The Use of Anti-Idiotypic Antibodies for the Correction of Autoimmune Reactions to Brain Protein S-100

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The possibility of eliminating hyperproduction of antibodies to brain protein S-100 in immunized mice through specific immunocorrection by anti-idiotypic antibodies to this protein or their Fab fragments is explored. Anti-idiotypic antibodies and their Fab fragments inhibit humoral immune response to S-100, which correlates with normalization of several behavioral responses.

Key Words: *anti-idiotypic antibodies; protein S-100; immunocorrection; autoimmunity; behavior*

As shown by enzyme-linked immunosorbent assays [8,10] and scratch tests [9], hyperproduction of autoantibodies and sensitization of the body to phylogenetically old brain protein S-100 are characteristic features of several mental and neurological diseases such as schizophrenia, epilepsy, multiple sclerosis, and some forms of dementia. Autoantibodies to S-100 make up to 2-3% of the total pool of circulating IgG in patients with epilepsy and up to 7-8% in patients with schizophrenia [3]. The pathogenetic significance of these autoantibodies and the mechanisms promoting their synthesis have not been elucidated. However, such high levels of autoantibodies to S-100 in the above-mentioned diseases do not correlate with the very low immunogenicity of this protein. It is believed that this property of S-100 is of adaptive nature and has developed in the course of evolution [1]. This apparent contradiction may be explained in terms of the immunoregulation theory. Anti-idiotypic antibodies (AIAb) against the epitopes of antigen-binding sites on primary antibodies or against lymphocyte receptors are one of the main regulators of the immune response [5]. It was reported that activation and suppression of the immune response to a particular antigen can be induced *in vivo* by administering AIAb in appropriate doses before immunization [4,7]; the mechanisms of this phenomenon remain unknown.

The aim of this study was to explore the possibility of correcting the immune response to S-100 proteins with the use of AIAb.

MATERIALS AND METHODS

S-100 protein (a mixture of S-100-a and S-100-b) was isolated from bovine brain as described previously [2]. Primary polyclonal antibodies to this protein were purified by immunoaffinity chromatography from sera of hyperimmune rabbits [1]. AIAb reacting with primary anti-S-100 antibodies were obtained by immunoaffinity chromatography on a column with immobilized primary antibodies against S-100. Fab fragments of the AIAb were prepared as described [6].

The production of autoantibodies to S-100 proteins was modeled in mice. The S-100:hemocyanin conjugate (molar ratio 6:1) prepared with the use of glutaraldehyde was mixed with an equal volume of complete Freund's adjuvant, and 120 adult mice were immunized with this preparation three times at 14-day intervals (10 μg of conjugated S-100 per mouse each time) intra- and subcutaneously into 8-10 points within the lymph node areas. Control

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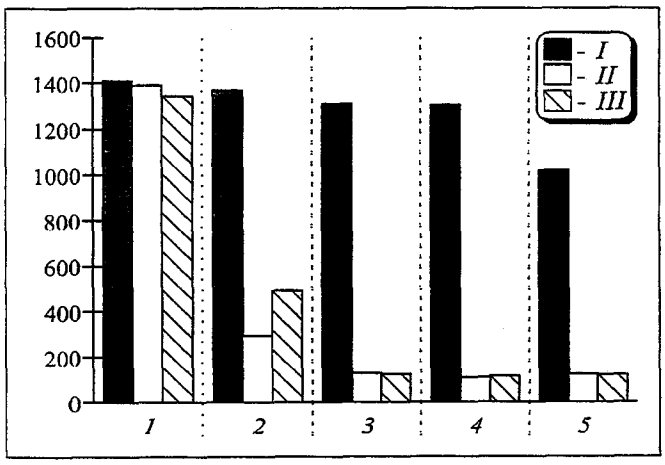

Fig. 1. Levels of anti-S-100 antibodies in mice injected with rabbit anti-idiotypic antibodies (AIAb). *1*) before AIAb injection; *2-5*) on days 2, 5, 7, and 28 after AIAb injection, respectively. Ordinate: anti-S-100 immunoreactivity (expressed in percent; anti-S-100 immunoreactivity in nonimmunized animals is taken as 100%). Here and in Fig. 2: *I*) mice given nonimmune rabbit IgG; *II* and *III*) mice given 1 μg and 100 μg of anti-S-100 AIAb, respectively.

mice (n=80) were immunized three times with the same doses of bovine serum albumin (BSA) conjugated with hemocyanin in complete Freund's adjuvant. Serum anti-S-100 reactivity was determined by the standard enzyme-linked immunosorbent assay (ELISA) on Nunc plates before and after immunization and after specific immunosuppression (on days 2, 5, 7, and 28). o-Phenylenediamine/H_2O_2 was used as the substrate. The reaction was read photometrically at a wavelength of 490 nm.

