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OF COMPLEX SYSTEMS

Some Approaches to the Activation of Antitumor Resistance Mechanisms and Functional Analogs in the Categories of Synergetics

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Abstract—The methods for activating antitumor resistance mechanisms that were developed using the concept of the periodic system of general nonspecific adaptational reactions of the body are briefly reviewed. The principles that allow effective impacts with electromagnetic radiation and biologically active substances are described. The criteria and concepts of the theory of adaptational reactions are compared to some of the concepts and categories of synergetics. The specific features in the dynamics of the studied parameters that are induced by effective impacts are considered. The antistress nature of the systemic effect that is caused by ferromagnetic nanoparticles on animals with grafted tumors is shown. Possible mechanisms that underlie the regression of large tumors that is caused by two different factors, viz., modulated electromagnetic radiation and magnetite nanoparticles, are discussed. The cases where the order parameter changed with the development of antistress unresponsiveness and regression of experimental tumors caused by a combined electromagnetic exposure are analyzed.

Keywords: antistress adaptational reactions, antitumor resistance, regulatory processes, order parameters, electromagnetic radiation, magnetite nanoparticles

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The search for efficient methods to inhibit malignant growths is one of the highest-priority biomedical challenges. An ideal solution would be technologies for the mobilization of the natural antitumor mechanisms that are arrested as a result of regulatory disturbances in the body and the activation of tumor-defense mechanisms. Unfortunately, a reductive approach is frequently prevalent here, which confines consideration of this problem to the processes that occur at individual structural and functional levels in the body. However, this problem undoubtedly requires an integrated holistic approach that implies insight into the principles that control the nonspecific resistance of the body. Note that the nonspecific resistance means *the resistance of the body to the effects of various disturbing factors based on the effective function of the body's regulatory systems.* Currently, this approach is to a certain degree implementable within activation therapy, that is, the technology that is based on the theory of the general nonspecific adaptational reaction (AR) of the body, as developed at the Rostov Research Institute of Oncology [1–3]. Three discoveries. viz., the discovery of the stress AR by Selye [4], the discovery of antistress ARs that are qualitatively different from both the stress AR and one another [5], and the discovery of the levels of the body's responsiveness (levels of reactivity) or a periodic pattern in the body's response as a whole to a change in the intensity of an acting factor of any nature in a wide range [6], form the background for his theory.

Abbreviations: AR, adaptational reaction; EMR, electromagnetic radiation; VLF, very low frequency; SHF, super high frequency; EHF, extremely high frequency.

MATERIALS AND METHODS

The discovery of antistress ARs and the subsequent development of the main principles that underlies the theory of the ARs is the result of long-term studies at the Rostov Research Institute of Oncology that are guided by Garkavi and Kvakina [1, 2]. The experimental part of this study was conducted with laboratory outbred and inbred (Wistar) rats (several thousand animals) that were obtained from the Rappolovo Animal Breeding Facility and the Central Laboratory Animal Breeding Facility with the Russian Academy of Medical Sciences (Andreevka) or the animals that were bred at the institute. The transplantable tumor lines were obtained from the Institute of Experimental Diagnostics and Therapy with the Blokhin Russian Cancer Research center and the bank of cell lines with the Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology of the National Academy of Sciences of Ukraine. The tumors were grafted using conventional protocols [7]. All experiments met the international regulations for manipulations with laboratory animals [8]. The clinical studies involved patients of the Rostov Research Institute of Oncology; various cases of chronic nontumor diseases that are treated in the hospitals of Rostov-on-Don, Rostov oblast, and several other Russian regions; and the staff of the Rostov Research Institute of Oncology and several institution of Rostov-on-Don and Rostov oblast.

The activation therapy was conducted using low-intensity electromagnetic radiation (EMR) of different frequency ranges, namely, very low frequency (VLF; devices of the Polyus, Spektr, and Gradient series), super high frequency (SHF; Akvaton device), extremely high frequency (EHF; Yav'-1 device with additional ferrite isolator and upgraded G6-37 generator unit), and optical (SpektrLTs device) ranges. In addition, dynamic neurostimulation therapy in the variant of SCENAR (self-controlled energo-neuro-adaptive regulator) therapy (the SCENAR-97.1 device with an external comb electrode). Only certified devices that have been approved by the Ministry of Public Health of the Russian Federation (or the Soviet Union) were used in the clinical experiments. In addition, the effects of systemic and local (directly on tumor) application of ferromagnetic nanoparticles (magnetite doped with maghemite, 10 ± 2 nm) in the form of an AM-01 magnetic water-based fluid with oleic acid as a surfactant were experimentally studied. The magnetic fluid was diluted with a physiological saline solution to the necessary concentration (3–12 mg/mL) and administered peritumorally.

The changes in human and animal bodies that occur at different structural and functional levels were examined using the modern methods of clinical laboratory diagnostics, histology, histochemistry (including fluorescence histochemistry), light and electron microscopy, cytology, cytochemistry, polarography, immunology, electrophysiology, experimental physiology, and radioisotope methods.

