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Thymus-Dependent and Thymus-Independent Lymophocyte Separation: Relation to Exposed Sialic Acid on Cell Surface

Abstract. *On preparative cell electrophoresis mouse lymph node lymphocytes separate into fast-moving (T, thymus-dependent) and slow-moving (B, thymus-independent) fractions. After treatment with neuraminidase all lymphocytes move as one very slow fraction, indicating that the difference in the mobility of the two kinds of cells is due to differences in the density of exposed sialic acid on their surfaces.*

Mouse lymphocytes from peripheral lymphatic organs can be separated by preparative free-flow electrophoresis into two populations (*1*, *2*). Zeiller *et al.* (*1*) studied the cooperation of separated cells in mounting an immune response in lethally irradiated mice and concluded that the low-mobility cells are B (thymus-independent) lymphocytes and the high-mobility cells are T (thymus-dependent) cells, but that B cells are present in the high-mobility fraction of spleen cells. We have characterized the separated cells both serologically with antiserum to theta (θ) antigen (*3*) and antiserum to mouse specific bone marrow derived lymphocyte antigen (MBLA), made in rabbits (*4*), and functionally by testing in vivo the graft-versus-host reaction and assaying in vitro for the presence of cytotoxic effector cells, plaque- and rosette-forming cells, and phytohemagglutinin-responding cells (*2*). Our conclusion was that the high-mobility cells are mainly T lymphocytes and the low-mobility cells mainly B lymphocytes, with no detectable overlap at the peaks (*2*). Furthermore, Bert *et al.* have shown that the mean electrophoretic mobility of mouse blood lymphocytes is reduced when the cell donor animal is first treated with antilymphocyte serum (*5*). Zeiller *et al.* (*6*) have demonstrated in the rat that the low-mobility cells are antibody-producing cells, whereas lymphocytes in the high-mobility fraction are able to induce graft-versus-host reactions.

Table 1. Effect of antiserum to θ and antiserum to MBLA on electrophoretically separated neuraminidase-treated C-SSI lymph node lymphocytes. The antiserums were tested in the presence of complement.

Lymphocyte	Peak	Cytotoxic index* with antiserum to	
		θ	MBLA
Unseparated		0.63	0.24
Untreated	Fast	0.91	0
Untreated	Slow	0	0.60
Neuraminidase treated	†	0.58	0.28

* The cytotoxic index = (% killed with antiserum − % killed with control serum)/(100 − % killed with control serum). The trypan blue dye exclusion test was used. Antiserums were assayed at "plateau" level (antiserum to θ, 1 : 8; antiserum to MBLA, 1 : 4). Guinea pig complement (C′) was used at a dilution of 1 : 10. For controls, AKR serum plus C′ was used for antiserum to θ and normal rabbit serum plus C′ was used for antiserum to MBLA. † Only peak.

Sialic acid is quantitatively the most important identified anionic group on the surface of most types of cells (*7*). We now report that differences in the number of sialic acid groups exposed on the surface of lymphocytes are responsible for their different electrophoretic mobilities.

Cell suspensions of lymphocytes were prepared from the lymph nodes of random-bred C-SSI white mice (*2*). Without further treatment, one lot of lymphocytes was separated by preparative electrophoresis. A second lot was treated with *Vibrio cholera* neuraminidase (Behringwerke, Marburg am Lahn, Germany) at 37°C for 30 minutes prior to electrophoresis. The lymphocytes were exposed at a concentration of 10^8 cells per milliliter to the enzyme solution, which contained 50 units of enzyme per milliliter in RMPI (Roswell Park Memorial Institute) 1640 medium, *p*H 7.2. Since this *p*H is well above the optimum (*p*H 5.5) for neuraminidase activity, we made the tests with bovine submaxillary mucin as substrate, and found that at *p*H 7.2 at least 50 percent of the neuraminidase activity is retained. The neuraminidase preparation was also tested for possible contamination with proteolytic enzymes (*8*) and with ribonucleases (*9*), which are known to alter the electrophoretic mobility of some cells (*10*). Contamination could not be detected. Prior to electrophoresis, a third lot of lymphocytes was treated in RPMI medium only. Electrophoretic separation was effected on a free-flow electrophoresis apparatus (*11*) (also commercially available as Model FF4 Desaga, Heidelberg, Germany) in a buffer of low ionic strength [as described by Zeiller *et al.* (*6*) with minor modifications (*2*)] in an electric field of 100 volt/cm.

