NATURAL AUTOANTIBODIES OF NEONATAL RATS DETECTABLE BY THE HYBRIDOMA TECHNIQUE

L. Z. Yakubov, A. V. Sakharova, N. V. Romanova,
V. V. Cherepakhin, and O. V. Rokhlin

UDC 612.112.94.017.1:
612.648].086.83

KEY WORDS: natural autoantibodies; hybridomas; ontogeny.

Autoantibodies detectable in the serum of unimmunized animals [10] and healthy human subjects [6, 8] are regarded as natural autoantibodies (NAA). Several hypotheses have been put forward to explain their functional role [6, 7], but additional factual material is required before final conclusions can be drawn. Prospects for the more penetrating study of the NAA phenomenon were opened up with the appearance of the hybridoma technique, whereby B lymphocytes producing monoclonal NAA (MNAA) can be cultured [2, 5, 7, 12, 13]. The presence of these B cells was found to be characteristic not only of adult, but also of newborn mice [2, 7].

The aim of this investigation was to detect potential NAA producers in the B-cell repertoire of neonatal rats.

EXPERIMENTAL METHOD

Splenocytes from 5-day-old (MSU × August)F_1 hybrid rats were provided by V. L. Yurin (All-Union Research Institute of Genetics and Selection of Industrial Microorganisms), and fused with myeloma P3/Ag8 653 cells [9] in the ratio of 1:1 as described previously [1]. The cells were seeded in selective HAT medium on eight 96-well plates (Flow Laboratories, England) at the rate of 150,000 splenocytes per well. RPMI 1640 culture medium and fetal calf and horse sera (Flow Laboratories) were used. The presence of immunoglobulins and their isotypes was determined in the wells with growing hybridomas by the radioimmunoadsorption inhibition method [4].

Antibodies (AB) used for testing the hybridomas were obtained as described previously [4]. Homogenates were prepared and hybridomas tested by radioimmunoassay (RIA) as in [6], with certain modifications. Tissue placed in 0.5% paraformaldehyde solution (Serva, West Germany) in phosphate-buffered saline (PBS) was minced in the cold in a glass homogenizer with ground-glass pestle and filtered through a sieve, washed 3 times in PBS with 2% bovine serum albumin (BSA, from Serva), and kept at −70°C. Culture media diluted 3 times with PBS containing BSA, were incubated for 1 h with homogenate, washed 3 times in PBS with BSA, and incubated for 1 h with the second AB, adsorbed beforehand on the target homogenate. Culture media from wells without hybridomas were used as the negative control, and hybridoma 2G10, revealed in preliminary hybridization by the intensive reaction with all homogenates, as the positive control. The usual values of the negative control were from 300 to 1500 cpm, and of the positive from 8000 to 20,000 cpm. The reaction with 2G10 was taken to be 100%. Hybridomas whose reactions with homogenate, after subtraction of the background, amounted to not less than 20% of the reaction of 2G10, were considered to be positive.

The immunocytochemical reaction with brain sections was carried out as described previously [2]. Immunofluorescence staining of mouse fibroblasts was done by the method in [3]. Solid-phase RIA on individual macromolecules followed the scheme described previously [11]. A reaction with values 5 times above the background level was considered positive.

EXPERIMENTAL RESULTS

Of 341 wells with hybridoma clones growing in them, a positive reaction in the test for immunoglobulin production was found in 81. The culture media of these hybridomas, which were found to contain monoclonal AB (MAB) of the IgM isotype only, were tested for the presence

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Institute of Neurology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 8, pp. 200-202, August, 1988. Original article submitted August 8, 1987.

 0007-4888/88/0008-1144$12.50

TABLE 1. Reactions of MNAA of Neonatal Rats in Different Screening Systems

Serial No.	Code numbers of hybridomas	RIA on homogenates			Immunocytochemical reaction on rat brain sections	Solid-phase RIA			Immunofluorescence staining on mouse fibroblasts
		rat brain	bovine brain	bovine liver		cardiolipin	G-action	BSA	
1	1G2	+	+	+	G	+	+	+	+
2	6G10	+	+	+	G	+	+	+	+
3	6C2	+	+	+	N	+	+	+	+
4	4D11	+	+	+	G	+	+	+	−
5	3D7	+	+	+	G	−	+	+	−
6	1C8	+	+	+	NG	+	+	−	−
7	7E5	+	+	+	N	−	+	−	+
8	5F9	+	+	+	NG	−	+	−	−
9	7G11	+	+	±	−	−	+	−	−
10	1B5	+	+	+	NG	−	−	−	−
11	8D6	+	+	+	E	−	−	−	−
12	7D7	+	+	+	NG	−	−	−	−
13	6D10	+	+	±	V	−	−	−	−
14	2B4	+	+	±	N	−	−	−	−
15	8D7	+	+	+	V	−	−	−	−
16	1E5	+	+	+	NG	−	−	−	−
17	3B3	+	+	−	NGE	−	−	−	−
18	4C2	±	+	−	−	−	−	−	−
19	5G8	+	+	+	−	−	−	−	−
20	4F8	+	+	±	−	−	−	−	−
21	7E9	+	+	+	−	−	−	−	−
22	6F6	±	+	+	−	−	−	−	−
23	1B9	−	−	−	E	−	−	−	−
24	1B11	−	−	−	E	−	−	−	−
25	2F4	−	−	−	E	−	−	−	−
26	2F8	−	−	−	E	−	−	−	−
27	6C2	−	−	−	G	−	−	−	−
28	8D7	−	−	−	ENV	−	−	−	−