THE MAIN BASIC AND APPLIED RESULTS

The concepts of the periodic system of general nonspecific adaptational reactions of the body. The theory of the adaptational reactions, which is based on analysis and generalization of experimental and clinical studies, contains several concepts on the structure and developmental patterns of the body's integral responses [1, 2]. As has been shown, at least three antistress ARs exist along with the AR to stress. These are the AR of training caused by the impact of relatively weak stimuli, as well as the ARs of calm and elevated activation, which are caused by "medium" strength stimuli (the intensity of the impact is somewhat higher in the case of the AR of elevated activation as compared with the development of the AR of calm activation). Similar to the stress AR, a specific set of characteristic changes in the neuroendocrine and immune systems, as well as at the level of cell energetics corresponds to each of the antistress ARs (Fig. 1). Thus, the AR can influence the state of the body's nonspecific resistance. As a rule, an especially pronounced increase in the nonspecific resistance is observed in the case of the AR of elevated activation. Note that although the ARs of calm and elevated activation of calm and elevated activation are noticeably similar, these responses still have systemic distinctions at the level of the hypothalamo–pituitary–adrenal system, which appears as different ratios of the glucocorticoid and mineralocorticoid hormones in the considered ARs; this actually determines the differences in the effects of these responses to acute and chronic inflammatory processes [1, 2]. Assays for biogenic amines in the blood and several neuroendocrine structures provided additional characterization of the systemic regulatory processes during the development of different ARs [9]. This also clarified the distinctive features of the ARs of calm and elevated activation, which are determined by higher activities of secretory and synthetic processes in monoaminergic structures and a more pronounced shift of the monoamine synthesis and secretion activities towards synthesis in the case of the AR of elevated activation.

Another important concept in the AR theory is associated with the levels of body reactivity at which the stress AR and antistress ARs can develop [1, 2, 6]. The impacts that induce various ARs are characterized by certain energy and strength ratios. The coefficient that reflects the ratio of the impacts that induce the neighboring ARs in the tetrad of the AR of training–AR of calm activation–AR of elevated activation–stress AR amounts to 1.1–1.3. The AR tetrads regularly repeat with a decrease or an increase in the intensity of the acting factor. Thus, the intensity of an impact is "assessed" by the body in both the relative "units" (AR type) and absolute "units" (the level of reactivity). Note that the ARs of the same type that belong to different levels of reactivity display certain distinctions along with the similarity in the directions of changes that are observed in the immune and neuroendocrine systems; the degree of these differences depends on the degree of differences between their levels of responsiveness.

In particular, the antistress ARs of higher levels of reactivity (corresponding to weak impacts) are the most favorable for the body, since the regulatory and energy processes during their development are balanced (harmonious), thereby enhancing the most pronounced increase in nonspecific resistance, espe-

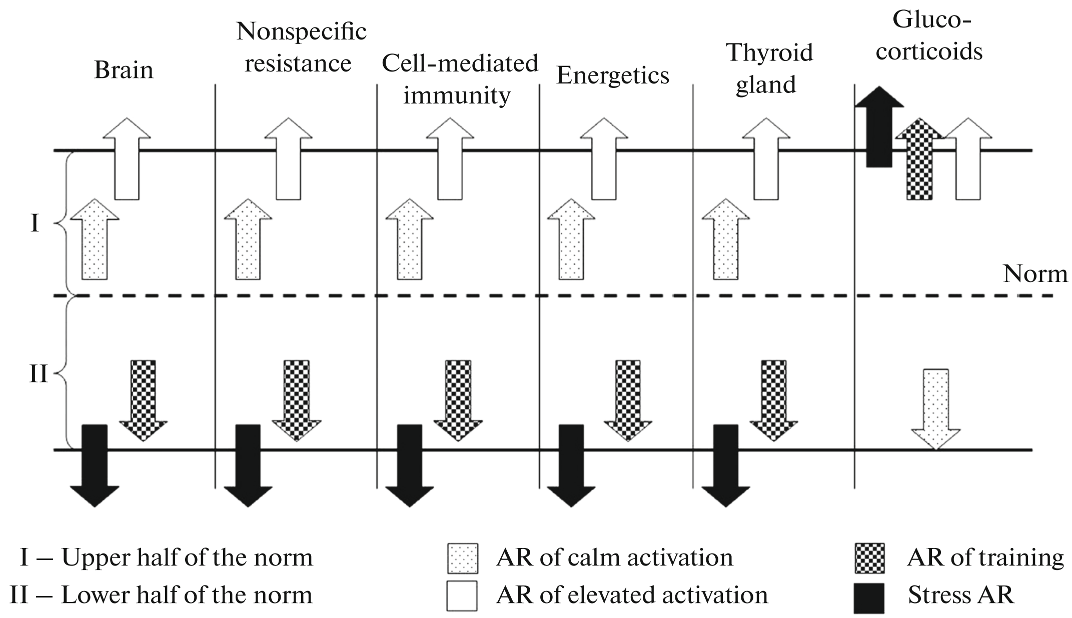

Fig. 1. Integrated changes in the regulatory systems of different levels during the development of nonspecific adaptive responses (ARs) of the body.

cially, in the case of the AR of elevated activation. The stress AR of lower responsiveness levels induced by the factors of extreme strength is the opposite variant for the antistress ARs. This AR is the most severe for the body and in the case of its long-term action causes depletion of energy and functional reserves and the development of multiple organ failure, which inevitably leads to death. The stress ARs of high responsiveness levels are considerably milder in their manifestations as compared with the stress ARs of low responsiveness levels; however, this does not make the AR in question not a stress AR and does not allow it to be regarded as an antistress AR, as a sort of "eustress" (in the later terminology by Selye). The direction of hormonal changes, immune depression, prevalence of catabolic processes, and disturbance of regulatory processes that are characteristic of the stress AR are also retained to a certain degree at the higher responsiveness levels. Signs of tension in the functioning of regulatory systems, a certain shift of metabolic processes towards catabolism, and development of individual functional disturbances on the background of preserved balance in the activities of immune and neuroendocrine system organs are characteristic of the antistress ARs of lower reactivity levels. The presence of distinct ARs and levels of reactivity where these ARs can develop suggests an analogy between the periodic system of ARs and the periodic system of chemical elements (Fig. 2).

