MIGRATION OF T LYMPHOCYTE PRECURSORS INTO THE THYMUS AND FACTORS AFFECTING THIS PROCESS

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A condition for maturation of T cells is migration of T cell precursors (TCP) into the thymus [7, 9]. Unlike recirculation of mature lymphocytes, with their specific "homing" into lymphoid organs [1, 5, 12], which has been studied in detail, the process of migration of TCP into the thymus remains virtually unstudied. The least clear aspect of the problem is the mechanism whereby TCP overcomes the blood-thymus barrier, which is impermeable not only for other cells, but also for macromolecules [8]. However, it can be tentatively suggested that for TCP to penetrate into the thymus, mutual recognition of the surface structures of TCP and of the cells forming the barrier is essential, like the recognition of lymphocytes by cells of the high endothelium of postcapillary venules in lymph nodes [5, 12].

The aim of this investigation was to study the effect of treatment of bone marrow cells with lectins and enzymes on migration of TCP into the thymus, and also the role of compatibility for certain membrane alloantigens in the mechanism of this process.

EXPERIMENTAL METHOD

Mice of lines CBA, C3H, AKR, and BALB/c, from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, and from the Central Nursery, Academy of Sciences of the USSR, aged 2-4 months, and kept on an ordinary diet, were used. The mice were irradiated with ^{137}Cs gamma-rays on the "Stebel" apparatus.

Cell suspensions were obtained by flushing out the femora and tibiae with medium 199. Viability of the cells was tested by absence of staining with 0.1% eosin solution. The cells were labeled by incubation in a solution of fluorescein isothiocyanate (isomer 1, from Sigma, USA and Reakhim, USSR, 300 μg/ml) for 20 min at 37°C [4]. In this way 100% labeling of the cells was obtained without any reduction of their viability (over 90%).

Labeled cells were injected intravenously into mice irradiated 3-4 h previously in a dose of 8.5 Gy. The mice were killed 3 h later, suspensions of thymocytes were prepared, and the number of labeled cells in them was determined on a Cytofluorograf 50-H continuous-flow cytofluorometer.

In some cases, the cells were treated before injection with neuraminidase from a non-cholera vibrio (25 U/ml, Gor'kii Institute of Epidemiology and Microbiology), with trypsin (0.125%, from Tomsk Research Institute of Vaccines and Sera), and with peanut and soy lectins (both were used in a subagglutinating concentration of 20 μg/ml, and were provided by M. D. Lutsik, L'vov Division of the A. V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR), with fraction 1 of thymosin (provided by G. K. Korotaev, All-Union Research Institute of Blood Substitute and Hormonal Preparations Technology), with antisera against Thy-1 antigens (obtained by immunizing rabbits with mouse brain followed by exhaustion with liver tissue and bone marrow cells) and with SC-1 (obtained by immunizing rabbits with mouse brain followed by exhaustion with liver tissue and mouse thymocytes [6]). Treatment with enzymes was carried out for 30 min at 37°C, with antisera for 30 min at 4°C

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TABLE 1. Effect of Treatment of Bone Marrow Cells with Lectins and Enzymes and of Genetic Compatibility of Donors (CBA) and Recipients on Migration of Cells into the Thymus

No. of experiments	Fraction of cells injected	Strain of recipient	Treatment of cells	Migration of labeled cells into thymus	
				absolute number per organ	fraction of control value
2	10^7	CBA (control)	Without treatment	17 160±1 040	1
		C3H	The same	15 840±990	0,92
		AKR		9 000±1 850*	0,52
		BALB/c	» »	260±670*	0,015
2	10^7	CBA	Without treatment (control)	55 680±1 300	1
		CBA	Soy lectin	11 880±1 600*	0,21
		CBA	Peanut lectin	4 238±680*	0,08
3	10^6	CBA	Without treatment (control)	1 100**	1
		CBA	Neuraminidase	1 980**	1,8
		CBA	Trypsin	506**	0,46

Legend. *P < 0.01, **) average of 3 measurements.

