Changes in Immunological Parameters in Patients with Opium Abuse Receiving ANAR Therapy

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We studied the effects of ANAR containing antibodies to morphine in ultralow doses on immunological parameters in patients with opium abuse. Changes in blood content of immunocompetent $CD3^+$, $CD16^+$, and $HLA\text{-}DR^+$ cells, lymphocytes carrying receptors for serotonin and dopamine, and humoral immune factors were evaluated. As differentiated from standard preparations, ANAR possessed immunomodulatory properties, increased the content of $HLA\text{-}DR^+$ lymphocytes and immunoglobulins in the blood, and normalized the number of $CD16^+$ cells.

Keywords: *immune system; opium abuse; ultralow doses; antibodies to morphine*

Drug abuse is accompanied by changes in neuromediator, neuroendocrine, biochemical, and immune processes in the organism, which impairs the relationship between nervous and immune systems, causes dysfunction of T lymphocytes, violates cytokine synthesis by immunocompetent cells, increases the amount of brain-specific proteins and their antigens in the serum, and affects elimination of immune complexes [1-4,6,7, 9,11,12,14].

The influence of psychotropic preparations on the immune system is determined by its close relationships with the nervous system. Psychotropic preparations modulate the immune system via central regulatory mechanisms and produce the direct effect on immunocompetent cells [5,8,10].

New preparations obtained by the method of homeopathic potentiation and containing antibodies to regulatory proteins in ultralow doses hold promise for the therapy of patients with addictions. Antibodies in ultralow doses may affect the activity of regulatory molecules. Potentiated antibodies to S100 protein (PAB-S100) entering the composition of Proproten possess particular neurobiological properties and relieve the dependence on psychoactive compounds without increasing the concentration [15]. Experimental and clinical observations indicate that the preparation ANAR containing potentiated antibodies to morphine (PAB-M) and synthesized at the "Materia Medica Holding" Research-and-Production Company may relieve the withdrawal syndrome after chronic morphinization [16].

Immune antibodies are used for the synthesis of new preparations. The immune mechanisms underlying action of these preparations are of considerable interest.

Here we studied changes in immunological parameters in patients with opium abuse and post-withdrawal disorders receiving ANAR monotherapy.

MATERIALS AND METHODS

We examined 46 patients with opium abuse that were divided into the ANAR (n=32) and reference groups (n=14). The control group included 36 mentally and somatically healthy donors.

The patients with opium abuse were comparable by the stage of disease, sex, and age (15-35 years). This disease is classified as a mental and behavioral disorder resulting from opioid consumption (withdrawal syndrome, F11.22.H, ICD-10).

ANAR containing affinely purified antibodies to morphine (mixture of homeopathic dilutions C30 and C200, equivalent concentration 10^{-60} wt %) was used for the therapy of post-withdrawal disorders after disappearance of acute manifestations of the opium withdrawal syndrome (OWS). On day 7 after opium withdrawal the preparation was given perorally in a dose of 1 tablet 5-8 times a day for the first 2 weeks of examination. Some patients received ANAR in a dose of 10-12 tablets a day, which depended on the severity of post-withdrawal psychopathological disturbances. Over the nest 3 weeks the preparation was administered in a single dose of 1 tablet 4-6 times a day. The

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patients with paroxysmal opium addiction received additionally 3 tablets of ANAR at 15-20-min intervals.

When acute manifestations of OWS were relived patients of the reference group received standard therapy, which included differential treatment with antidepressants, behavioral correctors, hypnotics, and nootropic and vegetostabilizing preparations.

Patient's immune status was evaluated before and 21-28 days after the start of therapy. In control patients immunological characteristics were determined 1 time.

To estimate parameter of the cellular immunity lymphocytes were isolated from the heparinized blood on a Ficoll-Paque density gradient (Pharmacia) by routine methods. Immunocompetent cells were phenotyped with monoclonal antibodies (Sorbent). The number of $CD3^+$ (mature T lymphocytes), $CD16^+$ (natural killer cells, NK cells), and HLA-DR^+ cells (B lymphocytes and activated T lymphocytes) was evaluated in the lymphotoxic test. The content of $CD4^+$ (T helpers/inductors) and $CD8^+$ cells (cytotoxic T killer/suppressor cells) was determined by the method of immunofluorescence [13].