The characteristics of the structure of the adaptational reactions (ARs). Order parameter. Principles of activation therapy. The characteristics of the AR structure are of great importance. As has been shown, the relative lymphocyte counts in human and animal peripheral blood is a signaling parameter that indicates the type of an AR. The values of this parameter were initially determined for the stress AR [4, 10] and later for antistress ARs [1, 2]. Figure 3 shows the ranges for the relative lymphocyte counts in the peripheral blood corresponding to different human ARs. The ratios of the considered parameter and type of AR for animals (white laboratory rats) are somewhat different, since their leukogram differs from the pattern that is characteristic of humans in the prevalence of lymphocytes over neutrophils [1]. Note that the correspondence of certain lymphocyte percentage rates to the AR type was found empirically and currently still has no theoretical explanation. The boundaries for the ranges of this parameter that correspond to different ARs are conditional. They may shift slightly (by 10–15%) to a

T_1	CA_1	EA_1	S_1
T_2	CA_2	EA_2	S_2
T_3	CA_3	EA_3	S_3
…	…	…	…

Li	Be	B	C	N	O	F	Ne
Na	Mg	Al	Si	P	S	Cl	Ar
K	Ca	Ga	Ge	As	Se	Br	Kr
…	…	…	…	…	…	…	…

Fig. 2. The periodic system of general nonspecific adaptational reactions (ARs) of the body: T, AR of training; CA, AR of calm activation; EA, AR of elevated activation; S, stress AR; 1, 2, 3,..., levels of body reactivity at which the analogous ARs develop (with an increase in the intensity of impacts).

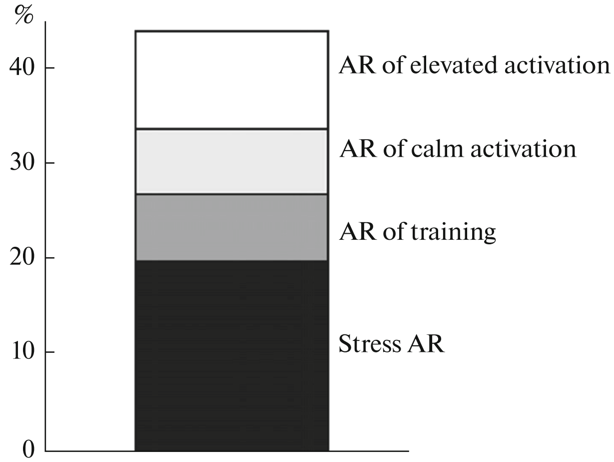

Fig. 3. The relative lymphocyte count in the peripheral blood is the signaling parameter that indicates the type of adaptational reaction (AR), the first order parameter.

particular side in some people or animals. If the relative lymphocyte counts display boundary values, the type of AR in experimental animals can be verified by histological examination for the state of immune and endocrine system organs. Thus, the percentage of lymphocytes in the peripheral blood reflects the type of AR as a particular variant of neuroendocrine interactions in the body and can be regarded as a generalized characteristic for the current functional state. Such integral characteristics for the state of complex nonlinear dissipative systems are referred to as *order parameters* [11, 12].

Hematological parameters also can to a certain degree characterize the level of reactivity at which ARs develop. In particular, a correlation has been shown between the AR intensity and deviations of the percentage rates of leukocyte lineage cells (except for lymphocytes) from the norm, as well as of the total counts of leukocytes, other blood cells, and hemoglobin content [2, 13]. Thus, the leukogram that was determined for 200 cells in combination with several other widely used hematological parameters makes it possible to assess the AR structure and the dynamics of the adaptive status for both the physiological norm and pathological processes developing in the body.

Currently, the integral parameters for assessing the adaptive status are searched for so that these characteristics would be obtainable in a noninvasive and instant manner. Rhythmic and quasi-rhythmic patterns of processes that occur at different structural and functional levels, the advent of new methods for assaying hematological processes, and the detection of highly informative diagnostic criteria [14–16] suggest that spectral and phase cardiometric and pulsemetric characteristics are promising for assessing the AR structure.

The concept of a multilevel AR system was used to formulate the principles of activation therapy that allow boosting the activation effects of the acting factors on nonspecific resistance of the human and animal bodies [2]. The most general and universal of these principles are

(1) targeted induction of the necessary antistress AR;

(2) the impact on central regulatory structures;

(3) minimization of the impact intensity (mild exposure and small doses);

(4) changes in exposure (dose) according to activation therapy algorithms;

(5) combination of central and local exposures; and

(6) the integrated use of various factors (including those of different modalities).

Some important principles reflect the particular nature of an acting factor, physical or chemical. They imply

—the use of electromagnetic radiation with biologically effective frequencies;

—exposure to multifrequency impacts;

—the use of effective modulation regimes;

—the use of different biologically active substances with multiple targets; and

—the use of solutions of biologically active substances and biological fluids modified by weak EMR.

