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THE DISTRIBUTION OF ELECTROLECTIN IN MOUSE: GENETIC AND ONTOGENIC VARIATIONS

Giovanni Levi and Vivian I. Teichberg

Department of Neurobiology
The Weizmann Institute of Science
Rehovot 76100, Israel

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Summary Electrolectins are β-D-galactoside binding lectins found in teleosts, avians and mammals. We have studied the distribution of electrolectin in several inbred strains of mice and have followed the changes in electrolectin activity occuring during development. Electrolectin activity is present in skeletal muscle, kidney, lung, heart, thymus and spleen of the young animal. The levels of electrolectin activity present in skeletal muscle, heart, lung, kidney and liver keep increasing up to 14 days after birth and then steadily decrease. In contrast, the level of electrolectin activity in the thymus and in the spleen remain constant during development. These results are discussed in view of the possible role of electrolectin in differentiation.

Extracts of a number of embryonic and adult animal tissues , of different origins, contain a β-D-galactoside specific lectin (1-7). Some of these lectins, designed here as electrolectins, have been purified and partially characterized, but their biological function remains still elusive. Electrolectins from different species and different organs share common physico-chemical characteristics : they have the same sugar specificity, similar molecular weight and need reducing agents for the maintenance of their activity; moreover electrolectins from different species crossreact immunologically (3,4,6,8) showing that at least some structural determinants are maintained during evolution. The wide distribution and the structural similarity of electrolectins in the animal kingdom strongly suggest that these proteins possess a biological function

We have recently observed that electrolectin has a prophylactic and therapeutic action on the experimental model of the autoimmune disease Myasthenia Gravis (9). To explain this effect, we have proposed a role of electrolectin in the process of maturation of immature thymocytes to

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mature T suppressor cells (10). This hypothesis is supported by observations that the mouse thymus contains an electrolectin that binds selectively to immature thymocytes (10). The mouse thymic electrolectin was purified and found to cross-react immunologically with a similar lectin from the electric organ of Electrophorus electricus (8).

In order to elucidate the role of electrolectin in the mouse immune system and to determine whether electrolectin levels can be correlated to the genetic susceptibility of mice to experimental Myasthenia Gravis (11), we have studied the distribution of this protein both in lymphoid organs as well as in the heart, lung, kidney and skeletal muscle removed from mice of different strains. We also present here the results of a study of the changes in electrolectin activity in these tissues during postnatal development.

MATERIALS AND METHODS

Animals Female mice aged 2-3 months (Jackson) were used throughout this study. The distribution of EL activity was assayed in female C57BL/6J mice of ages ranging from 2 to 18 days.

Preparation of extracts Organs were excised from two animals and cleaned from any extraneous tissue. Organs were then homogenized into 2 ml of cold phosphate buffer (pH 7.4) containing 14 mM 2-mercaptoethanol using an Ultraturax table homogenizer (2 min). The homogenates were then centrifuged in the cold for 30 min in an Eppendorf centrifuge. The supernatants were separated, centrifuged again as above for 30 min and immediately assayed for the presence of electrolectin activity and total protein concentration.

Assay of EL activity The activity of EL was assayed by measuring its ability to agglutinate trypsin treated erythrocytes. The procedure followed and the definition of agglutinating unit was essentially similar to the one described in (1) with the only difference that the final point of agglutination was determined by direct microscopic observation. For measuring the electrolectin activity in tissue homogenates containing less than 4 units/50 μl, the drop test described in (2) was used. All the observed agglutinating activity was lactose blockable. The specific activity was calculated by dividing the total agglutinating activity present in 1 ml of extract by the total protein concentration of the same extract.

Determination of protein concentration. The fluorescamine procedure described in (12) was followed using, as reference, standard solutions of bovine serum albumin.

RESULTS

Saccharide specificity of the mouse electrolectin. When extracts of various organs of the mouse are mixed with a suspension of trypsinized rabbit erythrocytes, agglutination occurs within 20 minutes. The clumps

formation can easily be followed under the microscope. No agglutination occurs with non-trypsinized erythrocytes. We tested a number of saccharides for their ability to inhibit the hemagglutinating activity of the mouse extracts. The saccharide concentration needed to inhibit 50% of the hemagglutinating activity is 0.2 mM for thiodigalactoside, 0.8 mM for lactose and 50 mM for galactose. The following saccharides were found to be ineffective in blocking the hemagglutinating activity at concentrations up to 50 mM : N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, D-mannose, D-glucose, D-fucose, L-fucose, sucrose, mellibiose, maltose, cellobiose.

Distribution of electrolectin in different organs and strains of mice.