Serum concentrations of IgM, IgG, and IgA were measured by the method of radial immune diffusion in agar gel. The amount of circulating immune complexes (CIC) in the serum was estimated by the polyethylene glycol method [18].

The state of neuromediator systems was determined by the number of peripheral blood serotonin-positive (S-RFC) and dopamine-positive lymphocytes (D-RFC) forming rosettes with sheep erythrocytes loaded with serotonin and dopamine, respectively [17].

Data processing and statistical analysis were performed using Statistica software [4]. We calculated the arithmetic mean (*M*), mean square deviation (S), and arithmetic mean error (*m*). The significance of differences was evaluated by Student's *t* test ($p<0.05$). Normality of the general population was determined by Kolmogorov-Smirnov test. Equality of general variances was evaluated by Fisher's *F* test.

RESULTS

Before the start of treatment post-withdrawal patients of the ANAR and reference groups differed from healthy donors. In these patients the relative number of mature T lymphocytes ($CD3^+$) and T helper/indictor cells ($CD4^+$) and content of NK cells decreased compared to the control (Table 1). The concentrations of serum IgM and CIC and number of lymphocytes carrying receptors for serotonin and dopamine increased in patients with OWS. The amount peripheral blood lymphocytes did not differ in patients of the main and reference groups (Fig. 1). Immunological parameters in control patients were taken as 100%. Parameters of patient's immune status before and after therapy are presented in percents of the control (Fig. 1).

ANAR monotherapy for 3 weeks relived clinical manifestations of post-withdrawal disorders. This preparation produced the vegetostimulating and normothymoleptic effects and modulated drug addiction. Clinical changes were accompanied by an increase in the content of peripheral blood $CD3^+$ and $CD4^+$ lymphocytes (tendency) and NK cells ($CD164^+$, statistically significant), which reached the control level. The number of lymphocytes carrying receptors HLA-DR increased compared to the control and parameters observed before the start of therapy. The concentration of serum Ig increased 3 weeks after the start of treatment. The content of CIC tended to decrease, but re-

TABLE 1. Changes in Immune Parameters in Patients with Opium Abuse before and after ANAR Therapy (*M*±*m*)

Parameter	Control (*n*=36)	Drug abusers	
		before therapy (*n*=32)	after therapy (*n*=17)
$CD3^+$, %	67.44±1.32	60.61±1.72*	62.14±2.87
$CD4^+$, %	38.86±1.19	30.82±1.05*	32.00±2.00
$CD8^+$, %	23.22±1.21	21.8±0.87	19.00±1.00
HLA-DR^+, %	17.97±1.15	22.86±1.52	29.50±2.01*
$CD16^+$, %	10.29±0.74	6.26±0.64*	10.07±1.30
IgM, g/liter	1.32±0.07	2.90±0.32*	3.24±0.27*
IgG, g/liter	15.60±0.54	16.20±1.08	20.83±1.38*
IgA, g/liter	2.08±0.11	2.35±0.16	2.72±0.24*
CIC, rel. U	103.89±3.89	209.93±26.46*	174.64±27.08*
S-RFC, %	3.62±0.36	6.05±0.93*	4.21±0.82
D-RFC, %	2.70±0.36	4.75±0.50*	5.07±0.46*

Note. *$p<0.05$ compared to the control.