Factors of activation therapy. Activation therapy of malignant process in clinical and experimental studies. Weak electromagnetic radiation of VLF, SHF, EHF, and optical ranges [17, 18] are most frequently used as the factors of activation therapy, as well as biologically active substances, including adaptogens, hormones, neurotransmitters, and regulatory peptides; substrates of cell energy processes and other tools of metabolic therapy [2, 3, 19]; biological fluids [20]; and so on. These factors of activation therapy can be used both independently and in various combinations. The systemic effects that are induced by iron oxide nanoparticles and associated with the development of antistress ARs [21] require a separate discussion. Low-intensity EMR and biologically active substances have been applied as tools of life-supporting therapy for cancer diseases [2, 3]. This allows an increase in efficiency of drug and radiation antitumor treatment with a considerable alleviation of their toxic effects, as well as a decrease in the rate of post-surgery complications.

In particular, magnetic therapy directed to the hypothalamus and the area of surgery in combination with chemical and radiation therapy of cerebral gliomas has a pronounced antistress effect, which enhances the improvement of the neurological dynamics, contributes to a direct clinical effect, and decreases (by more than three times) the rate of malignant glioma progress soon after treatment. In addition, both the total and recurrence-free survivals increase considerably (by more than two times) [22]. When an opto-magnetic impact was used in combination with a standard multiagent chemotherapy and autohemochemotherapy of women who are suffering from IIb–IIIb regional breast cancer, the development of antistress ARs was accompanied by convincing signs of activation of the blood lymphocyte metabolism and an increase in their energy supply, as well as an increase in the activity of their antioxidant systems. The integrated electromagnetic impact led to a statistically significant increase in the total regressive effect and a reduction in the number of the cases of progressive tumor growth (by at least 1.5 times) as well as an increase in total and event-free survival by 30% [23]. Analogous results have been obtained when using activation magnetotherapy in the treatment of lung and urological cancer cases [24, 25]. The exposure of patients with malignant breast, intestinal, and bladder tumors to EHF EMR with a low-frequency modulation of biologically effective frequencies also increased the efficiency of integrated antitumor treatments, as well as the use of combined EMR at the EHF and VLF ranges [26]. This occurred as the regression of axillary metastases in the breast cancer cases, stabilization of the extensive process in gastric cancer cases, and considerable alleviation of associated diseases despite the radiotherapy.

Some of the above listed EMR types, as well as their combinations, have been used as independent antitumor factors in experiments with rats that carry grafted tumors. In particular, the combined exposure of animal heads to EHF and VLF EMR without any other antitumor therapy caused a pronounced antitumor effect in over half of the rats. This effect consisted of a decrease in the tumor growth or its regression by two to four times in 17–40% of the cases [27]. Note that the degree of the antistress and antitumor effects of the used factors was distinctly correlated, which, in particular, is demonstrable based on the example of structural and functional shifts in the organ of central immunity and several other systemic changes.

In particular, Fig. 4 shows the changes in the thymus of male outbred white rats with grafted sarcoma 45 during tumor regression that was caused by exposure to combined EMR. Note that four principles of activation therapy were simultaneously implemented in this experiment, namely, a mild central impact (on the head of an immobilized animal; Fig. 4a), the use of biologically effective frequencies, modulation of the high-frequency signal, and integrated use of different EMR frequency ranges. Note that the changes that were observed in the thymus of control rats fit the micro pattern that is observed in chronic stress of lower responsiveness levels with considerable hypoplasia of the lymphoid tissue and pronounced signs of destruction in cells (Fig. 4b), which is characteristic of the late stages in tumor growth. The course of activation electromagnetotherapy resulted in a considerable increase in the lymphocyte proliferation activity in the thymus; in addition, the signs of intensified interactions between mast cells and thymocytes were observed, suggesting activation of the immune processes and fitting the thymus micro pattern that is characteristic of AR of elevated activation (Figs. 4c, 4d). Systemic changes of the analogous antistress direction have been observed when using the so-called resonance radiation of the SHF range containing a biologically effective frequency corresponding to the own EMR frequency of water-containing media [28]. The shifts in the recorded characteristics demonstrated that the antitumor effect of the considered factor was combined with pronounced antihypoxic, antioxidant, and detoxification effects (Fig. 5).

Thus, the changes in the state of the regulatory systems caused by activation therapy and associated with the AR type and pronouncedness in a general case distinctly correlate with the micro pattern of the animal-grafted tumor tissue. Note that the observed antitumor effects are of different scales up to a complete tumor regression without its recurrence in the case where AR of elevated activation was prevalent (Fig. 6).

As has been mentioned, one of the universal principles of activation therapy is the combination of a general impact and a local impact on the area that is affected by a pathological process. Figure 7 demonstrates the higher efficiency of a "double" exposure to resonance radiation that is applied to the head and peritumoral zone as compared with the variant of central exposure alone. As is evident, the combination of central and local exposures to resonance radiation considerably increased the rate of partial and complete tumor regression as compared with the exposure of the animal head alone to this EMR. Note that this difference in the antitumor effect also correlated with the differences in the rate of development of antistress ARs that were induced by double and central exposures. In the former case, the ARs of calm and elevated activation developed 1.3 times more frequently than in the latter case ($p < 0.05$).

