

Short communication

DEMONSTRATION OF MIGRATION INHIBITORY FACTOR (MIF) IN A MURINE SYSTEM USING MYELOMONOCYTIC LEUKEMIA CELLS (WEHI-3)

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(Received 2 November 1979, accepted 25 November 1979)

Cells of the myelomonocytic tumor cell line, WEHI-3, were used as indicator cells in the indirect capillary test for the detection of migration inhibitory factor (MIF). A migration inhibition of about 50% was found and the results were highly reproducible.

The indicator cells can be obtained in large quantities, as the myelomonocytic cells grow as an ascitic tumor in the peritoneal cavity of BALB/c mice.

INTRODUCTION

Large numbers of indicator cells are needed for the detection of migration inhibitory factor (MIF). For murine MIF usually mouse peritoneal macrophages are used as indicator cells. However, the number of mouse peritoneal cells which can be obtained from a single animal is relatively small even when exudates are induced by sterile irritants (thioglycollate, mineral oil, etc.). Moreover, mouse peritoneal cells show a wide variability with respect to their migration properties (Lohmann-Matthes, 1977; Adelman et al., 1978; Tagliabue et al., 1979). Macrophages, used as indicator cells in a MIF test, should have the following properties: (a) they should be obtainable in large quantities by an easy and reproducible method of isolation, and (b) they should have a constant migration behavior. Lohmann-Matthes (1977) described that the macrophages cultured from bone marrow were well suited as indicator cells.

In this study we have tested whether cells of the myelomonocytic leukemia, WEHI-3 (Warner et al., 1969; Sanel, 1973), could be used as indicator cells in the indirect MIF test. These leukemia cells fulfill both criteria and have the advantage that they can be obtained without *in vitro* culture procedures, as the tumor is maintained by weekly intraperitoneal transplantation.

MATERIAL AND METHODS

Mice and tumor

C57BL/10Sn mice, 6–10 weeks old, were obtained from Bomholtgård

(Denmark) and used for immunization. BALB/c mice, 6–10 weeks old, were obtained from Centraal Proefdierenbedrijf (CPB), TNO Zeist, The Netherlands, and were used for weekly i.p. transplantation of the myelomonocytic WEHI-3 tumor. The WEHI-3 tumor was a gift from Dr. F.T. Sanel (Baltimore, MD). From each mouse $1\text{--}2 \times 10^8$ cells could be obtained 8–10 days after i.p. transplantation of 5×10^6 cells.

Sheep red blood cells (SRBC)

SRBC stored in Alsever's balanced salt solution were obtained from RIV Biltoven, The Netherlands.

Immunization

C57BL mice were immunized subcutaneously (s.c.) at the chest with 0.1 ml of 10^6 SRBC in Freund's complete adjuvant (FCA). The MIF test is considered as an in vitro correlate of the delayed type hypersensitivity (DTH) reaction (Crowle, 1975). The data of a footpad swelling assay showed that an optimal DTH reaction was present 5 days after s.c. immunization with SRBC in FCA. These data were in agreement with the results of Kerckhaert et al. (1974). For this reason the draining peripheral lymph nodes (PLN), brachial and axillary, were collected 5 days after immunization and used for the MIF production.

Production of migration inhibitory factor (MIF)

The lymph nodes were minced on a metal sieve in Fisher's medium. The cell suspension was centrifuged and the cells were resuspended in Fisher's medium. Cell debris and dead cells were removed from the suspension by filtration over glass wool. The cell suspension was adjusted to a final concentration of 10^7 lymphocytes/ml in Fisher's medium supplemented with 10% fetal bovine serum (FBS). SRBC were washed several times and finally resuspended in Fisher's medium with 10% FBS (10^7 SRBC/ml). Five ml of lymphocyte suspension mixed with 5 ml SRBC suspension were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 24 h. The cells were spun down and the supernatants tested for MIF activity. Test supernatants were prepared from PLN lymphocytes collected after immunization. Supernatants from normal lymph node lymphocytes served as control.

