THE PERIPHERAL BLOOD LEUKOCYTE MIGRATION INHIBITION TEST IN SYSTEMIC LUPUS ERYTHEMATOSUS

T. G. Trayanova, I. T. Rass, and N. N. Prozorovskaya

UDC 616.5-002.525.2-031.81-07:616.155.3-07

Cellular hypersensitivity in systemic lupus erythematosus (SLE) was investigated by the migration inhibition test in the presence of different concentrations of native and denatured DNA. Inhibition of migration of peripheral blood leukocytes was found in 7 of 11 patients with SLE investigated.

In systemic lupus erythematosus (SLE) high titers of antibodies against various components of the cell and, in particular, against nuclear material and DNA, are found in the patients' serum [6, 9, 16]. Investigation of cellular immunity has been hampered for a long time by the lack of a suitable in vitro test, although the results of skin tests suggested the existence of cellular sensitivity to DNA in active forms of SLE [9, 10, 13].

In the present investigation to study cellular sensitivity in SLE the method based on inhibition of migration of peripheral blood leukocytes in the presence of the specific antigen [12] was used. The suitability of this method for detection of hypersensitivity of delayed type in SLE has also been described by Harpey et al. [8].*

EXPERIMENTAL METHOD

Heparinized venous blood, taken with sterile precautions in a volume of 12-15 ml, was kept for 1 h at room temperature. The leukocyte suspension was washed three times with Hanks's solution by centrifugation for 5 min each time at 1000 rpm. The cell residue was resuspended in 0.5-1.0 ml medium No. 199 and made up to a final concentration of approximately $1 \cdot 10^6$ cells per ml. The suspension was drawn in a volume of 0.05 ml into a series of glass capillary tubes with an internal diameter of 1.2 mm and length of about 7 cm. The capillary tubes were sealed, centrifuged for 5 min at 1000 rpm, and cut off at the level of the cell residue. By means of a mixture of mineral oil and wax the capillary tubes were fixed in the compartment of a transparent plastic chamber [3]. Into each compartment of the chamber 1 ml of medium No. 199 containing the antigen in the desired concentration was poured. Medium No. 199 without antigen was poured into the control compartments. Usually for each concentration of antigen there were 3 capillary tubes. The chamber was covered with a glass slide, the joint was made airtight with paste, and the specimens were incubated for 24 h at 37°C. At the end of incubation the pattern of migration was recorded by projecting the chamber on photographic paper by means of an enlarger. On the photograph obtained-the area of the migration zones was measured with a planimeter and the mean area calculated for the given concentrations of antigen. The migration index (I_m) was determined as described by David et al. [7]:

$$I_m = \frac{\text{Mean area of zone of migration in presence of antigen}}{\text{Mean area of zone of migration in control (without antigen)}} \times 100\%.$$

*This article was published during preparation of the present paper.

Department of Internal Medicine and Occupational Diseases, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR E. M. Tareev.)Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 2, pp. 61-63, February, 1973. Original article submitted January 11, 1972.

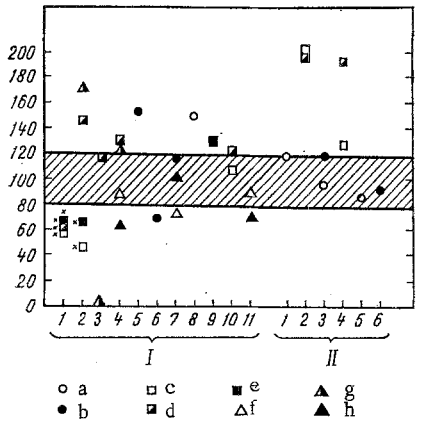

Fig. 1. Index of migration of peripheral blood leukocytes in the presence of various DNA preparations. Abscissa, No. of patient; ordinate, migration index (in percent). Titers of antibodies against formalinized DNA in patients with SLE: No. 1) absent; No. 2) 1:10; No. 3) not determined; No. 4) 1:15; No. 5) 1:40 and 1:60; No. 6) 1:80; No. 7) 1:1280; No. 8) 1:10; No. 9) 1:40; No. 10) 1:40; No. 11) 1:20. I) patients with SLE; II) patients with other diseases; a and b) native DNA, concentrations 100 and 200 μg/ml respectively; c-e) denatured DNA, concentrations 25, 50 and 100 μg/ml; f-h) formalinized DNA, concentrations 10, 20, and 40 μg/ml. Zone of migration indices corresponding to normal cell reactivity is shaded. Crosses denote results obtained in a mixed system of patients' peripheral blood leukocytes and normal guinea pig peritoneal macrophages.