Figure 1 shows the distribution profiles of the three lymphocyte preparations. The curves have been resolved into Gaussian distributions (shaded areas) by a semigraphic method (*12*). The mean mobilities and standard deviations were also determined by this method. Both untreated lymphocytes

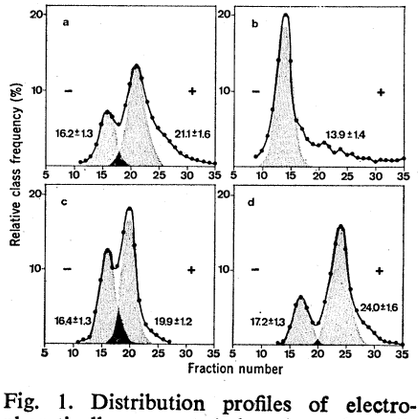

Fig. 1. Distribution profiles of electrophoretically separated lymph node cells from C-SSI mice: (a) untreated lymphocytes, (b) lymphocytes treated with *Vibrio cholera* neuraminidase, (c) lymphocytes treated with RPMI 1640, and (d) lymphocytes treated with *V. cholera* neuraminidase and EDTA at 4°C. Shaded areas represent the Gaussian distributions. The numbers indicate the mean mobility values ± standard deviations. The numbering of the fractions starts from the injecting point of the cell suspension and increases toward the anode (+).

and lymphocytes treated with medium only gave biphasic distribution profiles (Fig. 1, a and c). The neuraminidase-treated population gave a monophasic profile with a mean mobilty lower than that observed in either of the peaks of untreated cells (Fig. 1b). This experiment was repeated four times, with similar results.

A decrease in electrophoretic mobility may have been due to removal of charged groups, to adsorption of substances which thus covered charged groups, or to a rearrangement of the surface. The reaction mixtures were therefore also incubated in the presence of 10 m*M* ethylenediaminetetraacetic acid (EDTA) at 4°C, under which conditions *V. cholera* neuraminidase is enzymatically ineffective. This treatment did not disturb the diphasic distribution profile (Fig. 1d). Thus adsorption of neuraminidase cannot be the reason for the observed changes. These cells migrated somewhat faster than the untreated controls, perhaps because of the chelating action of EDTA, which alters the electrophoretic mobility of cultured cells (*13*).

There could have been selective loss of either B or T cells during neuraminidase treatment and the electrophoresis procedure. Therefore, samples from the peak fraction of the neuraminidase-treated and electrophoretically separated cells were tested with antiserum to MBLA and antiserum to θ (*3, 4*). In the presence of complement, 28 percent of the cells were killed by the antiserum to MBLA, as judged by the trypan blue dye exclusion test, and 58 percent were killed by antiserum to θ (Table 1). This roughly corresponds to the amounts of B and T cells in the untreated material and proves that selective loss has not taken place.

Thus, the difference in electrophoretic mobility and hence in the density of charged groups on the surfaces of B and T lymphocytes seems to be due to different amounts of sialic acid exposed at the electrokinetic plane of shear. The θ antigen cannot be responsible for the difference, since thymocytes on which the density of θ antigen is higher than on mature T cells (*14*) have the same electrophoretic mobility as B cells (*2*), and since both the MBLA and the θ antigen are still demonstrable on neuraminidase-treated lymphocytes. This indicates that sialic acid is not an essential part of these surface antigens.

Our findings, however, do not necessarily mean that the total amount of sialic acid on B and T cells is different. A definite possibility is that some of the sialic acid groups on the B cells are covered. Lymphocyte surface receptors are considered to be entirely immunoglobulins. They exist on the surface of both B and T cells, but are more abundant on B cells (*15*). The hypothesis that the low electrophoretic mobility of B cells is due to covering of some of the charged groups by receptor antibody molecules is supported by the fact that treatment of various types of cells with specific antibodies causes measurable reduction of the surface charge density, provided that the density of bound immunoglobulins is high enough (*16*). This does not explain why thymocytes have a low electrophoretic mobility (*2*). To obtain convincing evidence about whether or not the low electrophoretic mobility of B lymphocyte is due to the covering effect of surface immunoglobulins, it would be necessary to remove these immunoglobulins specifically.

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Acquisition of Key-Pecking via Autoshaping as a Function of Prior Experience: "Learned Laziness"?

Abstract. *A group of pigeons that had previously received noncontingent food delivery acquired the key-peck response (in autoshape training) more slowly than did a naive control group; key-peck acquisition was most rapid for a group given operant treadle-press training in the initial phase.*

Consider the following experiment reported by Seligman and Maier (*1*). In the initial stage of training, dogs were strapped in a Pavlovian harness and administered inescapable, unavoidable shocks on a random schedule. Twenty-four hours later they were placed in a shuttle box and given training during which the animal could escape or avoid the shock by jumping over a hurdle that separated the two compartments of the apparatus. About 65 percent of the subjects given the inescapable shocks failed to learn the escape response in the shuttle box, whereas only 6 percent of a naive control group failed to escape. The authors' interpretation was that during the first stage, the subject learns that instrumental behavior has no effect on either the onset of the aversive stimulus or its termination. When the subject is placed in the shuttle box with the same aversive stimulus present, he responds with the "expectation of helplessness" derived from previous experience with a problem that lacked an instrumental solution. After this and other demonstrations of "learned helplessness," this phenomenon was studied in an attempt to establish the necessary and sufficient conditions for its development (*2, 3*).

Maier *et al.* speculated (*2*) that a parallel phenomenon might exist in an appetitive situation; that is, a subject given repeated exposure to noncontingent reinforcement might be retarded in acquiring a response that now procures reinforcement. Most appetitive responses are acquired, however, through a shaping procedure involving reinforcement of successive approximations to the desired response. Since this is typically done manually, it is difficult