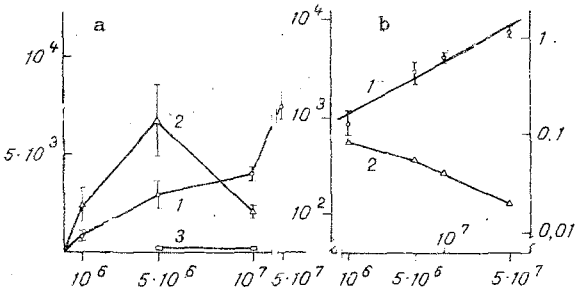

Fig. 1. Migration of bone marrow cells into thymus as a function of their dose. Abscissa (a, b): dose of cells injected; ordinate; on left (a, b) number of cells migrating into thymus, on right (b) number of cells migrating into thymus as a percentage of number of cells injected. Logarithmic axes in b. a) Migration of bone marrow cells: intact (1), treated with thymosin in a dose of 0.01 μg/ml (2), and treated with antiserum to antigen SC-1 (3); b) number of labeled cells migrating into thymus (1) and as a percentage of number of injected cells (2). Regression line 1 is described by the equation $\log y = -0.22 + 0.54 \log x$.

without complement or at 37°C with guinea pig complement, with lectins for 20 min at room temperature, and with thymosin for 60 min at 37°C. The final concentration of treated cells was 5×10^6/ml, except in the case of treatment with lectins when it was 5×10^7.

EXPERIMENTAL RESULTS

Only 0.01-0.2% of the number of cells injected was found in the thymus of the irradiated mice 3 h after injection of the labeled bone marrow cells. The fraction of cells migrating into the thymus decreased with an increase in the dose of cells injected, and accordingly, the curve showing the number of labeled cells in the thymus as a function of the number of cells injected was not linear, but became linear as a result of logarithmic transformation, in the form of a straight line with a slope of less than 1 (Fig. 1).

Treatment of the injected cells with anti-SC-1 serum abolished their migration into the thymus virtually completely (Fig. 1). The same effect was achieved with or without complement. Since the principal target for the action of antibodies to antigen SC-1 is TCP [3], this result can be interpreted as evidence that it is the cells of this type which constitute the majority of bone marrow karyocytes which migrate into the thymus from the

blood stream. It is an interesting fact that migration of cells is completely prevented if they are treated with anti-Thy-1-serum, although this antigen is contained in the membrane of TCP in very small quantities and is inaccessible for binding with antibodies [2]. The fact that treatment of bone marrow cells with thymosin in concentrations of 0.01 to 1 μg/ml potentiates their migration into the thymus, in a dose of (1-5) × 10^6 cells is also evidence that it is TCP which migrates into the thymus. Increasing the dose of cells to 10 × 10^6 has the result that the effect of thymosin changes to inhibition (Fig. 1).

The role of genetic compatibility of the thymus and cells migrating into it was demonstrated in experiments in which bone marrow cells of line CBA (H-2^k, Thy-1.2) were injected into mice of lines differing from them in several alloantigens, expressed on the lymphocyte surface. When C3H mice, differing from CBA in their weak non-H-2 loci, were used as recipients, no significant change in the level of migration of labeled cells into the thymus was recorded. When the cells were injected into AKR mice (H-2^k, Thy-1.1.) migration was reduced by half, but when they were injected into BALB/c mice (H-2^d, Thy-1.2) the virtually complete absence of migration of labeled cells into the thymus was observed (Table 1).

To characterize the nature of the surface structures of the cells that are important for their migration into the thymus, the effect of treating the bone marrow cells with lectins and enzymes was estimated (Table 1). Treatment with soy lectin, which interacts specifically with the terminal N-acetylgalactosamine of polysaccharide and glycoconjugates [11], reduced migration of the bone marrow cells into the thymus fivefold, whereas treatment with peanut lectin, interacting with the terminal disaccharide β-galactosyl-(β→3)-N-acetylgalactosamine, reduced it more than tenfold. Of the enzymes used to treat cells migrating into the thymus, neuraminidase potentiates but trypsin inhibits migration.

These results are evidence that the TCP receptors determining their migration into the thymus is a glycoprotein, whose carbohydrate moiety probably ends with partially sialated β-galactosyl-(β→3)-N-acetylgalactosamine, which is recognized by the lectin-like structures on the surface of cells of the blood-thymus barrier. Meanwhile the role of compatibility for alloantigens assumes the possibility of double control of migration into the thymus, determined not only by recognition of glycoprotein conductor molecules, but also by the possibility of prohibition of migration in the case of incompatibility for particular membrane structures (primarily for products of the principal histocompatibility complex). Similar patterns also are observed for migration of mature lymphocytes through the high endothelium of the postcapillary venules of lymphoid organs [1]. However, there are differences also between these two types of migration, manifested as the opposite effect of treatment of the cells with neuraminidase and with peanut lectin [10, 13], as the difference between classes of cells capable of overcoming the two types of barriers, and the absence of high endothelium of postcapillary venules in the thymus. This provides grounds for the conclusion that "homing" of cells into the thymus and into the peripheral lymphoid organs differs in its molecular and cellular organization, despite the similarity between the general principles of this organization.

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