REVIEWS

Dysregulation in Neuroimmunopathology and Perspectives of Immunotherapy

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Dysregulation of neuroimmune connections is a primary or secondary pathogenic factor of some CNS diseases. Autoimmune aggression is typical of multiple sclerosis, Alzheimer's disease, and epilepsy, while dysregulation characterized by enhanced production of autoantibodies to neurotransmitters and activation of cell factors is characteristic of alcoholism and drug abuse. In experimental models of alcoholism and drug addiction, protective effects of antiserotonin antibodies are mediated by immune cells stimulated by these antibodies. These effects can be used in the therapy of various forms of neuroimmunopathology by the method of adoptive immunotherapy.

Key Words: *immunoaggression; dysregulation; antibodies; lymphocytes; immunotherapy*

Dysregulation of neuroimmune connections can be either an impact or a cause of two forms of immunopathology in CNS. The first and most intensively studied form is related to direct cytotoxic effect of autoantibodies or immune cells on CNS followed by the development of neurogenic immunodeficiency [13]. The primary role of the immune system in CNS damage is proved by, first, focal accumulation of immune complexes or macrophage-lymphocytic infiltration in damaged neural structures, second, cytotoxic effect of neurospecific antibodies or immune cells sensitized to neuroantigens *in vitro*, and the third and most important proof is the possibility of adequate modeling of neuroimmunopathology with antibodies and immune cells. Three forms of neuroimmunopathology comply all these three criteria: multiple sclerosis (MS), Alzheimer's disease (AD), and epilepsy. At the same time, these criteria cannot explain the most salient feature of neuroimmune process — continuity of the disease course. Evidently, it results from persistent disturbances in the CNS-immune system connections producing a dysregulatory vicious circle. This assumption was confirmed by clinical and experimental data obtained during the last decade.

Immunological monitoring of the pathological process during MS [6,7] showed that active stage of the disease is characterized by increased number of lymphocytes expressing interleukin-2 (IL-2) receptors, activation of Th-1 cells producing interferon-γ, high level of tumor necrosis factor, and hyperproduction of IL-1 and IL-6. However, no direct correlation was found between clinical manifestation of the disease and *in vitro* myelin degradation by blood monocytes in MS patients [46]. The key role in the development of the autoimmune inflammatory process during MS is played by interferon-γ maintaining inflammation in the nervous tissue. This probably explains beneficial therapeutic effect of interferon-β, an antagonist of interferon-γ [47]. The mechanisms of dysregulation of neuroimmune relationships providing continuity of pathological process are extremely important for the pathogenesis of MS [3]. The involvement of neuroimmune processes in the pathogenesis of MS is confirmed by enhanced expression of

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opioid receptors on blood lymphocytes at various stages of the disease [30].

Severe disturbances of cerebral activity during AD result from degradation of acetylcholinergic neurons. Two basic concepts of the pathogenesis of morphofunctional disturbances in CNS of AD patients were advanced during two last decades. The autoimmune mechanisms of the disease were studied, and an adequate immunopathological model of AD was developed [33]. Much attention was focused on the role of biochemical and immunophysiological processes in the formation of senile plaques in cerebral tissues due to deposition of β-amyloid protein, the main factor inducing death of acetylcholinergic neurons [51,52].

A correlation was found between the severity of the disease and changes in immunological parameters, in particular, lymphopenia, enhanced T-lymphocyte proliferation, and increased IL-1 production. These changes in immunoreactivity correlated with changes in visual evoked potentials, which attested to pathological alternations in the limbic-reticular system [1,27].

A possible mean for neuroregulatory correction on the leading element of AD pathogenesis, *i.e*. formation of fibrillar neurotoxic β-amyloid protein deposits, is vasoactive intestinal peptide (VIP), which plays a key role in conscious activity in men. The content of VIP in the cerebral cortex markedly decreased during AD. It was demonstrated that lyophilized analog of VIP effectively protected neuronal cells against toxic effect of β-amyloid protein and prevented their death both in culture and in rats with modeled AD. Intranasal administration of VIP improved learning and memory in rats with AD [37]. Human T-lymphocytes have receptors to VIP, which inhibits *in vitro* response to mitogens [45].

The role of autoimmune processes and dysregulation of neuroimmune connections in the development of epilepsy was experimentally studied on various animal species. For instance, in monkeys injection of rabbit antihippocampal antiserum into the hippocampus provoked convulsions accompanied by epileptiform activity in the site of injection [44]. Similar effects were observed in monkeys receiving intracortical injection of splenocytes from rabbits immunized with simian cerebral antigens [32]. Antiserum against GM_1 ganglioside [40] or Fab fragments of antibodies [41] injected into cerebral ventricles also induced epileptiform activity in experimental animals. Recent data indicate that cells the immune system retain a kind of "memory" about the pathological process in CNS: being transferred to intact animals, these cells can reproduce some symptoms characteristic of this pathological process [18,26,42]. For instance, adoptive transfer of splenocytes from corazol-kindled mice (widely used model of chronic epileptogenesis [20]) increased sensitivity to corazol in recipient mice. This effect was demonstrated in experiments determining the threshold corazol dose inducing convulsions and tonic seizures with fatal outcome and on the model of corazol kindling [19].

