6. J. Hjelle and D. Petersen, Toxicol. Appl. Pharmacol., 70, 57 (1985).
7. A. L. Tappel, Free Radicals in Biology, ed. by W. A. Pryor, Vol. 4, New York (1980), pp. 2-47.

PHOSPHATASE ACTIVITY OF BLOOD AND WOUND EXUDATE LEUKOCYTES DURING HEALING OF AN EXPERIMENTAL ASEPTIC WOUND

L. A. Mamedov, A. V. Nikolaev, V. V. Zakharov, A. B. Shekhter, and Yu. R. Khrust

UDC 617-001.4-021.4-003.9-07:616.155.3-008.931:577.152.313

KEY WORDS: wound healing; acid phosphatase; alkaline phosphatase; leukocytes; wound exudate.

Cytochemical determination of alkaline phosphatase (AlP) and acid phosphatase (AcP) activity in some cases can be a reliable laboratory test of the character and course of the pathological process in surgical patients [2, 4, 8]. However, the dynamics of changes in the phosphatase activity of blood and wound exudate cells during healing of aseptic wounds has not been adequately studied [1, 8].

In this investigation the phosphatase activity of blood and wound exudate leukocytes was studied during healing of an aseptic experimental wound.

EXPERIMENTAL METHOD

Altogether 110 male Wistar rats weighing 190-200 g were used. Models of skin wounds were created by the method described previously [3]. Films of peripheral blood taken from the caudal vein and squash preparations of wound exudate were used as the test objects. Blood-and wound-exudate leukocytes were studied daily from the 1st through the 10th days, and again on the 12th and 15th days of the experiment; besides the times mentioned above, the cells also were studied before the operation (background). At each point of time 8-10 rats were used, and were decapitated after specimen taking. The leukocyte formula was determined on films stained by the Romanovsky–Giemsa method. AlP activity was detected by the method in [7] and AcP activity as in [10].

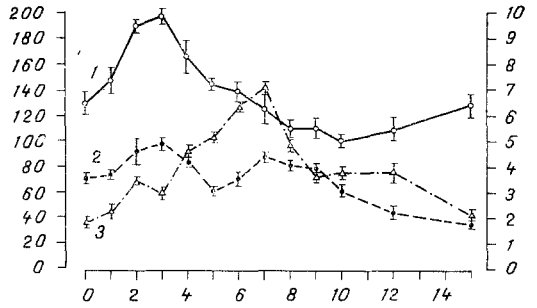

Fig. 1. Changes in phosphatase activity of peripheral blood neutrophils during healing of aseptic wounds in rats. Abscissa, time after wounding (in days); ordinate, AlP (in optical density units) and AcP (in conventional units [11]) levels; scale on right shows absolute number of neutrophils in 1 μl blood × 10^3). 1) AlP, 2) AcP, 3) number of neutrophils.

I. M. Sechenov First Moscow Medical Institute. M. V. Lomonosov Moscow State University.
(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 306-309, September, 1987. Original article submitted February 27, 1987.

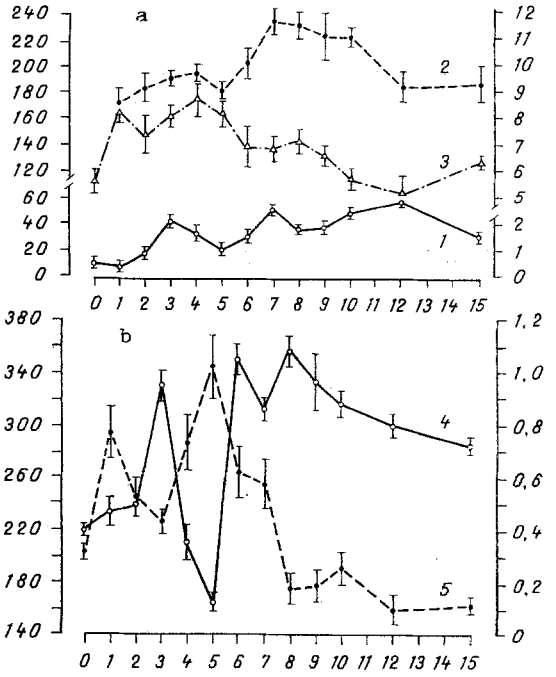

Fig. 2. AcP activity in peripheral blood lymphocytes (a) and monocytes (b) during healing of aseptic wounds in rats. 1) AcP level in small lymphocytes, 2) in large lymphocytes, 3) absolute number of lymphocytes in 1 μl blood ($\times 10^3$), 4) AcP level in monocytes, 5) absolute number of monocytes in 1 μl blood ($\times 10^3$). Remainder of legend as in Fig. 1.

