CORRELATION BETWEEN LEUKOCYTE MIGRATION ACTIVITY AND RNA AND DNA SYNTHESIS IN EXPERIMENTS in vitro

P. G. Nazarov, A. P. Volgarev, and S. A. Ermakov

UDC 612.112.2:612.112.94.015.36:547.963.32

In a parallel study of leukocyte migration in vitro in the presence of specific antigen and spontaneous synthesis of RNA and DNA the following significant correlations were found in lymphocytes in culture: 1) direct correlation between RNA and DNA synthesis; 2) close correlation between antigen-induced migration and the levels of RNA and DNA synthesis. The effect of the antigen was manifested as inhibition or stimulation of leukocyte migration. A high ratio between RNA synthesis and DNA synthesis corresponded to inhibition of migration, a low ratio to its stimulation. The value of the ratio itself varied mainly on account of changes in the level of DNA synthesis. The participation of T and B lymphocytes in the regulation of leukocyte mobility under the influence of antigen is discussed.

KEY WORDS: rheumatic fever; leukocyte migration; blast transformation of lymphocytes.

For the factor inhibiting migration of macrophages to be produced a definite level of activity of all cellular processes in the sensitized lymphocytes is necessary. This has been shown by experiments using inhibitors of RNA and protein synthesis: puromycin, actinomycin D, and mitomycin C prevented the secretion of the factor in the presence of antigen [5]. Most workers associate the production of migration inhibiting factor with T lymphocytes [6, 8]. However, some workers have observed that the factor can be produced in response to stimulation of lymphocytes by B-cell mitogens [9, 15].

In the investigation described below changes in leukocyte migration under the influence of antigen were studied parallel with spontaneous RNA and DNA synthesis. The relationship between these parameters was determined by correlation analysis. Besides the determination of correlation between pairs, multiple correlations between the varying parameters also were calculated [2].

EXPERIMENTAL METHOD

Observations were made on 34 patients with rheumatic fever in the inactive phase. The leukocyte migration inhibition test was carried out by Artemova's method [1] in Perfil'ev–Gabe capillary tubes. Blood mixed with antigen was introduced into capillary tubes, which were sealed with picein (working temperature about 40°C), centrifuged, and allowed to stand at 37°C for 18 h. Migration was measured in scale divisions of the ocular micrometer (MBS-1 microscope, magnification 8×), using the boundary with the erythrocyte layer as the origin. A change of 20% in the migration zone (compared with spontaneous) was assessed as either inhibition or stimulation. Hemolytic streptococcal allergen was used as the antigen in a final concentration of one skin dose to 0.2 ml blood. In this concentration it had no appreciable effect on the migration of leukocytes from healthy subjects.

DNA synthesis was judged from the incorporation of 5-methyl[^{3}H]thymidine (specific activity 15 Ci/mmole), and RNA syntheses from the incorporation of 5[^{3}H]uridine (specific activity 16.3 Ci/mmole) in 18-h cultures of whole blood by the method described in [3, 4]. The labeled precursors were added 5 h before the end of incubation. Incorporation of the label was estimated with the Isocap-300 scintillation counter (Nuclear Chicago, USA).

Department of Microbiology and Immunology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. Department of Propedeutics of Children's Diseases, Pediatric Medical Institute, Ministry of Health of the RSFSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Ioffe.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 721-723, June, 1978. Original article submitted October 3, 1977.

 0007-4888/78/8506-0790$07.50

TABLE 1. Incorporation of Labeled Precursors Depending on Character of Changes in Induced Migration (M ± m)

Change in induced migration	Number of observations	Incorporation of isotope, cpm		Uridine/thymidine ratio*
		[^{3}H]-thymidine	[^{3}H]-uridine	
Inhibition	10	190,9±32,9	819,6±150,8	4,660±0,783
No change	9	303,7±115,9	882,8±161,8	4,283±0,915
Stimulation	15	473,2±76,6	1342,6±326,4	2,687±0,376

*Ratio calculated as means of corresponding ratios in each individual case.