Until recently, primary dysregulation of neuroimmune connections was not considered as a cause of CNS pathology, although this approach is a biological antipode to psychoneuroimmunology, a novel interdisciplinary research branch. In this respect, the most interesting are autoantibodies against neurotransmitters, neuropeptides, and cytokines, which are biological regulators of the immune system and CNS. These autoantibodies were detected in some neuropathological states, specifically, in alcoholism and drug addiction.

Hyperproduction of autoantibodies against serotonin, catecholamines, and cerebrospecific antigens was demonstrated in animals subjected to chronic alcoholization and in alcoholic patients [12,17,25]. Active immunization with protein-conjugated serotonin or passive immunization with antiserotonin antibodies suppressed alcohol motivation and abstinence syndrome in alcohol-preferring C57B1/6 mice and rats [13,14]. Experimentally proved protective effect of antiserotonin antibodies does not contradict clinical observation on efficiency of serotonin receptors antagonists (fluoxamine and thianeptine) in the treatment of alcoholic patients [15,16]. In patients with fibromyalgia and depression, the concentrations of antiserotonin antibodies were comparable with that of antibodies against of serotonin receptor gangliosides [43,50].

The most intriguing problem is the mechanism of action of antineurotransmitter antibodies under conditions of active immunization and after systemic administration, because under natural conditions these antibodies cannot cross the blood-brain barrier. However, experiments showed that immunization with protein-conjugated neurotransmitters markedly changes the content of neurotransmitters and their metabolites in cerebral structures involved in the formation of alcohol dependence [5]. For evaluation of the effect of antiserotonin antibodies on CNS, behavioral activity (conditioned reflex of passive avoidance and open field test) were recorded and the contents of neurotransmitters in the sensorimotor cortex and hypothalamus were measured after intraperitoneal injection of the antibodies. A single systemic injection of antiserotonin antibodies to intact animals modulated the content of serotonin and dopamine and modified animal behavior in the immediate and delayed periods [29]. The delayed effect of antibodies on CNS attests to possibility of secondary activation of immune cells (macrophages and lymphocytes) producing neurotropic cytokines. This secondary activation of immune

cells with antiserotonin antibodies was demonstrated in experiments on cultured peritoneal macrophages and lymphocytes. Phagocytosis-stimulating effect of antibodies coincided with the dose-dependent effect of serotonin. Moreover, antiserotonin antibodies modulated the blastogenic effect of mitogens on T- and B-lymphocytes [8,22]. These data indicate that the pool of antiserotonin antibodies contains the anti-idiotypic antibodies binding with serotonin receptors on macrophages and lymphocytes [31,39,49]. These data stimulated further studies of the possible role of immune cells in the development of alcohol dependence. Splenocyte donors were alcoholized with ethanol spray for 10 days, then the abstinent syndrome was provoked, and serum and splenocytes were obtained and transferred to the animals of the same strain preferring alcohol under conditions of free choice. Blood serum increased alcohol consumption on postinjection day 1, while lymphocytes stimulated ethanol consumption for one week postinjection [26]. These effects could be mediated not only by cytokines crossing the blood-brain barrier, but also immunocytes penetrating into the brain. Activated immune cells can cross the blood brain barrier [4]. Therefore, a possible immune mechanism of alcohol abstinent syndrome is based on the capacity to augment alcohol motivation acquired by lymphocytes.

These findings and the fact that systemic administration of antiserotonin antibodies suppresses the major manifestations of abstinent syndrome led us to a conclusion that protective effect of antibodies is mediated by stimulated lymphocytes. To verify this hypothesis we studied the effect of cultured splenocytes treated by antiserotonin antibodies on alcoholized animals. Experiment showed that adoptive transfer of splenocytes incubated *in vitro* with antiserotonin antibodies reduced alcohol consumption in alcoholized mice [9]. Normal γ-globulin used as the control possessed no such properties. Evidently, this hypothesis can explain the therapeutic effects of mutual neuroimmune modulators such as interferon-α and vasopressin used to treat alcoholism [23,28]. Recent data attest to efficiency of adoptive immunotherapy of experimental alcoholism with lymphocytes stimulated *in vitro* by arginine-vasopressin [10]. The lymphocytes were isolated from abstinent mice, incubated in culture with arginine-vasopressin, and injected to alcohol-preferring mice of the same strain (at least 1 year under conditions of free choice). Alcohol motivation in recipients was suppressed for 3 weeks.