AlP activity was determined quantitatively by a cytophotometric method, using the MIF-k cytophotometer [6]. The content of the final reaction product in 50 cells was measured in each film. AlP activity was determined by a semiqualitative method [11] in neutrophils, lymphocytes, and monocytes. At the same time, and on the same days the wound tissues were studied histologically (stained with hematoxylin and eosin, by Van Gieson's method, with toluidine blue, and by Brachet's reaction). The significance of differences was determined by Student's t test.

EXPERIMENTAL RESULTS

The absolute number of neutrophils in the blood (Fig. 1) increased progressively after wounding to reach a maximum (7000 in 1 μl) on the 7th day, after which it fell, and by the 15th day it had almost regained its initial level (about 2000 in 1 μl). AlP activity in the blood neutrophils (Fig. 1) increased even on the first day, and reached a maximum on the 2nd-3rd days: during this period 1.5 times more of the end product of the reaction was detected cytophotometrically than initially. Starting with the 4th day AlP activity gradually declined, and by the 10th day it was lower than initially. Virtually all the blood neutrophils were AlP-positive. These data confirmed the results obtained by other workers [7, 12], and are evidence of high AlP activity in rats. The increased AlP activity in the blood cells during the first two days after wounding was evidently connected with the outflow of younger neutrophils, in which AlP activity is higher, from the bone marrow into the peripheral blood [12].

AcP activity in the blood neutrophils also increased starting with the first day after wounding (Fig. 1); until the 5th day inclusive, the curve of its changes almost completely reproduced the shape of the AlP curve, although the latter rose much more rapidly. However, the shape of these two curves later diverged: on the 6th-7th day AcP activity rose again, but later it fell below the control level, and thereafter remained unchanged until the end of the experiment.

AcP activity in the small blood lymphocytes (Fig. 2a) increased a little after the operation and remained higher than initially throughout the experiment. Starting with the first day after wounding the absolute number of lymphocytes in the blood was increased by 1.5 times and large lymphocytes with a wide cytoplasm containing azurophilic granules, and sometimes

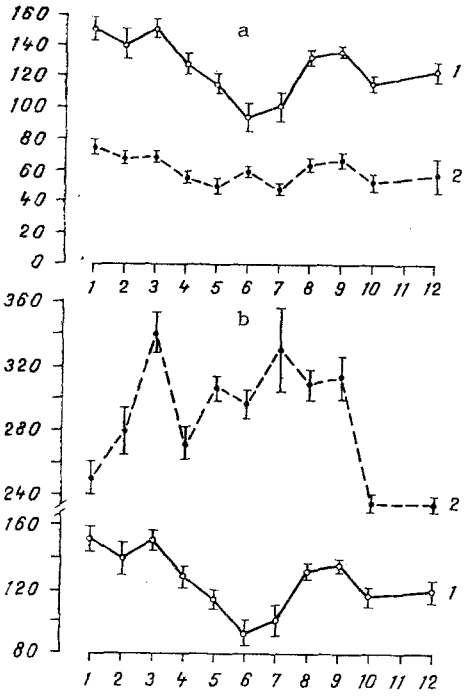

Fig. 3. Phosphatase activity of neutrophils (a) and macrophages (b) of wound exudates during healing of aseptic wounds in rats. Legend as to Fig. 1.

with a bean-shaped or segmented nucleus, appeared. They did not exceed 10% of the total lymphocyte population, but AcP activity in them was an order of magnitude higher than in the small lymphocytes. The appearance of large lymphocytes was an indicator of activation of the lymphoid immune system, which usually is accompanied by increased AcP activity [5].

High AcP activity also was observed in the peripheral blood monocytes: it was increased on the 3rd day, then fell to a minimum on the 5th day, after which it again increased and thereafter remained much higher than initially until the end of the experiment (Fig. 2b). The absolute number of monocytes in the blood also followed a wave-like course. The rise of this parameter on the first day was probably attributable to operative trauma, and that on the 5th day to increased release of monocytes into the tissue (the macrophagal phase of inflammation), leading to an increase in their migration from the bone marrow into the blood. Clear negative correlation also was observed between the absolute number of monocytes in the blood and AcP activity in them until the 8th day of the experiment (Fig. 2b). This was evidently connected with the fact that lysosomal enzyme activity is lower in immature monocytes released from the bone marrow into the blood.

