Distribution of Immunoregulatory Carbohydrate Receptors for Lectins on Membranes of Neurons and Syngeneic Peripheral Lymphocytes

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Common immunoregulatory carbohydrate receptors on the membranes of neurons and sysngeneic peripheral lymphocytes of mice are detected by using lectins. Brain neuron membranes possess no receptors characteristic of immature lymphocytes. The common immunoregulatory receptors on brain neurons and mononuclear cells of peripheral immune organs are shown to represent one of the mechanisms of integration of the nervous and immune systems.

Key Words: *carbohydrate lectin receptors; neurons; lymphocytes; immunoregulation*

According to current views, many immunoregulatory effects: proliferative, suppressor, and contrasuppressor, are mediated through the binding, expression, or hindrance of certain carbohydrate determinants of lymphokines and membrane receptors of immunocompetent cells by sialic acids [3-5,8]. Several types of carbohydrates, namely D-galactose, D-mannose, N-acetylglucosamine, N-acetylgalactosamine, a-fucose, and neuraminic acid, are known to be typically involved in various regulatory reactions between cells [3]. It is known, in particular, that the proliferative response of lymphocytes is primarily induced by mannose-specific receptors, starting from the production of IL-1 by macrophages whose activity is determined by the expression of D-mannose [9], with the subsequent production of IL-2, another mannose-specific glycoprotein, by T lymphocytes [11], and including the production and secretion of IgM, where D-mannose is a constituent of the carbohydrate core of the Fc-fragment [5]. Expression of N-acetylglucosamine on the membrane of T and B lymphocytes is required for the realization of the suppressor effect [4], whereas lymphocytes adherent for *Vicca villosa* lectin, which specifically binds N-acetylgalactosamine, possess a contrasupressor effect [8]. At the same time, the above carbohydrate have been found to be constituents of brain gangliosides [1].

Accumulated experimental data imply the commonness of immunoregulatory receptors on cell membranes in the nervous and immune systems. The identification of common immunoregulatory receptors on neurons and peripheral blood mononuclear cells may provide a basis for deliberate modification of their structure for the creation of new preparations and methods of diagnosing and treating various CNS disorders. In this connection, the aim of the present investigation was a parallel study of the expression of lectin receptor on neurons and syngeneic lymphocytes in experimental mice in order to clarify one of the mechanisms of integration of the nervous and immune systems.

MATERIALS AND METHODS

Brains of C57Bl/6 mice were aseptically removed into Eagle medium, dissected with a needle, and left at room temperature during 1 hour. Then the

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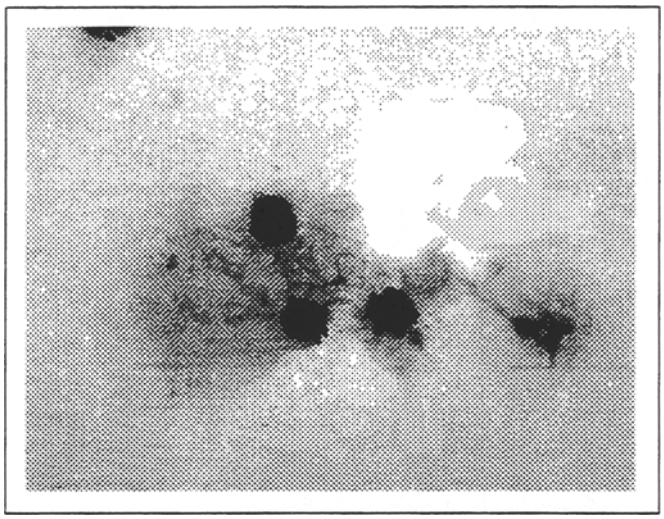

Fig. 1. Neurons isolated on sucrose gradients and stained with horseradish peroxidase–labeled WGA lectin and additionally stained with methyl green. Shadows of peroxidase–negative neurons are seen. ×600.

obtained cell suspension was layered onto a sterile gradient of 2, 1.32, 1.2, and 0.9 M sucrose and centrifuged at 200 g for 1 hour in a horizontal rotor. The cell fraction above 2 M is enriched with neurons by 85%, and between 1.32 and 1.2 M with glia cells by 70-75%. Syngeneic lymphocytes were routinely isolated from the spleen, thymus, and mesentery lymph nodes in a 1.077 Ficoll-Verographin gradient. Carbohydrate receptors on lymphocytes and neurons were assayed by the direct immunoperoxidase method using horse-radish peroxidase-labeled lectins (Lektinotest, Lvov) according to recommendations described earlier [2] on "dried drop" preparations (Figs. 1 and 2). The following lectins were used: soybean (SBA), laburnum (LCA), peanut (PNA), elder (SNA), wheat-germ (WGA), and lentil (LCL). The cells with a dark-brown rim were morphologically counted (×90 objective, ×11 ocular) and photographed using an Ibos microscope in an immersion system (×600).