Anti-S-100 AIAb were injected once intraperitoneally in a dose of 100 μg or 1 μg in 200 μl of normal saline. Some experimental and control rats were injected intraperitoneally with nonimmune rabbit IgG, Fab fragments of anti-S-100 AIAb, or Fab fragments of nonimmune rabbit IgG (Table 1).

The behavior of mice was studied in an open field: a 70×70×70 cm box with nontransparent walls and the bottom divided into 49 equal squares and having 36 holes 3 cm in diameter. Behavioral reactions were evaluated before and after immunization with S-100 or BSA and after injection of AIAb (after 3 h and on days 2, 5, and 28). The following parameters were recorded: the total motor activity (the number of squares crossed), frequency of upright postures, frequency of exploring the holes, and grooming. Each session lasted 10 min.

The results were analyzed by Student's t test.

RESULTS

This study was designed to explore the possibility of suppressing humoral immune response to S-100 in mice with the use of specific AIAb or their Fab fragments.

As a result of three immunizations, mice developed high titers of anti-S-100 Ab (1:10,000 or higher), after which they received a single injection of rabbit anti-S-100 AIAb in a dose of 1 μg or 100 μg. As Fig. 1 shows, AIAb are an effective tool for correcting humoral immune response to S-100 proteins. By day 5 after the injection of anti-S-100 AIAb, serum immunoreactivity to S-100 was normalized and remained stable at least for 28 days. In control mice, neither nonimmune rabbit IgG (Fig. 1) nor its Fab fragments had any significant effect on serum immunoreactivity to S-100.

On day 2 after injection of 1 μg AIAb, the immunosuppressive effect was significantly greater than that of 100 μg AIAb (p<0.05). This may result from dose-dependent differences in the sensitivity to AIAb of immunocompetent cells (for example, T helpers and T suppressors) involved in modulating the immune response. A similar but less pronounced effect was observed in mice injected with Fab fragments of anti-S-100 AIAb (data not shown).

The results of the open field experiments are shown in Fig. 2. The mice immunized with anti-S-

TABLE 1. Preparations Administered to Test and Control Mice

Preparation	Conjugate-immunized mice			
	S-100—hemocyanin		BSA—hemocyanin	
	dose, μg	No. of mice	dose, μg	No. of mice
AIAb to S-100	100	20	100	10
	1	20	1	10
Nonimmune IgG	100	10	100	10
	1	10	1	10
Fab fragments of AIAb to S-100	70	20	70	10
	0.7	20	0.7	10
Fab fragments of nonimmune IgG	70	10	70	10
	0.7	10	0.7	10

