SIMPLIFIED MODIFICATION OF THE MACROPHAGE MIGRATION INHIBITION METHOD IN MICE

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UDC 616-006-07: 616.155.3-008.11-076.5

Simplified modification of the macrophage migration inhibition method is suggested. The simplification is achieved by omitting the capillary centrifugation stage.

KEY WORDS: macrophage migration inhibition method.

The macrophage migration inhibition (MMI) method in vitro reflects the state of cell-mediated immunity and correlates with the hypersensitivity of delayed type (HDT) test in vivo [6]. The MMI method is most widely used in its "classical" variant [7], based on the use of glass capillary tubes. The advantages of this method are its high sensitivity and reproducibility. However, the "classical" variant of the method is laborious and requires a large quantity of cellular material. To simplify the MMI method, both capillary and noncapillary modifications have been suggested [1, 8-10], but for various reasons they have not yet achieved wide popularity.

A simple micromodification of the "classical" capillary variant of the MMI method is suggested below. Simplification is achieved by omitting the capillary centrifugation stage.

(CBA × C57BL/6)F_1 mice weighing 20-22 g were used. The animals were sensitized by intradermal injection of 10^8 sheep's red blood cells (SRBC) in 40 μl Freund's complete adjuvant (FCA) into the hind footpad [12]. The level of HDT was determined by skin tests [11]. At various times after sensitization, 10^8 SRBC in 40 μl physiological saline were injected into the hind footpad of immune mice and also of normal (control) mice. The results of the tests were read 24 h after the reacting injection. After measurement of the thickness of the paws with a micrometer, the skin reaction index (SRI) in the experimental and control groups was calculated by the equation:

$$\text{SRI} = \left(\frac{\text{Mean thickness of paws in experimental group}}{\text{Mean thickness of paws in control group}} - 1\right) \cdot 100.$$

The spleen cell migration inhibition test was carried out at the same time. To remove red blood cells the spleen cells were sedimented at 300g for 7 min and resuspended in 0.17M NH_4Cl-Tris buffer, pH 7.2, cooled to 4°C [4]. The cell suspensions were washed twice in medium No. 199 with 10% fetal calf serum (FCS) and transferred to test tubes 30 mm long, 16 mm in diameter, with a conical bottom. The cells were washed for the last time at 220g for 5 min. The supernatant was carefully removed and the residue broken up by means of a cell suspension mixing apparatus (the Maxi Mix TM, from Sybron Corp., USA). A massage apparatus with a spherical head, commercially available, can be used for this purpose.

A capillary tube 25 mm long, with a bore of 0.5 mm, was filled by means of a steel wire, the diameter of which was the same as the bore of the capillary tube. To avoid artifacts due to lowering of the capillary tube into the cell suspension and removal of the wire, the ends of the capillary tube were cut off by 2-3 mm with an ampul file. The middle part of the capillary tube was used in the experiments and was divided into 5 or 6 equal segments, each 3 mm long. The segments were placed, as described previously [3], on the bottom of wells in microplates (Microplate II No. 3040, from Falcon Plastics, USA), filled with incubation medium (complete medium for cell culture in vitro [5], generously provided by A. E. Gurvich, or medium RPMI-1640 with the addition of 10% ECS and antibiotics (penicillin and streptomycin, 100 units/ml of each), containing

Laboratory of Immunochemistry and Diagnosis of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Laboratory of Immunologic Tolerance, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 8, pp. 236-238, August, 1979. Original article submitted November 8, 1978.

0007-4888/79/8808-0944 $07.50

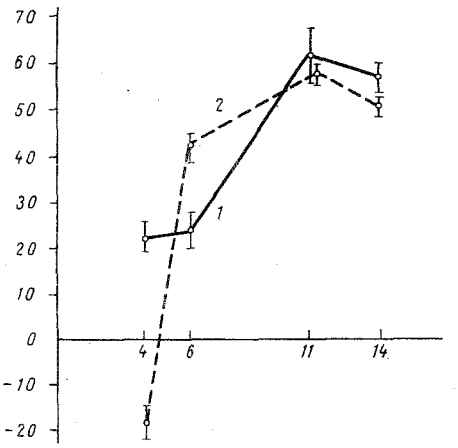

Fig. 1. Dynamics of intensity of HDT reactions determined by skin tests (1) and by the MMI test (2). Abscissa, times after immunization (in days); ordinate, MMI and CRI (in %). Vertical lines give standard error.

