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

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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].

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TABLE 1. Comparison of Mean Relative Weight of PLN in Local GVHR in F_1 Hybrid Mice Induced by Ford's Method and by the Writer's Suggested Modification

Series of experiments	Group No.	Number of mice	Number of lymphocytes in 1 ml ($\times 10^8$)	Volume of suspension injected, ml	Weight of PLN, mg (M ± m, limits of variation)
I	1	10	2	0,05	2,39±0,0799 (1,88—2,69)
	2*	10	5	0,02	2,12±0,2521 (1,34—3,74)
	3†	10	—	—	1,02±0,0306 (0,89—1,18)
II	4	10	2	0,05	2,46±0,0677 (2,06—2,68)
	5*	10	5	0,02	2,62±0,1505 (1,96—3,23)
	6	10	—	0,05	1,01±0,0599 (0,82—1,31)

*Cell suspension injected by method of Ford et al. [4].
†Trauma by needle.

To eliminate these unfavorable factors the writer has developed a new method of injecting the cell suspension into the mouse foot, based on the use of the Achilles' tendon as a natural "shutter" closing the lumen of the wound canal and preventing leakage of the suspension.

Male (CBA × C57BL/6)F_1 mice weighing 20 g, obtained from the Stolbovaya Nursery of Inbred Animals, Academy of Medical Sciences of the USSR, were used as recipients. Male C57BL/6 mice weighing 20 g served as donors. The cell suspension was prepared from the spleen in the usual way in medium No. 199 with a final concentration of $2 \cdot 10^8$ living cells/ml. A syringe graduated in divisions of 0.01 and a No. 1 needle were used to inject the suspension.

The foot of a hind limb was extended at the ankle to an angle of 120°. With the tip of a needle located medially to the Achilles' tendon, the tendon was displaced toward the lateral surface of the foot and the skin punctured. The needle was passed beneath the skin as far as the middle third of the dorsum of the foot so that the ankle joint continued to remain medially to the needle. A single injection of 0.05 ml of a suspension containing $1 \cdot 10^7$ living cells was given and the needle withdrawn. The Achilles' tendon returns to its former position and securely covers the lumen of the wound canal, thus preventing any of the suspension from escaping. The suspension should be injected slowly in the course of 5-8 sec, so as not to tear the skin of the foot.

Two series of experiments were carried out on 60 mice in order to compare the two methods of injection of the cell suspension: into the footpads by the method of Ford et al. [4], and beneath the skin of the dorsum of the foot, according to the suggested modification. The effect of trauma by the needle and injection of 0.05 ml medium No. 199 on hypertrophy of PLN also was investigated as a control.

The results in Table 1 show that additional trauma by the needle, as a result of lengthening the wound canal (group 3), and also injection of 0.05 ml medium No. 199 by themselves did not cause hypertrophy of PLN. However, in the first case considerable variation was observed in the weight of PLN (the sum of the standard deviations in groups 2 and 5 was 5.7224 and 2.0032 respectively), whereas in the second case variations were very small (the sum of the standard deviations in groups 1 and 4 was 0.5753 and 0.4139 respectively). Furthermore, the working suspension with a low cell concentration can be kept for a long time on ice without the risk of rapid death of the cells (the number of dead cells after 60 min did not exceed 25% of the total number of cells). With the new method, up to 0.1 ml of cell suspension can be injected on one occasion without loss of cells. For these reasons the experimental conditions can be standardized, the number of animals in the experimental groups can be reduced, and induction of the local GVHR on PLN in the mice is greatly simplified.

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