Regression of large tumors caused by magnetite nanoparticles and low-intensity modulated EHF EMR. Changes in the central and effector components of the immune system. "Regimes with exacerbation"? The fact that a central impact is advantageous for effective development of the integral response by no means denies the possibility of AR development in the case of local exposure involving peripheral zones. In this process, the systemic changes that are induced by peripheral impacts can, in turn, influence the effects of this impact on a site. In our opinion, the effects of magnetite nanoparticles in the rats with grafted sarcoma 45 or Pliss lymphosarcoma belong to the phenomena of this type. As mentioned, magnetite nanoparticles (doped with maghemite) were used as a water-based magnetic fluid, which was diluted to the necessary concentration and applied peritumorally, i.e., at a small distance from the tumor edges. The degree of the antitumor effect distinctly matched the antistress effect of this factor, which was assessed according to the peripheral blood leukogram and micro patterns of the immune organs. As a rule, cases of complete tumor regression

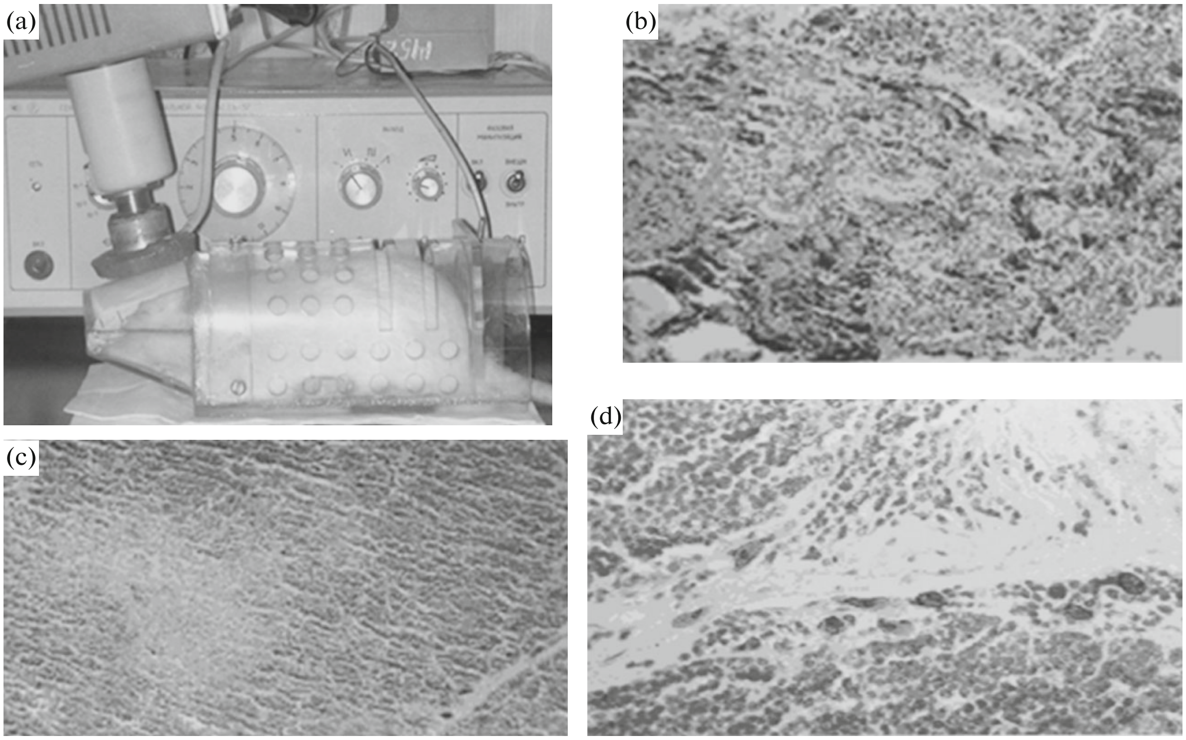

Fig. 4. Changes in the state of the central immunity organ that were induced by the effective combined impact of low-intensity EMR of EHF and VLF ranges on rats with transplanted sarcoma 45. (a) The device that was used for combined EMR. The coaxially located inductor coil and the guide of a Yav'-1 device for EHF therapy. (b) The control group, which displays hypoplasia of the lymphoid tissue as the focal degeneration of cell elements at the late stages of tumor development; staining according to Brachet. (c) Exposure to combined EMR; pronounced lymphocyte proliferation in the thymus of an animal with a regressed tumor; staining according to Brachet. (d) Exposure to combined EMR; activation of interactions between cells in the thymus of an animal with a regressed tumor: abundant tissue basophils in the interlobular connective tissue and their contacts with thymocytes; staining according to Brachet.

were observed when an AR of elevated activation was prevalent (Figs. 8a, 8b). An electron microscope examination of the tissues in regressing tumors demonstrated the signs of manifold intercellular interactions, suggesting the activation of effector component of the immune system (Fig. 9).

In several cases rats with Pliss lymphosarcoma developed extremely pronounced antitumor effects, namely, complete regression of very large tumors with a volume of over 10 and even 20 cm^3. An interesting specific feature in the dynamic of Pliss lymphosarcoma volume in these cases was that the growth rate of the tumors initially was the same as in the control despite the regular administration of magnetite nanoparticles; however, when the tumor reached a large size, it switched to a complete regression. In this process, no signs of intoxication were detectable despite the rather short period of regression (1–2 weeks), although intoxication by the products of tumor tissue necrosis is typical when large tumors are damaged with specialized antitumor drugs. Several months after this complete regression the animals appeared to be completely healthy (Fig. 10).