Migration inhibition assay

WEHI cells were collected by lavage of the peritoneal cavity of a tumor-bearing BALB/c mouse with Fisher's medium. The tumor cells were washed, adjusted to a concentration of 5×10^7 cells/ml and drawn into 1.0 mm \times 75 mm capillary tubes (microhematocrit tubes, BS 4316, Bilbate, U.K.). The tubes were sealed at one end with Clay-Adams Seale-ease and centrifuged at $125 \times g$ for 2 min. The capillary tubes were cut at the cell-fluid interface. The cell portion was anchored in a migration chamber (2 capillaries per chamber) with inert silicone grease (High Vacuum grease, Dow Corning).

The chambers were made as follows: siliconized perspex plates (3 mm × 25 mm × 75 mm) with two holes (Ø 20 mm) were fixed on glass slides. The wells, formed in this way, were filled with test supernatant, control supernatant or medium (Fisher's medium supplemented with 10% FBS) and sealed with a coverslip and silicone.

The migration chambers were incubated at 37°C for 24 h. The fan formed by the cells that migrated from the capillary tube was projected with the use of an overhead projector on a sheet of graph paper. The areas of migration were determined and the migration inhibition percentage (% MI) was obtained as follows:

$$\% \text{ MI} = \left(1 - \frac{\text{area of migration with supernatant tested}}{\text{area of migration with Fisher's medium + 10\% FBS}} \right) \times 100\% .$$

Footpad swelling assay

The method of Kerckhaert et al. (1974) was used. Briefly: at different times after immunization delayed type hypersensitivity reactions were determined by measuring the increase in footpad thickness of C57BL mice after stimulation by an injection into the left footpad of 4×10^6 SRBC in 0.05 ml phosphate-buffered saline. The footpad thickness was measured with the paw meter as described by Bonta and Vos (1965).

RESULTS AND DISCUSSION

The draining peripheral lymph nodes were collected 5 days after immunization and used for MIF production. Myelomonocytic leukemia cells, WEHI-3, were tested as indicator cells for the presence of MIF in the supernatants from cultures of lymphocytes and target cells. The area of the fan formed by the migrated WEHI cells in Fisher's medium with 10% FBS varied less than 10% within separate experiments.

TABLE 1

PERCENTAGE MIGRATION INHIBITION (% MI) OF WEHI-3 CELLS BY LYMPHOCYTE SUPERNATANTS

Experiment ^a	% MI ^b	
	Control supernatant	Test supernatant
1	18 ± 3	55 ± 9
2	10 ± 2	45 ± 5
3	19 ± 3	51 ± 9

^a % MI are mean values of 8 samples ± S.D.

^b % MI was determined compared to the migration of WEHI-3 cells in Fisher's medium with 10% FBS.

Supernatants of immune lymphocytes stimulated *in vitro* with SRBC induced about 50% migration inhibition of the WEHI-3 cells after 24 h. This result was reproduced in 3 consecutive experiments (Table 1). The control supernatants from cultures of normal lymphocytes and SRBC showed some migration inhibition (20%) compared to the migration in Fisher's medium with 10% FBS (Table 1).

The advantages of the use of WEHI-3 cells for the MIF test

Large numbers of WEHI-3 cells can be obtained easily by simple lavage of the peritoneal cavity of a tumor-bearing mouse ($1-2 \times 10^8$ cells/mouse).

Although the tumor is not purely a monocytic one but also contains granulocytic precursor cells (Warner et al., 1969), the population of WEHI-3 cells is more homogenous (>90% tumor cells) than a population of normal peritoneal cells or induced peritoneal exudate cells.

Variability in migration properties of peritoneal cells due to activation (Ando et al., 1972) will not be encountered with cultured cells such as the SV40-transformed macrophage cell line IC-21 (Defendi, 1976) or cultured bone marrow cells (Lohmann-Matthes, 1977). However, the culturing of cells is laborious while transplantation of tumor cells is a simple procedure. As shown the migration of the WEHI-3 cells is constant.

Mineral oil-induced peritoneal exudate cells from different inbred mouse strains responded differently to MIF. The responses varied from good, to intermediate and refractory (Tagliabue et al., 1979). The 50% MI found with WEHI-3 cells can be considered as a good response.

In summary, WEHI-3 cells are suitable indicator cells for the indirect capillary MIF test as (a) they can be easily obtained in large quantities, and (b) they have stable migration properties and are sensitive to MIF.

ACKNOWLEDGEMENT

We thank Coby Heinen for her technical advice.

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