General features in the pathogenesis of alcoholism and drug abuse established by I. P. Anokhina [2] and other Russian scientists open prospect for the use of adoptive immunotherapy for the treatment of drug abuse. Convincing arguments for this conclusion are provided by experimental immunoneurobiological studies of N. Dafny *et al*. [34] and P. M. Dougherty *et al*. [34-36], which were the first attempts to use adoptive immunotherapy for the treatment of drug abuse: inhibition of abstinent syndrome after systemic administration of interferon-α, cyclosporine, cyclophosphamide, cortisol, and after γ-irradiation; transfer of the protective effect of immunomodulators (suppression of the abstinent syndrome) by *in vitro* activated lymphocytes; induction of the abstinent syndrome suppressed by γ-irradiation with normal lymphocytes. At present, adoptive immunotherapy with extracorporeally stimulated lymphocytes is effectively used in the treatment of allergic, infection-allergic, and oncological diseases [21,24,38,48].

In summary, it should be stressed that the dysregulation concept of neuroimmunopathology opens new vistas in the therapy of such diseases as alcoholism and drug abuse. Current methods of immunotherapy in various CNS pathologies do not provide complete recovery, probably due to the formation of a vicious circle that can be broken only after normalization of regulatory interactions between CNS and the immune system. Namely, these interactions are persistently "invalidated" in such neuroimmunopathologic states as MS, AD, and epilepsy. Therefore, the adoptive immunotherapy with autolymphocytes extracorporeally stimulated with various immunomodulators can be a treatment of choice under these condition.

REFERENCES

1. L. V. Androsova, T. P. Sekirina, N. D. Selezneva, *et al., Zh. Nevrol. Psikhiatr*., **1**, 24-27 (1995).
2. I. P. Anokhina, *Vopr. Narkol.,* No. 2, 27-31 (1995).
3. V. P. Barkhatova, I. A. Zavalishin, and B. T. Khaidarov, *Zh. Nevrol. Psikhiatr*., **11**, 51-54 (1998).
4. I. A. Belyaeva, E. I. Gusev, V. P. Chekhonin, *et al., Ibid.,* **8**, 57-62 (1999).
5. N. V. Bobkova, L. A. Plakkhinas, L. A. Basharova, *et al., Pat. Fiziol.,* No. 5, 25-29 (1988).
6. E. I. Gusev, T. L. Demina, and A. N. Boiko, *Multiple Sclerosis* [in Russian], Moscow (1997).
7. E. I. Gusev, A. N. Boiko, Zh. M. Salmasi, *et al.,* in: *Allergy and Immunopathology*, Ed. G. V. Poryadin [in Russian], Moscow (1999), pp. 226-238.
8. T. V. Davydova, V. A. Evseev, V. G. Fomina, *et al., Pat. Fiziol.,* No. 2, 10-13 (1998).
9. T. V. Davydova, V. A. Evseev, V. G. Fomina, *et al., Byull. Eksp. Biol. Med.,* **126**, No. 9, 328-330 (1998).
10. T. V. Davydova, V. A. Evseev, V. G. Fomina, *et al., Ibid.,* **129**, No. 6, 617-619 (2000).
11. V. A. Evseev, *Immunologiya*, No. 2, 4-9 (1990).
12. V. A. Evseev and S. V. Magaeva, *Vestn. Ross. Akad. Med. Nauk*, No. 11, 85-92 (1988).
13. V. A. Evseev, V. G. Fomina, T. V. Davydova, *et al., Immunologiya*, No. 5, 77-78 (1989).