The results of our experiments and the relevant published data on the roles of iron and iron-containing proteins in proliferation, differentiation, and the regulation of cell death [29–31], their immunomodulatory effects [32–34], and association with the macrophage functional profile [35] suggest that the events that occur in the body and tumor could follow the pattern of exacerbation that is known from synergetics, with a positive feedback as an essential condition [36]. Figure 11 shows the scheme of possible processes in the neuroendocrine and immune systems that enhance the formation of the conditions for the regression of large tumors. The applied magnetite nanoparticles could induce pronounced cytokine and magnetochemical responses of the leukocytes and fibroblasts in the peritumoral zone that are intensified by the effects of humoral factors that are released by neuroendocrine structures via the activation of these structures by both the signals from the periphery and the centers of vegetative regulation as a result of the developed antistress ARs. Eventually, this could change the functional activity of effector immunocompetent cells, in particular, reprofiling of tumor-associated macrophages [37, 38] and/or transformation of monocytes and macrophages into

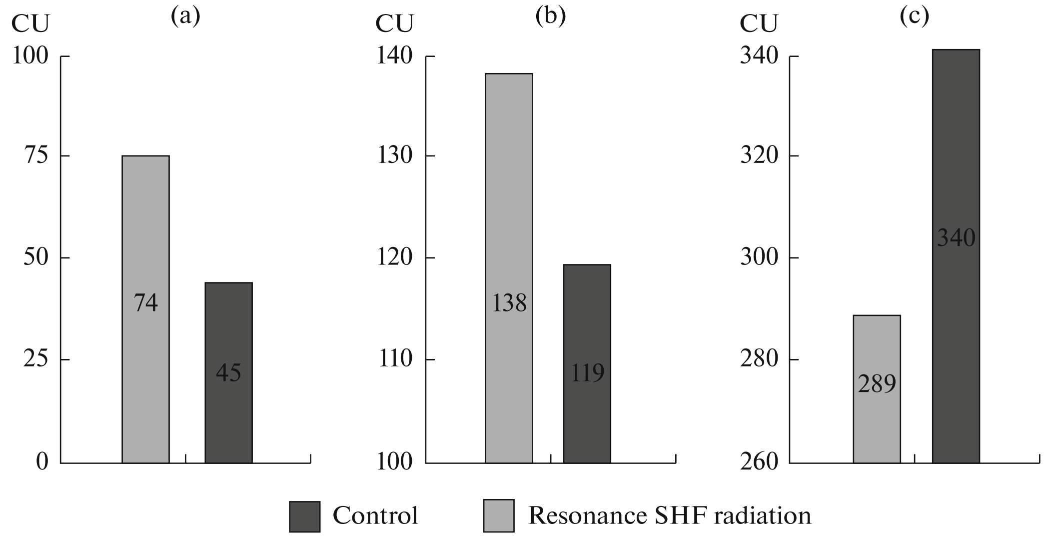

Fig. 5. Changes in several systemic biochemical characteristics induced by the effective combined impact of resonance radiation in the SHF range on rats with sarcoma 45 ($p < 0.05$): (a) increase in the level of hemoglobin (antihypoxic effect); (b) increase in erythrocyte catalase activity (antioxidant effect); and (c) a decrease in the level of the middle-molecular-weight peptides (detoxification effect).

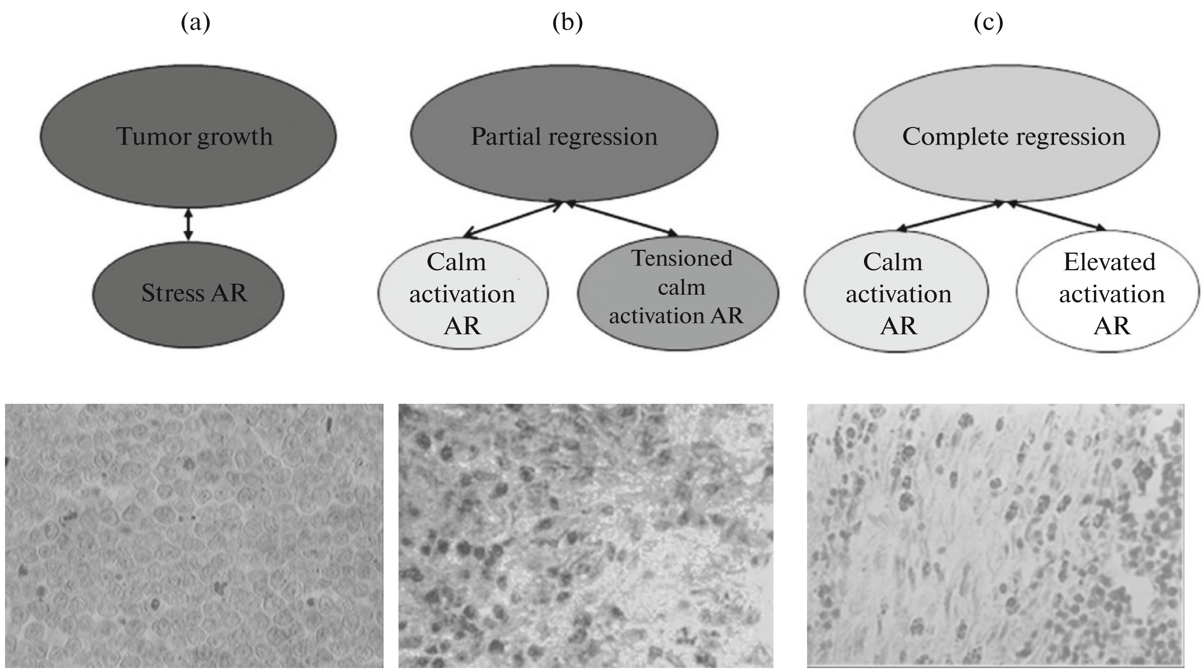

Fig. 6. The degree of the antitumor effect of the activation therapy and the pattern of prevalent ARs (Pliss lymphosarcoma; staining according to Brachet): (a) tumor growth; densely arranged cells with numerous mitotic figures; (b) partial tumor regression; pronounced infiltration with plasma cells; and (c) final stages in a complete tumor regression; replacement of tumor tissue by connective tissue; pronounced infiltration with macrophages, lymphocytes, and plasma cells.

dendrite cells [39], as well as the development of antigen presentation processes, leading to antigen-specific tumor cell killing via induced apoptosis.