We have measured the titer of lactose-blockable agglutinating activity in extracts of different organs and strains of adult (>60 d) mice. As seen in Table 1, electrolectin is present in the thymus, spleen, lungs,

TABLE 1
DISTRIBUTION OF ELECTROLECTIN ACTIVITY IN ADULT MICE

		Agglutinating units / mg protein					
STRAIN	H-2 Haplotype	THYMUS	SPLEEN	LUNG	KIDNEY	MUSCLE	HEART
A/J	a	25	11	12	6	ND	ND
B10.A	a	16	9	12	3	7	8
C57BL/6J	b	22	17	ND	2	3	8
C3H/SW	b	25	15	9	1	6	9
CWB	b	20	17	11	3	2	5
BALB/C	d	18	8	7	4	5	ND
DBA/2	d	22	10	9	3	5	ND
C3H/Hej	k	28	15	12	4	5	ND
CKB	k	21	8	11	3	4	ND
AKR/Cu	k	27	15	ND	3	4	ND
DBA/1	q	25	12	5	2	5	ND
SWR	q	30	28	10	4	6	4
SJL/J	s	17	19	11	2	6	ND
ASW	s	18	13	20	5	5	4

ND : not detectable.

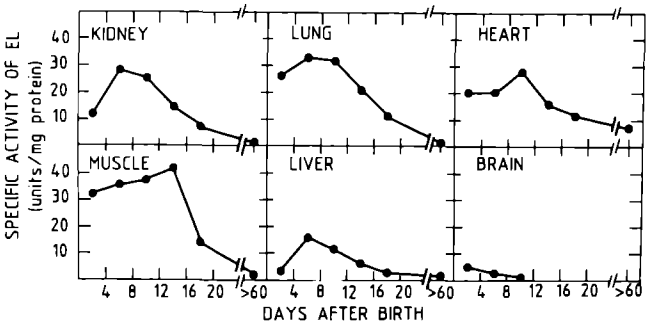

Figure 1. Ontogenic variations of electrolectin activity in selected organs of C57BL/6J strain of mice.

kidney and skeletal muscles of most of the strains analyzed. In the heart, electrolectin activity can be detected only in some of the strains; we could not detect any electrolectin activity in the brain and liver of adult mice of any strain. In adult mice, the specific activity of electrolectin is systematically higher in the immune system (thymus, spleen) than in any other tissue. Differences in the specific activity of electrolectin were more pronounced between different organs than between different strains.

Distribution of electrolectin activity in developing mouse tissues.

We have measured the variation in the titer of electrolectin specific activity during postnatal development of newborn C57BL/6J mice. As shown in Figure 1, the electrolectin titer in the kidney, lung, heart, liver and skeletal muscles is strongly developmentally regulated. In the kidney, lung, liver and heart it reaches a maximum between day 6 and day 10 of postnatal development and then decreases. In skeletal muscles, the titer of electrolectin remains high up to day 14 and than sharply decreases, the specific activity of electrolectin being 20 times higher in a 14 days old mouse than in a 60 days old animal.

Figure 2 shows the ontogenic evolution of electrolectin activity in the thymus and spleen of C57BL/6J mice. In contrast to all the other tissues studied, electrolectin activity remains constant during postnatal development and even increases in adult animals.

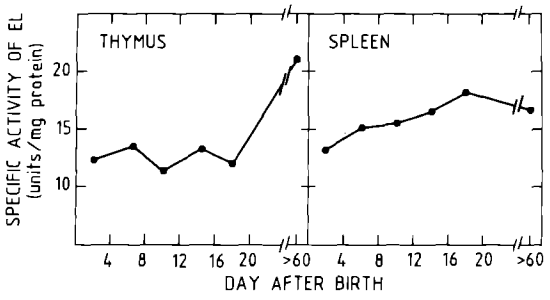

Figure 2. Ontogenic variations of electrolectin activity in the immune system of C57Bl/6J strain of mice.

DISCUSSION

In this study, we report the distribution and ontogenic variations of electrolectin activity in various strains of mice. Electrolectin activity could be detected in the fourteen strains of mice tested. In all strains, the level of electrolectin activity found in adult tissues was highest in the thymus and in the spleen and lowest in the kidneys and skeletal muscles. No obvious correlation was observed between the titer of electrolectin and strains representing different haplotypes of the major histocompatibility complex (H-2). There was also no correlation between the electrolectin levels and mouse susceptibility to experimental autoimmune Myasthenia Gravis (11). The electrolectin activity was found to be strongly developmentally regulated. The levels of electrolectin in skeletal muscle, heart, lung, kidney and liver increase during postnatal development and than steadily decreases. In contrast, the levels of electrolectin in the thymus and in the spleen remain constant and even increase during development. A similar postnatal regulation of electrolectin activity has been observed in the rat lung (13); in the rat brain however, the titer of a lectin with a sugar specificity similar, but not identical, to that of electrolectin reaches a maximum ten days after birth (14) while in the mouse brain, electrolectin is virtually absent. In the chick, the concentration of electrolectin is maximal between day 8 and day 14 of embryonal development (15,16).

The wide distribution of electrolectin activity and its developmental regulation suggest a general role of this protein in terminal

differentiation. Indeed, the tissue concentration of electrolectin clearly drops when organ differentiation is completed; in the immune system, where lymphocyte differentiation continues in adulthood, the titer of electrolectin remains high. Electrolectin represents one of the few cases in which it is possible to correlate the expression of a protein to the developmental state of a tissue. The presence of electrolectin in the immune system further supports the suggestion of a specific role of this lectin in the maturation of lymphocytes (10).

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