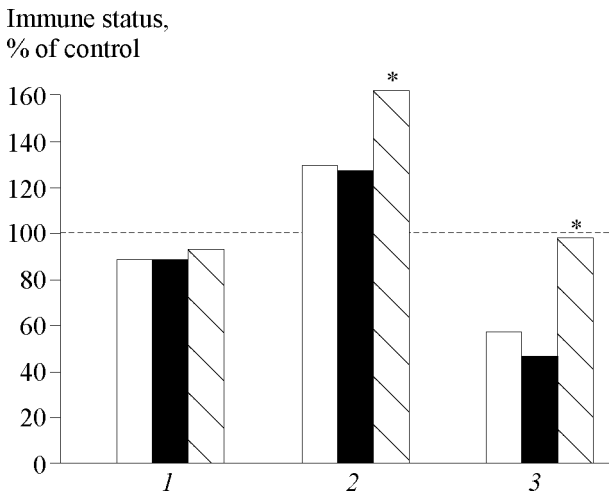

Fig. 1. Changes in the content of lymphocytes in patients with opium abuse receiving ANAR and standard preparations. Light bars: before therapy. Dark bars: after standard therapy. Slant shading: after ANAR therapy. Dotted line: control. $CD3^+$ (*1*), HLA-DR+ (*2*), and $CD16^+$ lymphocytes (*3*). Ordinate: number of peripheral blood lymphocytes (% of normal). Her and in Fig. 2: **p*<0.05 compared to the control.

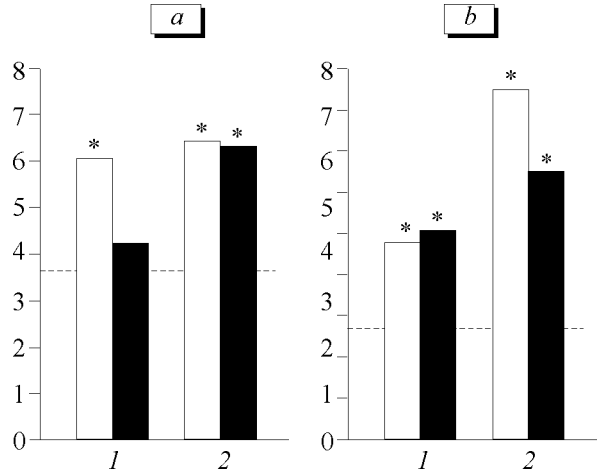

Fig. 2. Changes in the content of lymphocytes carrying receptors for serotonin (*a*) and dopamine (*b*) in patients with opium abuse receiving ANAR and standard preparations. Ordinate: number of serotonin-positive (S-RFC, *a*) and dopamine-positive lymphocytes (D-RFC, *b*) in the peripheral blood (%).Light bars: before therapy. Dark bars: after therapy. Dotted line: control. ANAR (*1*) and standard preparations (*2*).

mained above the control. The number of serotonin-positive lymphocytes in the peripheral blood returned to normal. The amount of lymphocytes carrying receptors for dopamine remained high.

Cellular immunosuppression was preserved in patients of the reference group receiving standard therapy. The number of T lymphocytes ($CD3^+$) in post-withdrawal patients after and before treatment and in healthy donors was 60.25±3.70, 60.21±1.74, and 67.44±1.32%, respectively (p<0.05). The content of NK cells in these patients after and before treatment and in healthy donors was 4.75±0.94, 5.85±0.86, and 10.07±1.30%, respectively (p<0.05).

The number of lymphocytes carrying receptors for serotonin and dopamine showed that the activity of neuromediator systems remained high on days 21-28 of therapy (Fig. 2). It should be emphasized that standard preparations decreased CIC concentration in patients to normal (before therapy: 181.85±24.11 rel. U; after therapy: 95.00±20.83 rel. U, p<0.05; control level: 103.89±3.89 rel. U).

Our results demonstrate changes in immunological parameters during treatment with ANAR and standard preparations. PAB-M are involved in the psychoneuroimmune interaction in patients with drug abuse. These antibodies produce a strong effect on the cellular immunity, normalize the contents of T lymphocytes, T helper/inductor cells (main immunoregulatory cells), and NK cells (most significantly), and modulate metabolism of neuromediators. ANAR therapy increases the number of immunocompetent cells expressing receptors HLA-DR that play an important role in recognition and activation of the immune response.

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