The hypothesis that the processes that determine the regression of a large Pliss lymphosarcoma induced by magnetite nanoparticles are also of the central genesis associated with the development of calm and elevated activation ARs is also indirectly supported by the fact that analogous results were obtained earlier in an experiment with activation electromagnetotherapy [40]. In that work, the animal head was exposed to low-intensity modulated EHF EMR 3 days before the tumor was transplanted (sarcoma 45). The fact that the treatment started before tumor development was

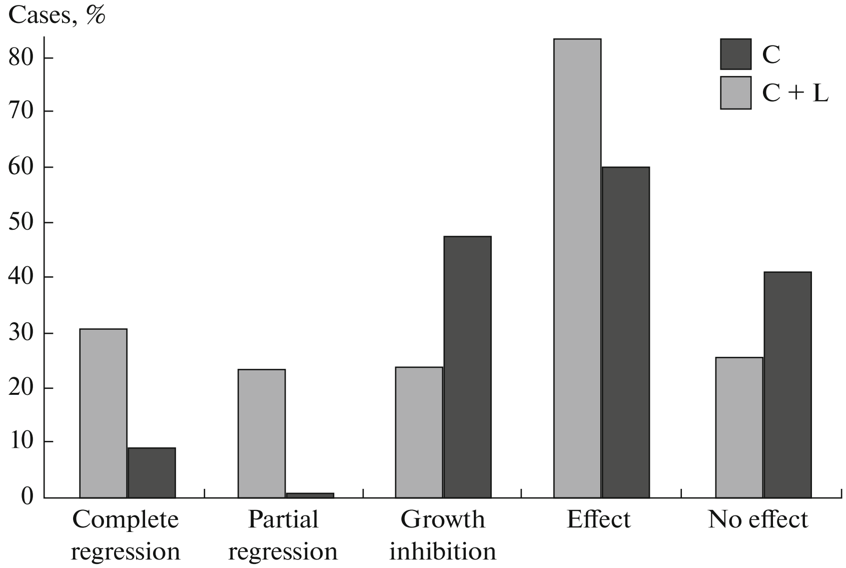

Fig. 7. An increase in the efficiency of resonance radiation by exposure of the head and peritumoral zone of the rates with sarcoma 45: C, central exposure (head); L, local exposure (peritumoral zone); and C + L, combined exposure of the head and peritumoral zone.

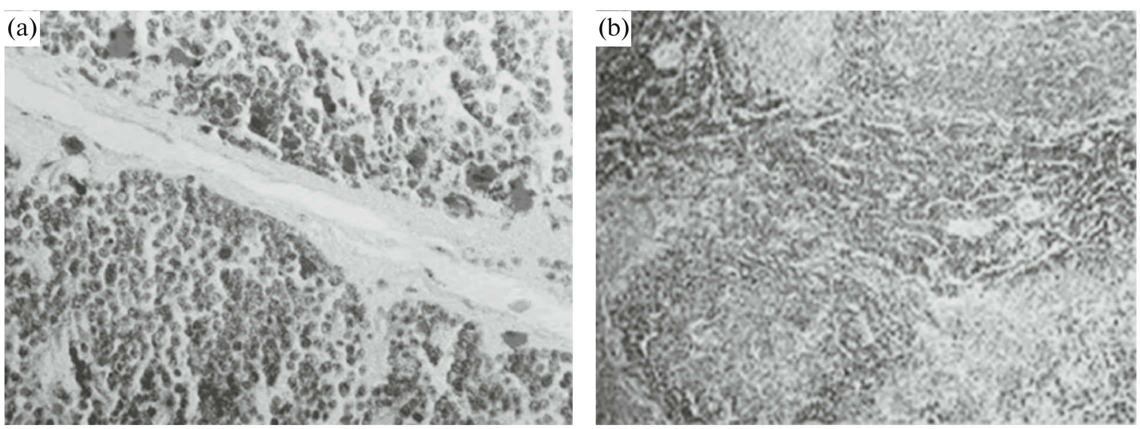

Fig. 8. Changes in the organs of the rat immune system in the sarcoma 45 regression induced by magnetite nanoparticles (staining according to Brachet): (a) thymus; densely arranged thymocytes (increased activation AR); signs of contacts of tissue basophils with thymocytes of subcapsular regions of the thymic lobes and (b) spleen; high lymphocyte proliferation activity (increased activation AR); increase in the number and size of follicles; expansion of thymus-dependent zones.

of fundamental importance, since no noticeable antitumor effect of EHF EMR had been earlier reported when the exposure commenced after tumor formation. When the activation electromagnetotherapy began before transplantation of sarcoma 45, some of the animals (over 30%) displayed a lag in their response similar to the situation with magnetite nanoparticles: tumors with a volume of 5–13 cm^3 actively developed for 1.0–1.5 weeks and then began to regress. We note that histological examination of the site of a regressed tumor did not show any signs of tumor cells in the cases of complete Pliss sarcoma regression (Fig. 12), which is similar to the effect of magnetite nanoparticles; no tumor recurrence was later observed in these animals.