50 μg/ml water-soluble SRBC antigen, prepared by the method of Semon et al. [13]. The cells were incubated at 37°C for 18 h in an atmosphere of 5% CO_2. After incubation, the capillary tubes were removed, the migration zones were projected on paper, and the projections of the zones were cut out and weighed. The migration inhibition index (MII) was calculated by the equation:

$$\text{MII} = \left(1 - \frac{\text{Mean weight of migration zones in experiment}}{\text{Mean weight of migration in control}}\right) \cdot 100.$$

The kinetics of the intensity of the skin reactions and the intensity of the migration inhibition reaction, revealed by the suggested modification, after sensitization of mice with 10^8 SRBC in FCA, is illustrated in Fig. 1. Both reactions were similar in character and reached a maximum on the 11th day. The results agree with those of Ohmichi et al. [12], who studied inhibition of migration of peritoneal exudate cell in the "classical" variant of the method.

The results of three experiments to compare the sensitivity of the suggested modification without centrifugation with the usual method showed that the sensitivity of the suggested modification is not lower, but actually a little higher than the sensitivity of the "classical" method (MII 61 ± 5 and 51 ± 8 respectively). Comparison of the migration zones of spleen cells of intact animals showed that in both case, whether centrifuged or uncentrifuged capillary tubes were used, the weight of the migration zones was the same, namely 8.6 ± 0.37 mg (n = 17) and 8.2 ± 0.35 mg (n = 18) respectively.

Further experiments showed that a capillary tube 3 mm long contains on average 0.68-0.7 million cells, i.e., about 6 million cells are needed to fill a capillary tube 25 mm long. This means that the suggested modification can be used for individual analysis of HDT in mice when the MMI test is used not only on spleen cells, but also on other cells of lymphoid tissue, such as peritoneal exudate cells.

The suggested modification thus preserves all the advantages of the "classical" method (high sensitivity, reproducibility, correlation with cellular immunity in vivo) and, at the same time, it is much simpler because it omits the centrifugation stage, with the results that the time taken for the test is approximately halved. If macrophages labeled with ^{51}Cr are used [2], the suggested modification can serve as the basis for development of a quantitative MMI method.

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MODIFIED METHOD OF INJECTING PARENTAL LYMPHOCYTES TO INDUCE A LOCAL GRAFT VERSUS HOST REACTION

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UDC 612.6.02.017.1

A new method of injecting parental lymphocytes into the foot of F_1 hybrid mice to induce a local graft versus host reaction, based on the use of the Achilles' tendon as a natural "shutter" covering the lumen of the wound channel, is suggested. The new method of injection greatly simplifies the test and enables the conditions for its performance to be standardized. The low cell concentration in the working suspension enables it to be kept on ice without any significant increase in the percentage of dead cells.

KEY WORDS: graft versus host reaction; popliteal lymph nodes.

Local forms of the graft versus host reaction (GVHR) are widely used experimentally to test the immunocompetence of lymphocytes, to study the effect of lymphoid organs and biological preparations on reactions of cellular immunity, and to simulate autoimmune diseases [1-3, 6, 7, 8].

Ford et al. [4] developed a model of local GVHR in the popliteal lymph nodes (PLN) of F_1 hybrid rats. The test is based on hypertrophy of drainage PLN developing after injection of parental cells into the hindlimb footpads of the hybrids. A local GVHR can be induced similarly in PLN of F_1 hybrid mice. A suspension of parental lymphocytes in a concentration of $5 \cdot 10^8$ living cells/ml is injected from a microsyringe into the hindlimb footpad, inserting the needle through the 5th interdigital space. A single injection of 0.02 ml of suspension containing $1 \cdot 10^7$ living cells is given. If a larger number of cells has to be given, the injection is repeated after an interval of 15 min, during which time the first portion of the cells is absorbed. The reaction is read on the seventh day: The mice are killed, PLN removed and dehydrated in acetone, and weighed with an accuracy of 0.1 mg. The strength of the GVHR, which depends on the dose of cells injected and on their immunocompetence, associated with differences between donor and recipient for H-2 [1, 4, 6, 8], is judged from the degree of hypertrophy of PLN. The method of Ford et al. [4] has been extensively used in various laboratories of the world engaged on the study of cellular immunity [2, 5, 8].

In the course of my experimental work I found several factors which interfere with standardization of the experimental conditions during induction of the local GVHR in PLN of F_1 hybrid mice. Rapid agglutination and death of the cells is observed in a small volume of working suspension with a concentration of $5 \cdot 10^8$ living cells/ml (as many as 60-80% of cells die while kept on ice for 40-60 min); the viability of the cells was determined by staining with trypan blue. Even the use of microsyringes does not completely rule out errors in the dose of cells injected (an error of 0.002 ml gives an error of $1 \cdot 10^6$ living cells). These factors lead to fluctuations in weight of the PLN, and these in turn reduce the reliability of the results, for hypertrophy of PLN depends on the dose of cells injected [4].

Department of Microbiology, Smolensk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 8, pp. 238-239, August, 1979. Original article submitted July 14, 1978.

 0007-4888/79/8808-0946 $07.50