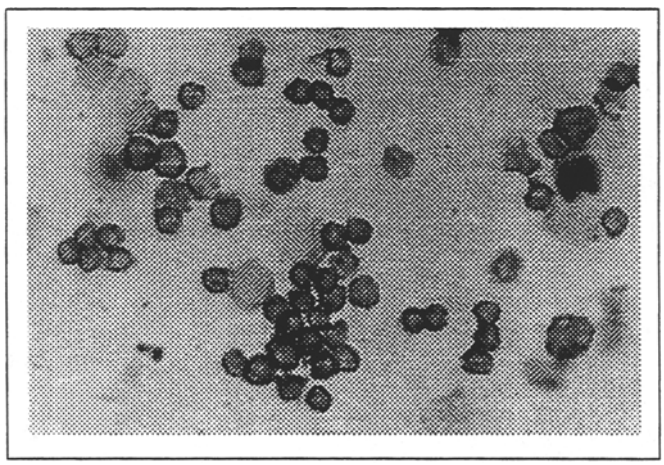

Fig. 2. Lymphocytes of C57Bl/6 mouse isolated from the thymus and stained with horseradish peroxidase–labeled WGA lectin and additionally stained with methyl green. ×600.

RESULTS

Different distributions of immunoregulatory receptors were found on thymus, spleen, and mesentery lymphocytes of the C57Bl/6 mice (Table 1). Thus, D-galactose- (PNA^+) and D-mannose- (LCL^+) specific receptors reliably prevailed in the thymus membranes; a-fucose (LCA^+) was more often presents in splenocytes (Table 1). No receptors for peanut lectin (PNA^+) and only 9% mannose-specific (LCL^+) receptors were expressed on the mesentery lymphocytes (Table 1). Receptors for soybean lectin (SBA^+), which are N-acetylgalactosamine-specific receptors, were approximately equally expressed in the thymus and spleen lymphocytes, and reliably less expressed in the mesentery lymphocytes. N-acetyl-neuraminic acid-N-acetylglucosamine disaccharide (WGA^+-receptor) were determined in 70-85% of immunocompetent cells, while receptor containing neuraminic acid (SNA^+) was expressed on 70-84% of thymocytes and splnocytes, only on 28% of lymphocytes from the mesentery lymph nodes. Moreover, no receptors for peanut lectin (PNA^+), a marker of immature cortical lymphocytes [6], were determined on the mesentery lymphocytes.

Many lymphocytes from the thymus and spleen supposedly possess receptors involving both D-sugar and N-amino-sugar moieties. It may be assumed that the quantitative ratio between various types of receptors determines the expression of the carbohydrate determinant, which acquires specificity for realizing the corresponding function. Neuronal membranes have not been found to possess SBA^+ and PNA^+ receptors, markers of immature lymphocytes [6], LCA^+ receptor has been determined on about 32% of cells, this being similar to its percentage on thymocytes (Table 1). D-mannose is expressed on 16% of neurons. D-mannose has been shown to determine the regulatory effect of IL-1 [9]. It has also been found that the blood-brain barrier (BBB) in the hypothalamic area is permeable for IL-1 [7], and some neurosecretory neurons of the hypothalamus possess IL-1 receptors [7]. In this connection, it may be surmised that one of the mechanisms of integration of the nervous and immune systems is mediated via mannose-specific receptors of peripheral mononuclears, lymphokines, and neurons. It should be emphasized that in our study the majority of neurons and immune cells expressed N-acetyl neuraminic acid-N-acetylglucosamine disaccharide

TABLE 1. Distribution of Immunoregulatory Carbohydrate Receptors for Lectins on Neurons and Syngeneic Peripheral Immune Cells

Type of cells	Distribution of Lectin Receptors, %					
	soybean (SBA)	laburnum (LCA)	peanut (PNA)	elder (SNA)	wheatgerm (WGA)	lentil (LCL)
Cells of brain neuronal fraction ($n=5$)	–	32.1±1.5	–	43.8±19.6	60.9±5.5	16.3±1.8
Spleen lymphocytes ($n=5$)	56.5±10.5	72.1±2.3	13.5±2.5	70.6±5.7	85.7±3.4	28.0±3.5*
Thymus lymphocytes ($n=5$)	62.0±2.6	49.5±4.5	78.9±9.0*	84.0±6.4	78.0±8.0	55.6±3.0*
Mesentery lymphocytes	27.0±2.3	12.5±3.1	–	28.5±4.5*	70.5±3.1	9.0±0.9*

Note. *: The differences between cell types are reliable ($p<0.01$).

(WGA$^+$). Receptors for WGA have also been found to be expressed on supressor T and B lymphocytes [4,8], and on brain capillary endotheliocytes [10]. In line with this the obtained data suggest, on the one hand, a possible immunoregulatory mechanism of nervous and immune system integration, effected through common carbohydrate receptors expressed on brain neurons and immune cells and mediated by lymphokines, and, on the other, the existence of a reliable local protection of the brain cells, mediated through a predominance of markers of suppressor cells (WGA receptors) on neurons.

It may be surmised that the WGA receptors, markers of suppressor cells, detected on neuronal membranes regulate the proliferative response of lymphocytes crossing the BBB and, together with the BBB, represent a system guaranteeing the immunological isolation of the brain.

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