CYTOPHOTOMETRIC ASSAY OF ALKALINE PHOSPHATASE IN POLYMORPHS AND FIBROBLASTS DURING WOUND HEALING IN RATS

M. A. Lushchitskii, K. K. Zaitseva,
V. A. Bubnov, A. P. Utochkin,
and V. V. Grigor'ev

UDC 617-001-003.9.092.9-07:616.153.1:
577.152.313

KEY WORDS: cytophotometry; alkaline phosphatase; wound; polymorph; fibroblast.

Changes in alkaline phosphatase (AP) activity in peripheral blood polymorphs are an informative indicator of the development of necrobiotic and inflammatory processes in the body [4]. AP activity in polymorphs and fibroblasts, which are among the principal cellular components of wound tissues, also changes substantially in the course of wound heating [3]. However, published reports of studies of wound healing make no reference to attempts to evaluate correlation between local inflammatory and regenerative processes and the responses of the blood cells as reflected in similar quantitative parameters.

The aim of this investigation was to determine the most informative parameters of AP activity in peripheral blood polymorphs and in wound polymorphs and fibroblasts and to use these parameters to identify correlation between responses of the blood system and inflammatory and regenerative changes in the wound tissues.

EXPERIMENTAL METHODS

Experiments were carried out on 60 noninbred male rats weighing 180-200 g. An incised wound was inflicted under ether anesthesia in the lumbar region by removal of a full-thickness skin graft 3 cm^2 in area. AP was demonstrated in polymorphs in peripheral blood films by the method in [1], and in wound polymorphs and fibroblasts in cryostat sections [1]. AP activity was assayed by a cytophotometric method, using a microspectrophotometer with programmed control, based on the LYUMAN-IZ microscope, the SFN-10 spectrophotometric attachment, and the Élektronika DZ-28 computer. Reference wavelengths were chosen on the basis of the spectral characteristics of the cells in the visible region of the spectrum. In polymorphs in the peripheral blood of the rats, classified visually as cells with varied AP activity, the greatest changes in optical density were observed within the 460 ± 10 nm waveband (Fig. 1). Spectral characteristics of polymorphs and fibroblasts in frozen sections obtained from wound tissues were rather different in appearance. However, in this case also characteristic staining was revealed in the blue region of the spectrum, with a maximum at a wavelength of 440 nm. These wavelengths were chosen as reference wavelengths for the cytophotometric investigations of AP. The total number of leukocytes and of polymorphs was determined in the animals' blood, the number of polymorphs and fibroblasts per unit area (0.016 mm^2) in the zone beneath the regenerating epidermis was counted in frozen sections, and the area of the wound was measured. The peripheral blood investigations were carried out before wounding and simultaneously with determination of the other parameters mentioned above, namely 3, 7, 14, and 21 days after trauma.

EXPERIMENTAL RESULTS

Comparison of AP activity in the peripheral blood of intact rats estimated cytophotometrically and visually showed that the cytophotometric data in the cell sample studied were not directly proportional to the classification in [3]. Besides, visual subdivision of the cells into groups III and IV of activity, i.e., into groups containing polymorphs with high

Department of Naval and Military Surgery, Research Laboratory of Electron Microscopy and Histochemistry, S. M. Military Medical Academy, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Kolesov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 486-488, April, 1988. Original article submitted June 3, 1986.

 0007-4888/88/0004-0586$12.50

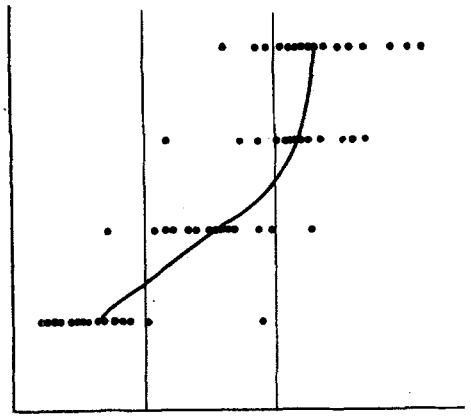

Fig. 1

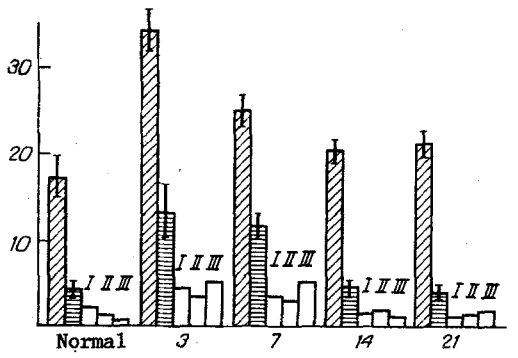

Fig. 2

Fig. 1. Comparison of AP activity in peripheral blood polymorphs of intact rats estimated cytophotometrically and visually. Abscissa, optical density (d, in relative units); ordinate, AP activity estimated visually.

Fig. 2. Histograms characterizing total number of leukocytes and quantitative distribution of polymorphs in peripheral blood among groups based on AP activity after wounding. Abscissa, time after trauma (in days); ordinate, number of leukocytes and polymorphs ($\times 10^3$). Obliquely shaded columns) total number of leukocytes in 1 μl blood; horizontally shaded columns) number of polymorphs in 1 μl blood. I, II, III) Number of polymorphs with low, average, and high AP activity respectively.