EXPERIMENTAL RESULTS

The following correlations were discovered by statistical analysis of the results: 1) positive correlation between RNA and DNA synthesis ($r = 0.704$, $P = 0.0005$); 2) close correlation between three parameters: DNA and RNA synthesis and migration in the presence of the allergen (combined coefficient of correlation 0.521, $P = 0.0005$).

The action of the allergen was manifested as inhibition or stimulation of migration, or there was no effect. The corresponding levels of DNA and RNA synthesis in these three groups were determined.

As Table 1 shows, the highest incorporation of labeled thymidine coincided with stimulation of migration and the lowest incorporation with its inhibition ($P = 0.01$). A tendency for a similar pattern is also noted for the incorporation of [^{3}H]uridine. The results of a comparison of the ratios between the incorporation of labeled uridine and thymidine and the type of change in the induced migration merited serious attention. As can be seen from Table 1, when migration was stimulated, the indicated ratio was 2.687 ± 0.376, while in the case of inhibition it increased to 4.660 ± 0.783; the difference proved significant ($P = 0.01$).

Incorporation of labeled uridine and thymidine are known to be associated with different populations of lymphocytes: uridine is incorporated by T cells [11, 13] and thymidine by B cells [7, 10, 12, 14]. The present results confirming the role of T cells in this phenomenon show that inhibition or stimulation of leukocyte migration is determined not only by T-cell activity, but also to a large extent by a change in B-cell activity also.

As regards the problem of what lies at the basis of the changes observed in leukocyte migration in the presence of antigen, only a few suggestions can be made at this stage. First, it may be that the production of two different mediators is observed: a migration inhibiting factor (MIF) by the T cells and a stimulating factor by the B cells; the balance between them determines the change in leukocyte migration. Second, the possibility cannot be ruled out that inhibition and stimulation of leukocyte migration are associated with the action of the same factor, namely MIF synthesized by T cells and which, in large doses, depresses migration, whereas in small doses it stimulates migration; the end result may depend on fluctuations in B-cell activity. Third, activation of B cells may also indicate an increased level of antibodies capable of neutralizing the antigen partially; under these circumstances a low antigen concentration induces insufficient MIF production and this, in turn, leads to stimulation of the migratory activity of the leukocytes.

LITERATURE CITED

1. A. G. Artemova, Byull. Éksp. Biol. Med., No. 10, 67 (1973).
2. G. F. Lakin, Biometrics [in Russian], Moscow (1968).
3. P. G. Nazarov and V. I. Purin', Lab. Delo. No. 4, 195 (1975).
4. V. I. Purin' and P. G. Nazarov, Vopr. Okhr. Mat., No. 5, 44 (1977).
5. B. R. Bloom and B. Bennett, Science, 153, 80 (1966).
6. B. A. Clinton, T. J. Magoc, and R. L. Aspinall, J. Immunol., 112, 1741 (1974).
7. M. P. Dabrovski, Transplantation, 48, 531 (1974).
8. A. Dimitriu, M. Dy, N. Thomson, et al., Clin. Exp. Immunol., 18, 141 (1974).
9. E. G. Elfenbeim and J. Green, Fed. Proc., 32, 877 (1973).
10. V. Glik and M. R. Schwarz, Immunol. Commun., 4, 123 (1975).
11. J. W. Hollingsworth and J. Carr, Cell. Immunol., 8, 270 (1973).
12. H. Mellstedt, Clin. Exp. Immunol., 19, 75 (1975).
13. D. W. Scott and S. H. Josepus, Cell. Immunol., 20, 64 (1975).
14. Y. Wybran, S. Chantler, and H. H. Fudenberg, J. Immunol., 10, 1157 (1973).
15. T. Yoshida, H. Sonozaki, and S. Cohen, J. Exp. Med., 138, 784 (1973).