14. V. A. Evseev, T. V. Davydova, V. G. Fomina, and L. A. Vetrile, *Byull. Eksp. Biol. Med.,* **112**, No. 7, 33-34 (1991).
15. N. N. Ivanets, I. P. Anokhina, T. L. Shibakova, *et al., Vopr. Narkol.,* No. 4, 6-10 (1993).
16. N. N. Ivanets, Yu. V. Valentik, and A. M. Barinov, *Ibid.,* No. 1, 18-20 (1995).
17. G. N. Kryzhanovskii and V. A. Evseev, *Vestn. Akad. Med. Nauk SSSR*, No. 3, 10-14 (1988).
18. G. N. Kryzhanovskii, T. V. Davydova, and V. G. Fomina, *Byull. Eksp. Biol. Med.,* **117**, No. 3, 233-234 (1994).
19. G. N. Kryzhanovskii, M. N. Karpova, T. V. Davydova, *et al*., *Byull. Eksp. Biol. Med.,* **119**, No. 3, 249-251 (1995).
20. G. N. Kryzhanovskii, A. A. Shandra, L. S. Godlevskii, and R. F. Makul'kin, *Usp. Fiziol. Nauk,* **19**, No. 4, 12-33 (1988).
21. M. M. Mendelenko, S. A. Kravtsov, and E. R. Rodionov, *Rus. J. Immunol.,* **2**, No. 3-4, 191-198 (1997).
22. O. I. Mikovskaya, V. G. Fomina, L. A. Basharova, and V. A. Evseev, *Byull. Eksp. Biol. Med.,* **124**, No. 12, 663-665 (1997).
23. G. V. Morozov and A. M. Ivanitskii, *Vopr. Med. Khim.,* **30**, No. 3, 63-68 (1984).
24. A. A. Ostanin, E. R. Chernykh, O. Yu. Leplina, *et al., Rus. J. Immunol.,* **2**, No. 3-4, 167-175 (1997).
25. L. F. Panchenko, S. G. Morozov, and B. B. Gnedenko, *Vopr. Narkol.,* No. 1, 60-66 (1996).
26. K. D. Pletsityi, T. V. Davydova, and V. G. Fomina, *Vopr. Med. Khim.,* No. 6, 48-49 (1995).
27. N. V. Ponomareva, V. F. Fokin, L. V. Androsova, *et al., Vestn. Ross. Akad. Med. Nauk,* No. 12, 27-32 (1995).
28. F. I. Sluchevskii, S. M. Tikhomirov, and V. D. Bakharev, *Zh. Nevropatol. Psikhiatr*., **86**, No. 2, 244-247 (1986).
29. N. A. Trekova, T. G. Khlopushina, L. A. Basharova, *et al., Zh. Vyssh. Nervn. Deyat*., **48**, No. 2, 251-259 (1998).
30. A. A. Shutov, A. A. Bykova, and E. V. Volodina, *Zh. Nevrol. Psikhiatr*., No. 8, 35-37 (1999).
31. T. M. Aune, H. W. Golden, and K. M. McGrath, *J. Immunol.,* **153**, No. 2, 489-498 (1994).
32. M. L. Baltz, G. Eulinger, K. Parrish, *et al., J. Neurol. Sci.,* **49**, 335-340 (1981).
33. J. Chapman, G. Alroy, Z. Weiss, *et al., Neuroscience,* **40**, 297-305 (1991).
34. N. Dafny, J. R. Lee, and P. M. Dougherty, *J. Neurosci. Res.,* **19**, 130-139 (1988).
35. P. M. Dougherty, J. Aronowski, D. B. Drath, and N. Dafny, *J. Neuroimmunol.*, **13**, 331-342 (1987).
36. P. M. Dougherty, N. R. Pellis, and N. Dafny, *Neuroscience*, **36**, No. 2, 285-289 (1990).
37. I. Gozes, A. Bardea, A. Reshef, *et al.*, *Proc. Natl. Acad. Sci. USA*, **93,** 427-432 (1996).
38. E. A. Grimin, A. Mazninder, H. Z. Zhang, and S. A. Rosenberg, *J. Exp. Med.*, **155**, 1823-1841 (1982).
39. K. Iken, S. Cheng, A. Fargin, *et al.*, *Cell. Immunol.,* **163**, No. 1, 1-9 (1995).
40. S. E. Karpiak, L. Graf, and M. M. Rapport, *Brain Res.,* **59**, 303-310 (1976).
41. S. E. Karpiak, S. P. Mahadic, L. Graf, and M. M. Rapport, *Epilepsia,* **22**, 189-196 (1982).
42. E. S. Kimbal and R. B Faffo, *J. Neuroimmunol.,* **22,** 185-192 (1989).
43. R. Klein, M. Bansch, and P. A. Berg, *Psychoneuroendocrinology*, **17**, No. 6, 593-598 (1992).
44. L. J. T. Mihailovic and D. Cupic, *Brain Res.,* **32**, 97-124 (1971).
45. M. S. O'Dorisio, C. L. Wkod, and M.S. O'Dorisio, *J. Immunol.*, **135**, No. 2, 792-796 (1985).
46. S. J. Owen, C. M. Watson, and A. N. Davison, *Clin. Exp. Immunol.*, **82**, 86-92 (1990).
47. J. Purez-Miranda and J. L. Parrila-Ramerez, *Rev. Neurol.*, **27**, 117-121 (1998).
48. S. A. Rosenberg, *Immunol. Today*, **9**, 58-62 (1988).
49. T. L. Roszman, J. C. Jackson, R. J. Cross, *et al.*, *J. Immunol.,* **135**, No. 2, 769-772 (1985).
50. K. Schott, A. Batra, R. Klein, *et al.*, *Eur. J. Psychiatry,* **7**, 209-215 (1992).
51. B. Solomon, R. Koppel, E. Hanan, and T. Katzav, *Proc. Natl., Acad. Sci. USA*, **93**, 452-455 (1996).
52. B. Solomon, R. Koppel, D. Frankel, and E. Hanan, *Ibid.*, **94**, 4109-4112 (1997).