Preparations of native double-helical DNA, of heat-denatured partially coiled DNA, and of single-stranded DNA heat-denatured in the presence of formaldehyde [1] were used as the antigen. The original DNA preparation was obtained from calf thymus [11]; its DNA concentration, determined by Spirin's method [4], was 860 μg/ml, and the residual protein content did not exceed 1.5% [12].

Antibiotics could not be used in this investigation because the patients might have been sensitized to them, and the presence of antibiotics in the culture fluid could have affected the results of the tests.

In 2 experiments a mixture of washed patients' leukocytes with an equal number of guinea pig peritoneal macrophages, obtained after intraperitoneal injection of 10 ml sterile 2% solution of glycogen in 0.1% peptone in physiological saline, was used as the migrating system. The concentration of the resulting mixture was adjusted to $1 \cdot 10^6$ cells/ml and the suspension was introduced into capillary tubes as described above. The tubes were incubated in the presence of 5% inactivated guinea pig serum.

EXPERIMENTAL RESULTS

Altogether 20 tests were carried out with blood from 17 patients: 11 patients with SLE and 6 with other diseases (1 with allergic reactions to therapeutic substances, 1 with chronic colitis, 4 with various diseases of the kidneys). The results of the tests are shown in Fig. 1. Inhibition of migration in the presence of various types of DNA was found with material from 7 patients with SLE suffering from a relapse at the time of the investigation. In 6 of them, however, when other concentrations of DNA were used, both normal (migration index 80-120% [15]) and increased migration were observed. In 4 patients with SLE the migration was within normal limits or higher. The results of the migration test did not correlate with the titers of serum antibodies against formalinized DNA, determined by the passive hemagglutination method [2]. Among the patients with other diseases, in 4 migration was normal and in 2 it was increased in the presence of DNA.

The preliminary results thus obtained suggest that cellular hypersensitivity to DNA is present in at least some patients with SLE. More reliable results may be obtained by the use of a mixture of heterologous cells (sensitized leukocytes and peritoneal macrophages from a normal guinea pig) as the migrating system, for in this way it is possible to work with a larger quantity of material.

LITERATURE CITED

1. A. M. Poverennyi, T. L. Aleinikova, and A. S. Saenko, Biokhimiya, 3, 1160 (1966).
2. A. M. Poverennyi and M. I. Levi, Biokhimiya, 29, 80 (1964).
3. O. V. Protasova, I. M. Pozina, O. S. Sergeeva, et al., Lab. Delo, No. 11, 667 (1966).
4. A. S. Spirin, Biokhimiya, 23, 656 (1958).
5. F. J. Azoury, H. E. Jones, V. J. Derbes, et al., Ann. Intern. Med., 65, 1221 (1966).
6. R. Ceppellini, E. Polli, and F. Celada, Proc. Soc. Exp. Biol. (New York), 96, 572 (1957).
7. J. R. David, S. Al-Ascari, H. S. Lawrence, et al., J. Immunol., 93, 264 (1964).
8. J. P. Harpey, B. Caille, R. Moulias, et al., Lancet, 1, 292 (1971).
9. H. R. Holman and H. G. Kunkel, Science, 126, 162 (1957).
10. H. E. Jones, V. J. Derbes, O. B. Gum, et al., Arch. Derm., 95, 559 (1967).

11. E. R. Kay, N. S. Simmons, and A. U. Daunce, J. Am. Chem. Soc., 74, 1724 (1952).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. R. O. Ores and K. Lange, Am. J. Med. Sci., 248, 562 (1964).
14. M. Soborg and G. Bendixen, Acta Med. Scand., 181, 247 (1967).
15. M. Soborg and P. Halberg, Acta Med. Scand., 183, 101 (1968).
16. M. Seligman, C. R. Acad. Sci. (Paris), 245, 243 (1957).