CHANGES IN NUMBER OF NEUROTRANSMITTER RECEPTORS ON MOUSE LYMPHOCYTES DURING ONTOGENY

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Neurotransmitters play an important role in the development of immune reactions by their effect on functional activity of immunocompetent cells [1-3, 6-8], which is connected with the presence of specific receptors for them on the lymphocyte membrane. The number of receptors for neurotransmitters and the associated reactivity of lymphocytes to neurotransmitters can be modified by various procedures and also in the course of the immune reactions themselves [1-3].

Immunologic reactivity is known to undergo considerable fluctuations during ontogeny: it increases in the postnatal period of development, then stabilizes, and declines with the onset of old age [5]. The problem arises of the connection between changes in immunologic reactivity during ontogeny and expression of lymphocyte receptors for neurotransmitters.

In the investigation described below the number of receptors on lymphocytes of the mouse spleen to β-adrenergic and muscarinic cholinergic agonists and to the neuropeptide Met-enkephalin, and also reactivity of lymphocytes to adrenalin were investigated during ontogeny.

EXPERIMENTAL METHOD

Experiments were carried out on BALB/c mice aged 1-2 days and 1, 3, and 12-15 months (old). Suspensions of cells were obtained from the spleen of separate groups of mice, and layered above a solution of Ficoll–Verografin with a density of 1.09 g/cm^3, and centrifuged with acceleration of 200 g for 30 min in the cold. The lymphocytes were removed from the interphase, washed by centrifugation, and suspended in medium 199. The number of living cells was counted with the aid of trypan blue, and it was usually over 95%. To determine the number of β-adrenergic receptors on a lymphocyte, a labeled blocker of receptors of this type was used, namely ^{3}H-dihydroalprenolol, with specific activity of 82 Ci/mmole ("Amersham," England). Samples each containing $5 \cdot 10^6$ lymphocytes were incubated with the radioligand in a concentration of 8 nM without (to determine total binding) and in the presence of the β-adrenoreceptor blocker propranolol, in a concentration of 10^{-5} M (to determine nonspecific binding) at 23°C for 20 min, after which the number of binding sites, i.e., the number of β-adrenoreceptors on a lymphocyte was determined by the method developed previously [3]. To determine the number of muscarinic acetylcholine receptors, the labeled blocker ^{3}H-quinuclidinyl, with specific activity of 36 Ci/mmole ("Amersham") was used. The samples contained $70 \cdot 10^6$ lymphocytes, and were incubated with the radioligand in a concentration of 8 nM without and in the presence of atropine in a concentration of 10^{-4} M at 23°C for 60 min, after which the number of muscarinic acetylcholine receptors on a lymphocyte was determined by a method developed by the present writers [1]. To determine receptors for the neuropeptide, binding of labeled ^{125}I-enkephalin with specific activity of 2000 Ci/mmole ("Amersham") was studied. Samples each containing $5 \cdot 10^6$ lymphocytes were incubated at 23°C for 20 min with the radioligand in a concentration of 0.2 nM, which, according to preliminary data, was found to be optimal for determination of total binding without, and in the presence of the unlabeled analog – dalargin. By using ordinary calculations to determine the number of binding sites of the radioligands on a lymphocyte [1, 2] the number of enkephalin receptors was determined.

To determine all types of binding of the radioligands with the cells, bound radioactivity was recorded after the cells had been washed twice on a liquid β-scintillation counter.

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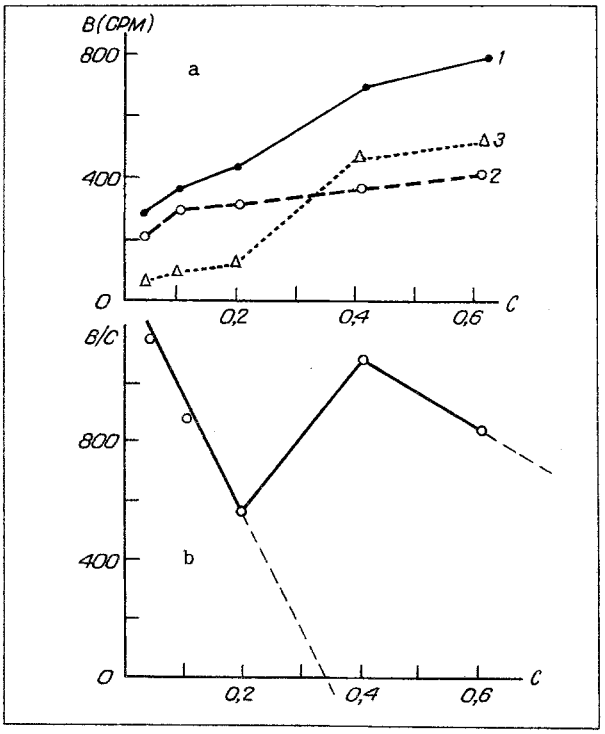

Fig. 1. Dose-dependent binding of ^{125}I-Met-enkephalin with splenic lymphocytes from adult animals. Abscissa, concentration of added ligand (in nM); ordinate, bound radioactivity: initial data (a) or ratio of bound radioactivity to concentration of added ligand (b). 1) Total binding; 2) background radioactivity; 3) specific binding.