TABLE 1. Parameters Characterizing Time Course of Healing of Incised Wounds in Rats

Time after wounding, days	Area of wound, cm^2	No. of polymorphs in wound (in a 0.016 m^2 area)	No. of fibroblasts in wound (in a 0.016 m^2 area)	AP activity in wound polymorphs (OD units)	AP activity in wound fibroblasts (OD units)	No. of leukocytes in 1 μl of peripheral blood	No. of polymorphs in 1 μl of peripheral blood	AP activ. in peripheral blood polymorphs (OD units)
3	4,55±0,120	25,6±2,36	12,4±1,33	0,64±0,35	0,43±0,57	34 722±1411,6	13 889±511,6	0,7±0,04
7	2,28±0,067	16,4±1,03	15,4±0,75	0,57±0,049	0,6±0,04	25 620±1410,1	12 093±696,8	0,89±0,047
14	0,69±0,043	17,6±1,03	16,6±1,63	0,42±0,049	0,27±0,029	20 960±1128,6	4737±318,8	0,77±0,067
21	0,40±0,043	10,4±1,40	6,8±1,42	0,19±0,008	0,12±0,004	21 920±930,8	438,4±261,4	1,04±0,170

and very high AP activity, was not statistically significant. It was accordingly decided to classify the peripheral blood polymorphs with respect to AP activity in three groups (Fig. 1): I) cells with low activity (d = 0-0.6), II) cells with average activity ($0.6 < d \leq 1.2$), and III) cells with high activity ($d > 1.2$). That this approach was soundly based was confirmed by subsequent experiments. For instance, the study of the peripheral blood polymorph population showed that it is heterogeneous as regards the qualitative composition of the cells in both intact and wounded animals. At all times of investigation after wounding there was a significant increase in the relative number of group III cells with high AP activity (evaluated by the Kolmogorov–Smirnov test) [2]. It can be concluded from a comparison of these data with the other blood parameters (Fig. 2) that the most marked changes in composition of the polymorphs were observed among cells with high AP activity, with respect to both their total number (Fig. 2) and their relative number. The data confirm the results obtained in [4, 5], in which it is pointed out that during inflammatory processes the increase in the number of polymorphs in the blood takes place chiefly on account of young cells with high AP activity. Cytophotometry of polymorphs and fibroblasts in frozen sections showed that at different times after wounding, although cells with different values of AP activity appeared, their distribution at each time was sufficiently homogeneous and obeyed the normal law. Thus at each period of development of the wound, cells (both polymorphs and fibroblasts) with definite AP activity are functioning, and their quantitative characteristics are reliably expressed by the mean value of the cytophotometric parameter reflecting their activity which was determined in the present investigation.

Investigation of the number of polymorphs and their AP content in the wound tissues showed that these parameters characterizing the state of the inflammatory process in the wound reached maximal values 3 days after wounding, and thereafter fell gradually (Table 1). A similar tendency is characteristic also of the total number of leukocytes and polymorphs in the peripheral blood. However, the number of polymorphs with high AP activity (group III), both as a percentage and in absolute terms, reached a maximum by the 7th day. By this time the phase of inflammation in the wound was replaced by the phase of proliferation, the area of the wound was reduced, and the number of fibroblasts and the AP activity in them were increased (Table 1). By the 21st day, when wound healing was virtually complete and the total number of polymorphs was the same as normal, their distribution by AP activity still did not agree with the data characteristic of intact animals. During the period when regeneration begins to predominate over inflammation in the wound, and later in the proliferation phase of wound healing also, the peripheral blood system and, in particular, the leukocyte pool, may perhaps play the role of a buffer system.

Cytophotometric analysis of the distribution of polymorphs by AP activity in the peripheral blood of rats thus showed the advantage of dividing these cells into three functional groups. The time course of development of inflammation and regeneration in the wound tissues was adequately reflected in changes in both the total and the relative numbers of polymorphs of functional group III, characterized by high AP activity, circulating in the blood. The results of the investigations are evidence of the high informativeness of the cytophotometric method and they can be used to objectivize the course of wound healing in clinical practice and also to assess the effectiveness of treatment.

LITERATURE CITED

1. M. S. Burstone, Enzyme Histochemistry and Its Applications in the Study of Neoplasms, Academic Press, New York (1965).
2. E. V. Gubler, Computational Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leiningrad (1978).
3. M. I. Kuzin and L. L. Shimkevich, Wounds and Wound Infection [in Russian], Moscow (1981), pp. 114-160.
4. M. G. Shubich and B. S. Nagoev, Alkaline Phosphatase of Leukocytes under Normal and Pathological Conditions [in Russian], Moscow (1980).
5. L. S. Kaplow, Blood, 10, No. 10, 1023 (1955).
6. D. M. Williams, Br. J. Haemat., 31, No. 3, 371 (1975).
7. D. M. Williams, R. Gillett, and J. E. Linder, J. Histochem. Cytochem., 30, No. 4, 323 (1982).