The principal type of cells in the wound exudate was neutrophils, the relative proportion of which decreased from 77.6% at the beginning of the experiment to 48.1% toward the end. Starting with the 2nd day, degenerative forms of neutrophils with extensive areas of translucency of the cytoplasm, loss of granules, pycnosis of the nucleus, and so on, predominated in the exudate. From the first and until the last day of the experiment macrophages were present in the wound exudate: the proportion of them increased from 5.5% at the beginning of the experiment to 29.3% on the 8th day, after which it fell a little. The number of lymphoid cells in the exudate did not exceed 10% in the course of the experiment, until the last day, when it rose to 30%. The number of fibroblasts varied from 0 to 1.8% between the 1st and 9th days, but on the 10th and 12th days it increased to 4.1 and 6.0% respectively.

Enzyme activity was determined only in morphologically intact neutrophils. AlP activity in neutrophils of the wound exudate fell progressively until the 6th day, and then rose a little (Fig. 3a). This increase was perhaps caused by contamination of the film with blood neutrophils. Attention is drawn to two circumstances: first, AlP activity virtually throughout the experiment was observed in 90-100% of neutrophils, and second, it was discovered not only in morphologically intact cells, but also in degenerating cells. The role of this

persistent AlP activity in dying neutrophils of the wound is not yet clear. Some workers [8] have emphasized the important role of AlP in regulating the rate of repair processes in tissues: it inactivates glucocorticoids, which inhibit proliferation of fibroblasts and collagen synthesis by them.

AcP activity of the neutrophils fell a little during the first five days, and thereafter remained at between 50 and 60 conventional units until the end of the experiment. AcP activity in the macrophages was high and in the morphologically intact cells it remained throughout the experiment at between 200 and 300 conventional units (Fig. 3b). The high AcP activity in the cells of the monocytic-macrophagal system is in good agreement with data in the literature [9].

Comparison of the results of the cytochemical study of blood and wound exudate leukocytes with the results of a parallel histological study of tissues from the wound regions shows that the first rise of AlP activity in the blood neutrophils coincided closely with the duration of the inflammatory phase of wound healing. During this period neutrophilic infiltration of the tissues reached a maximum, when the outflow of neutrophils from the blood into the tissues evidently led to the greatest release of young cells with high AlP activity from the bone marrow into the blood stream. It is an interesting fact that AlP activity of the leukocytes responded earlier to a decrease in the intensity of the local inflammatory process than the absolute number of neutrophils in the blood, which continued to rise until the 7th day. AlP activity in the blood neutrophils must evidently be regarded as an important prognostic sign in wound healing.

Distinct patterns of AlP and AcP activity are thus observed in leukocytes in the blood and wound exudate during aseptic wound healing. These patterns reflect complex reactions of the body to surgical trauma, they are evidence of activation of different components of the immune system of the body, and they can be used both to develop more effective ways of stimulating wound healing and of evaluating them.

LITERATURE CITED

1. A. V. Gavril'chak, A. M. Shapiro, A. V. Nikolaev, et al., Experimental–Clinical Aspects of Repair Processes and Methods of Stimulating Them [in Russian], Moscow (1977), p. 41.
2. B. S. Kaplan and V. I. Solov'ev, Voen. Med. Zh., No. 4, 60 (1970).
3. L. A. Mamedov, N. Yu. Kosaganova, G. T. Rikhireva, et al., Vopr. Med. Khimii, No. 6, 82 (1986).
4. I. S. Peterson, Lab. Delo, No. 6, 328 (1966).
5. F. G. J. Hayhoe and D. Quaglino, Hematological Cytochemistry, London (1980).
6. Yu. R. Khrust, L. L. Litinskaya, S. A. Cheptsov, et al., Tsitologiya, No. 8, 997 (1975).
7. M. G. Shubich, Tsitologiya, 8, No. 3, 420 (1966).
8. M. G. Shubich and B. S. Nagoev, Alkaline Phosphatase of Leukocytes under Normal and Pathological Conditions [in Russian], Moscow (1980).
9. S. G. Axline, J. Exp. Med., 128, 1031 (1968).
10. M. S. Burstone, J. Histochem. Cytochem., 7, 1 (1959).
11. L. S. Kaplow, Blood, 10, 1023 (1955).
12. D. M. Williams, Br. J. Haemat., 31, 371 (1975).