TABLE 1. Number of Receptors for Neurotransmitters on Mouse Spleen Lymphocytes during Ontogeny (M ± m)

Type of receptor	Number of binding sites per cell at age of			
	1-2 days	1 month	3 months	1 year
β-adrenoreceptors	2740±320*	4470±415	3600±335	2260±219*
Muscarinic acetylcholine receptors	———	440±52	320±26	140±12*
Receptors for Met-enkephalin	———	21±2	14±5	11±2*

Note. Asterisk indicates significant differences compared with group of 1 month ($p < 0.05$).

TABLE 2. Reactivity of Lymphocytes of Young and Old Mice to Adrenalin (M ± m)

Type of receptor	Number of binding sites per cell at age of		
	1	3	12
β-adrenoreceptors	1,10±0,12	0,77±0,10	0,58±0,04*
Muscarinic acetylcholine receptors	2,75±0,31	1,60±0,15*	1,10±0,01*
Receptors for Met-enkephalin	1,65	0,83	0,52

Note. M) Arithmetic mean calculated from 6-8 measurements. Number of animals for each group was 55-70 (but 45 for newborn group).

Reactivity of lymphocytes from mice of different age groups to adrenalin was estimated by accumulation of CAMP in the cells after incubation for 10 min with adrenalin in a concentration of 1 μM at 37°C [3]. cAMP was determined with the aid of the appropriate kit ("Amersham").

EXPERIMENTAL RESULTS

Determination of the absolute number of neurotransmitter binding sites on lymphocytes from mice of different ages showed that their number varies sharply in the course of ontogeny (Table 1). For all types of neuroreceptors studied, their largest numbers per cell were noted in young mice (aged 1 month), after which a decline continued through maturity into old age. In newborn mice the number of β-adrenoreceptors also was lower than in the young age group, evidently indicating immaturity of the immune system in this period.

For Met-enkephalin, the number of binding sites given is a conventional value, for the dose-dependent binding curve does not give one binding site when analyzed by Scatchard plot: the curve is complex in character and intersects the axes of coordinates at two points

(Fig. 1). This result is in agreement with data in the literature and it evidently indicates the presence of several binding sites for the neuropeptide, with different dissociation constants [4].

Reactivity of the cells, recorded as accumulation of cAMP in the lymphocytes, showed a similar fall with age (Table 2). A decrease was observed both in the background cAMP level and in the adrenalin-induced increase of cAMP after exposure of the cell to adrenalin.

Thus during ontogeny the sensitivity of mouse lymphocytes to neurotransmitters falls uniformly, except in the newborn period; this may be one mechanism of the change in immunity in old age observed both in man and in animals.

The main cause of the decrease in the number of receptors to neurotransmitters in old age may be, for example, depression of cellular metabolism, leading to slowing of resynthesis of receptors on the cells.

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PHENOTYPE AND FUNCTION OF LYMPHOCYTES FROM COLITIS PATIENTS

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Research by many workers aimed at studying the immune status of patients with nonspecific ulcerative colitis (NSUC) have yielded evidence of changes in the functional powers of T lymphocytes [8, 13]. The writers showed previously [1] that in NSUC there is disproportion between the ratios of subpopulations posessing helper/suppressor activity: T_G^+/T_G^-, theophylline-sensitive/theophylline-resistant (tps-1/tpr-1) lymphocytes. In severe forms of pathology not only are changes found in the proportions of alternative subpopulations, but unity between the parameters tps-1 and T_G^+, and trp-1 and T_G^- is absent: a decrease in tpr-1 and an increase in tps-1 coexist with an increase in T_G^- and a decrease in T_G^+. In accordance with data obtained in [4, 12], trp-1 and T_G^- and tps-1 and T_G^+-lymphocytes belong to immunoregulatory subpopulations and possess a common range of functions. The functional capacity of T_G^- and T_G^+ was assessed previously [2].

The aim of the present investigation was to test the functional activity of tpr-1 and tps-1 with the aid of the local heterologous graft versus host reaction (GVHR) and to determine the cytochemical status of the lymphocytes, i.e., methods which were used to study T_G^+ and T_G^--lymphocytes.

EXPERIMENTAL METHOD

Mononuclear cells were obtained from human peripheral blood in the usual way [6] by gradient centrifugation. To obtain the lymphoid population, monocytes were removed from a mononuclear suspension by adsorption on plastic Petri dishes for 60 min. Nonadherent cells were added to an equal volume of sheep's erythrocytes (3% suspension made up in medium 199 with 40% fetal calf serum – FCS) and the ordinary E-RFC test was carried out, followed by

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