procedure for obtaining a pure population of human neutrophils.

KEY WORDS: human neutrophils; chamber variant of the "skin window" method.

The "skin window" method [12], with the use of chambers, was developed for quantitative analysis of the migrating capacity of human leukocytes [10, 11]. The principal cells in chamber exudates were shown to be neutrophils [6, 14]. However, information on their functional state is very limited and contradictory [3, 5].

This paper gives the characteristics of the morphological composition, viability, and functional activity of neutrophils migrating through a skin window.

METHODS

Observations were made on 34 healthy male volunteers aged 20-40 years. A skin window 0.3 cm^2 in area was obtained by an abrasive method [13] by means of dental polishing heads and the BÉRP-10 drilling machine. Teflon chambers with an internal volume of 1 ml were treated with silicone, filled under sterile conditions with medium No. 199 with 30% autologous serum, and mounted in the middle third of the palmar surface of the forearm. The chamber was removed 18-20 h later and the number of cells counted on a Picoscale PS-4 apparatus (Hungary). The morphological composition was determined in films stained by Pappenheim's method. The cells were washed 3 times (5 min at 200g) at 4°C with Hanks's solution containing 0.1% medicinal gelatin and resuspended in Hanks's solution (pH 7.2) containing 10% group IV serum (pool from six donors) in a concentration of 10^7 cells/ml. The viability of the cells was assessed by the trypan blue test. The functional state of the neutrophils was determined by the nitro-BT reduction reaction [1] with intact cells and during stimulation by heated *Salmonella marcescens* vaccine ($6 \cdot 10^8$ bacterial cells/ml). The percentage of activated neutrophils containing reduced nitro-BT (diformazan) was calculated [9].

RESULTS

The mean number of migrating cells was $(52.0 \pm 5.3) \cdot 10^6$ per square centimeter of the skin window. Individual counts varied from $7.8 \cdot 10^6$ to $123.0 \cdot 10^6$, with a coefficient of variation of 77.2 ± 8.2%. When two chambers were filled simultaneously, differences between the number of cells did not exceed 20%.

The number of viable cells in all the experiments exceeded 92% (96.5 ± 0.8%). Neutrophils accounted for 95-100% of the cell exudate: 93.4 ± 1.6% of them were polymorphs and 5.2 ± 0.3% stab cells. Solitary monocyte-macrophages, lymphocytes, and reticulum cells were seen.

In the nitro-BT reduction reaction without the stimulator the number of activated neutrophils did not exceed 11% (6.9 ± 0.9). On the addition of *S. marcescens* vaccine a high level of activation of the neutrophils was observed in all cases: The number of diformazan-positive cells was 21-83% (51.7 ± 3.6%; $P < 0.001$).

Department of Bacterial Allergy, Research Institute of Epidemiology and Microbiology, Kazan'. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 11, pp. 623-624, November, 1979. Original article submitted March 3, 1979.

0007-4888/79/8811-1375$07.50

By the use of the skin window principle it is thus possible to obtain a virtually pure population of mature, functionally normal neutrophils, similar in their reactivity to peripheral blood neutrophils [2]. It is interesting to note some significant differences between the composition of the cell exudate in these chambers and the composition of cells migrating on a coverslip in the original method [12]. In the latter case, a high proportion (about 50%) of macrophages was obtained after 18-20 h [7, 8].

The present observations do not confirm the functional injury to the neutrophils noted by Hellum and Solberg [5] during migration into a skin window chamber. Evidently the technique which those workers used (the formation of a skin blister) does not enable physiologically intact neutrophils to be obtained.

Isolation of neutrophils by the skin window method has many advantages over methods of obtaining them from blood. The method is technically simple and yields neutrophils with a high degree of purity. If modern flotation methods are used to fractionate peripheral blood cells, considerable loss of neutrophils and their contamination with other cells takes place (greater than 10%; [4]). Furthermore, if the neutrophils are purified in successive stages it is more difficult to preserve their integrity.

On the basis of the observations described above the chamber variant of the skin window method can be recommended as a technically simple and physiological procedure for obtaining neutrophils.

LITERATURE CITED

1. M. E. Viksman and A. M. Mayanskii, Kazan' Med. Zh., No. 5, 99 (1977).
2. A. N. Mayanskii, M. E. Viksman, P. N. Kotel'nikov, et al., Zh. Mikrobiol., No. 6, 108 (1977).
3. M. L. Bell, R. C. Clay, C. W. Howe, et al., J. Reticuloend. Soc., 11, 167 (1972).
4. K. B. Hellum and C. O. Solberg, Acta Path. Microbiol. Scand., Sect. C, 85, 1 (1977).
5. K. B. Hellum and C. O. Solberg, Acta Path. Microbiol. Scand., Sect. C, 85, 1 (1977), pp. 413-423.
6. J. F. Holland, H. Senn, and T. Banerjee, Blood, 37, 499 (1971).
7. P. Jansa and J. Kratky, Blut, 18, 218 (1969).
8. P. Jansa, Folia Haemat. (Leipzig), 101, 928 (1974).
9. B. H. Park, S. M. Fikrig, and E. M. Smithwick, Lancet, 2, 532 (1968).
10. O. E. Perillie and S. C. Finch, J. Clin. Invest., 43, 425 (1964).
11. W. P. Peters, J. F. Holland, H. Senn, et al., New Engl. J. Med., 282, 342 (1972).
12. J. W. Rebuck and J. H. Crowley, Ann. New York Acad. Sci., 59, 757 (1955).
13. H. Senn, J. F. Holland, and T. Banerjee, J. Lab. Clin. Med., 74, 742 (1964).
14. C. M. Southam and A. G. Levine, Blood, 27, 734 (1966).