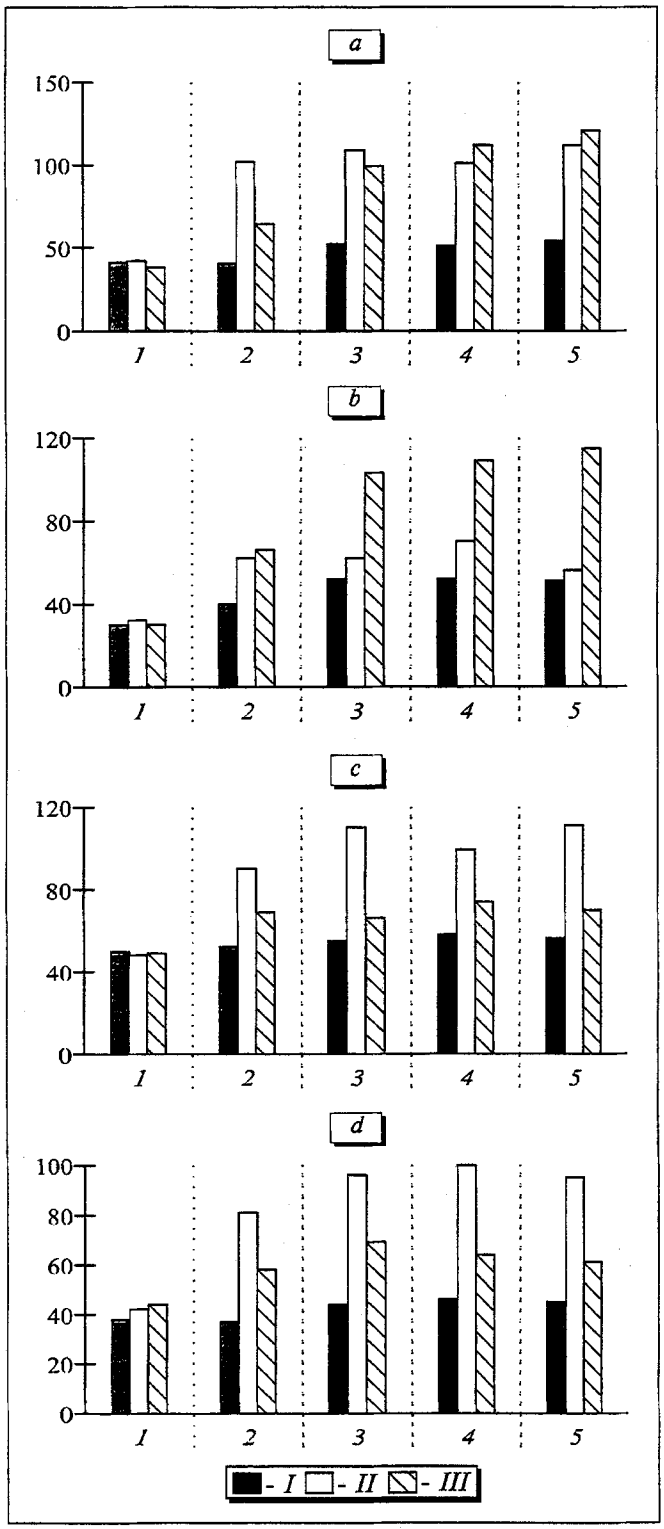

Fig. 2. Changes in the behavior of mice in the open field test: *a*) total motor activity; *b*) grooming; *c*) frequency of upright postures; *d*) frequency of exploring holes. Testing times: *1*) before AIAb injection; *2-5*) at 3 h and on days 2, 5, and 14 after AIAb injection, respectively. Ordinate: behavioral parameters (expressed in percent; behavioral parameters of nonimmunized animals are taken as 100%).

100 AIAb showed low motor and exploratory activities, whereas no appreciable changes in behavioral reactions were observed in mice immunized with BSA. The total motor activity, the number of upright postures, and the number of holes explored were similar to the initial levels as early as 3 h after injection of anti-S-100 AIAb in a dose of 1 μg ($p>0.2$ compared with the initial levels) and remained unchanged for at least 28 days. The level of grooming remained slightly increased. In mice given anti-S-100 AIAb in a dose of 100 μg, the number of upright postures and the number of hole explored did not reach the initial levels, but the total motor activity and grooming behavior were normalize on day 2 after the AIAb injection. Changes in the behavior of mice injected with Fab fragments of anti-S-100 AIAb were less pronounced. Neither IgG from nonimmune rabbits nor its Fab fragments had any appreciable effect on the behavior of control mice.

Our results indicate that immunoregulatory mechanisms may be implicated in the pathogenesis of a number of neurological and mental diseases. It was shown that the hyperimmune response to S-100 can be corrected with specific AIAb. This effect may be associated with specific stimulation of immunocompetent cells. Changes in the behavior of mice correlated with hyperproduction of anti-S-100 AIAb. Some nervous system disorders may be caused by impairments of the mechanisms underlying the tolerance to particular antigens of the nerve tissue as a consequence of congenital or acquired defects of the immune system.

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REFERENCES

1. S. V. Belyaev, T. I. Kupriyanenko, N. P. Lysova, *et al.*, *Biokhimiya*, **46**, 2193-2201 (1981).
2. A. B. Poletaev, "Brain-specific proteins of group S-100, their endogenous acceptors and ligands, and the regulation of metabolic processes in nervous tissue," Author's synopsis of Doct. Med. Sci. Dissertation [in Russian], Moscow (1987).
3. A. B. Poletaev and O. P. Selifanova, *Neirokhimiya*, No. 6, 572-580 (1987).
4. I. M. Roitt, in: *Essential Immunology*. 6th ed., Oxford (1988).
5. R. V. Colvin and K. A. Olson, in: *Concepts of Immunopathology*, Eds. J. M. Cruse *et al.*, Vol. 1, Basel (1985), pp. 133-172.
6. S. Demignot, M. C. Garnett, and R. W. Baldwin, *J. Immunol. Methods*, **121**, No. 2, 209-217 (1989).
7. S. L. Epstein, S. O. Sharrow, and D. H. Sachs, *Ann. Inst. Pasteur. Immunol.*, **139**, 689-700 (1988).
8. B. D. Jankovic and D. Djordjijevic, *Int. J. Neurosci.*, **60**, 119-127 (1991).
9. B. D. Jankovic, S. Jakulic, and J. Horvat, *J. Biol. Psychiatry*, **17**, 687-698 (1982).
10. P. Mecocci, L. Parnetti, and G. Romano, *J. Neuroimmunol.*, **57**, 165-170 (1995).