Legend. −) No reaction; ±) magnitude of reaction less than 20% of the magnitude of control hybridoma 2G10, but more than 3 times above background level. G) Glial; N) neuronal type of reaction; E) reaction with ependyma; V) reaction with vessels.

of a reaction with autoantigens (AAG) by the following methods: a) RIA on homogenates of isogenous brain; b) the immunocytochemical reaction on isogenous brain sections. A positive reaction with brain homogenate was found for 22 hybridomas (Table 1), or 27% of the number tested. A similar number of positively reacting hybridomas was found in the immunocytochemical test on rat brain sections. The following groups of MNAA can be distinguished depending on the types of reactions observed in the immunocytochemical test: 1) those reacting with neuronal structures of nerve tissue; 2) those reacting with glial structures; 3) those reacting with ependymal cells; 4) those reacting with the vascular component of the brain. Some MNAA gave a mixed type of reaction. This diversity of reaction types is not characteristic of MNAA panels of neonatal and adult mice [2].

Besides testing on targets of isogenous origin, the MAB panel chosen for study was tested on heterologous targets: in RIA on bovine brain and liver homogenates, in the indirect immunofluorescence test with staining of the cytoskeleton of mouse fibroblasts, and in the solid-phase RIA on individual macromolecules (BSA, actin, cardiolipin; see Table 1).

A reaction also with bovine brain homogenate was given by 22 hybridomas (those which were found to be positive in the reaction with isogenous brain homogenate). This indicates the species-nonspecificity of those AAG epitopes, against which the NAA discovered were directed.

Testing all culture media in reactions with bovine liver homogenates revealed 20 positive — all belonging to the number of those reacting with brain homogenates. In other words, AAG determinants to which rat NAA are directed are not only species-nonspecific, but also organ-nonspecific.

Testing the hybridoma panel by the indirect immunofluorescence test with a primary mouse fibroblast culture revealed five MAB reacting with the cytoskeleton. They all belonged to the number of those reacting in the RIA test on homogenates and the immunocytochemical test on brain sections.

In some investigations MAB have been allotted to the NAA group on the basis of the presence of a reaction with individual isogenous macromolecules in tests of the ELISA type [7]. We also studied interaction of the MAB obtained with three individual macromoles, namely actin, cardiolipin, and BSA, in the solid-phase RIA test. We considered that substances of

nonisogenous origin could be used, for as the previous tests showed, all determinants of AAG against which the NAA which were found were directed, were species-nonspecific. Seven of the 22 MNAA which reacted with homogenates were found to be positive in this test. Four of them reacted with all three targets, thus exhibiting polyspecificity, which also was characteristic of MNAA from newborn mice [7]. However, it is not yet clear what precisely is meant by the term "polyspecificity," for it was found in the immunocytochemical test, for example, that the majority of these polyspecific MNAA give a reaction of glial, and not of mixed type.

It was thus found, in confirmation of data obtained previously on mice [2], that a significant proportion of neonatal rat hybridomas produces NAA. According to the results obtained by all types of screening, this proportion is 35% of the total number of immunoglobulin producers.

LITERATURE CITED

1. M. N. Petrosyan, A. V. Chervonskii, A. R. Ibragimov, et al., Dokl. Akad. Nauk SSSR, 256, 509 (1981).
2. A. V. Sakharova, L. Z. Yakubov, N. V. Romanova, et al., Zh. Obshch. Biol., 47, 625 (1986).
3. T. M. Svitkina, I. S. Tint, A. D. Bershadskii, and V. I. Gel'fand, Nonmuscular Motor Systems [in Russian], Moscow (1981), p. 76.
4. V. V. Cherepakhin, L. Z. Yakubov, A. R. Ibragimov, et al., Mol. Biol., 20, 1672 (1986).
5. R. J. Cote, D. M. Morrissey, A. N. Houghton, et al., Proc. Natl. Acad. Sci. USA, 80, 2026 (1983).
6. A. S. Daar and J. W. Fabre, Clin. Exp. Immunol., 45, 37 (1981).
7. G. Dighiero, P. Lymbery, D. Holmberg, et al., J. Immunol., 134, 765 (1985).
8. B. Guilbert, G. Dighiero, and S. Avrameas, J. Immunol., 128, 2279 (1982).
9. J. F. Kearney, A. Radbruch, B. Liesengang, et al., J. Immunol., 123, 1548 (1979).
10. S. E. Martin and W. J. Martin, Cancer Res., 35, 2609 (1975).
11. V. G. Niciforov, L. Z. Yakubov (L. Z. Jackubov), G. T. Bogachova, et al., FEBS Lett., 158, 113 (1983).
12. B. S. Prabhakar, J. Saegusa, T. Onodera, et al., J. Immunol., 133, 2815 (1984).
13. J. W. Yoon, S. Y. Shin, and C. J. Bachurski, Lancet, 2, 641 (1984).