An increase in antitumor resistance during the development of antistress areactivity state. Order parameter change. The integrated exposure that combined EMR of the EHF range and SCENAR therapy led to very interesting results [41]. In the case of SCENAR therapy, the pulse electric impact had multiple central and peripheral localizations, including the head, zones of backbone projections, tumor, and liver. The combination of SCENAR therapy and exposure of the head to EHF EMR according to the strategies of activation therapy strengthened the central component of the integrated impact, provided an additional modality,

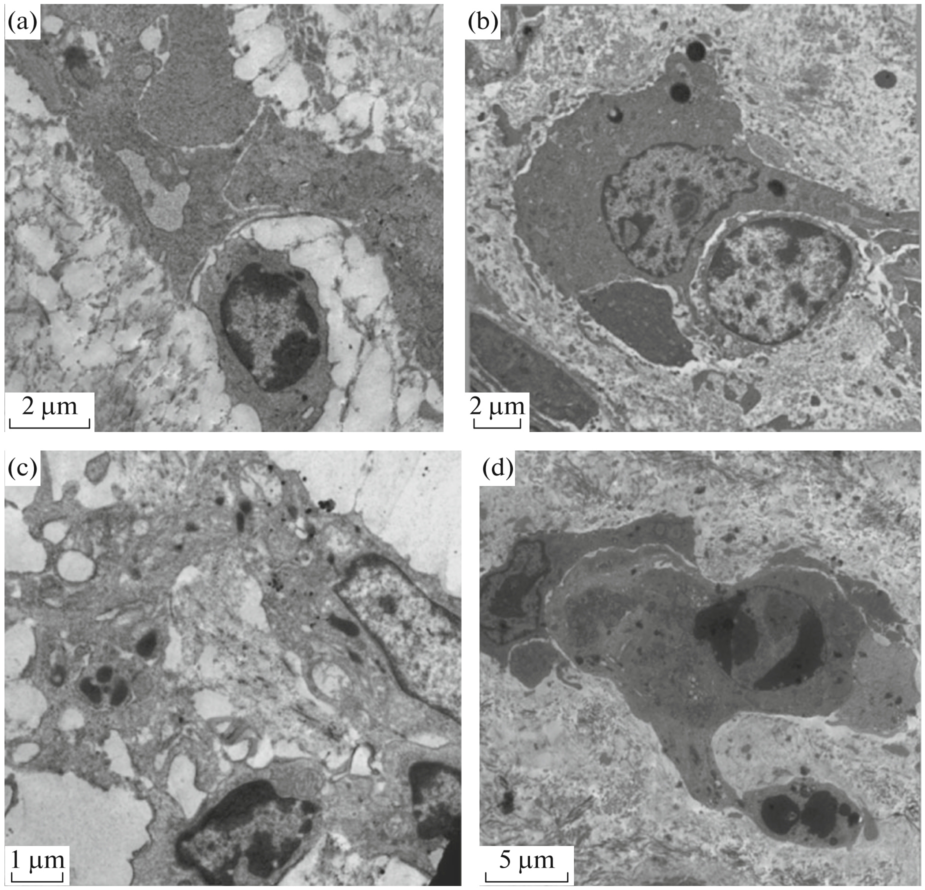

Fig. 9. Electron microscopy images of sarcoma 45 demonstrating partial regression induced by magnetite nanoparticles and activation of cell interactions in the tumor zone: (a) the contact of an activated lymphocyte with a tumor cell; (b) phagocytosis of tissue basophil granules by a macrophage; (c) complex contacts between neutrophils and lymphocytes; and (d) a tumor cell undergoing apoptosis and a macrophage interacting with it.

and increased the number of channels for the input of wave information. This allowed for a considerable increase in the efficiency of the impact on the rats with tumors as compared with SCENAR therapy alone (Fig. 13). An antitumor effect of SCENAR therapy was recorded in approximately half the animals (53%) versus almost all of the experimental animals after the integrated electromagnetic exposure; the displayed effect was of different degrees. This effect consisted in a slowdown of tumor growth (by 3.5 times) and tumor regression. By the end of experiment, the average tumor volume in the group with combined exposure was 3.0 times smaller (Fig. 13a) and complete regression was 1.9 times more frequent (Fig. 13b) as compared with SCENAR therapy alone. We note that the antistress effect of the combined electromagnetic irradiation was also more pronounced as compared with SCENAR therapy, since the cases of elevated activation AR were more frequent (Fig. 13b).

One of the specific features of the effective combined electromagnetic impact was that some of the animals developed stable antistress calm and elevated activation ARs. In this process, the most interesting shifts in the ratio of thyroid hormones were observed, which suggested multilevel regulatory rearrangements in these animals and the development of a specialized functional state, viz., antistress areactivity state.

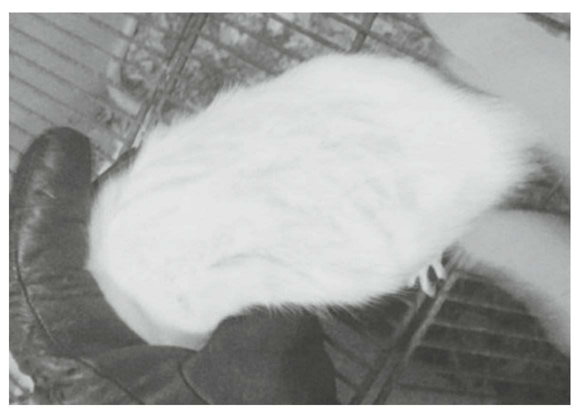

Fig. 10. Examination of an animal with completely regressed Pliss lymphosarcoma with a volume of over 20 cm^3 8 months after the completion of the experiment; no sign that indicates the recurrence of the malignant growth.

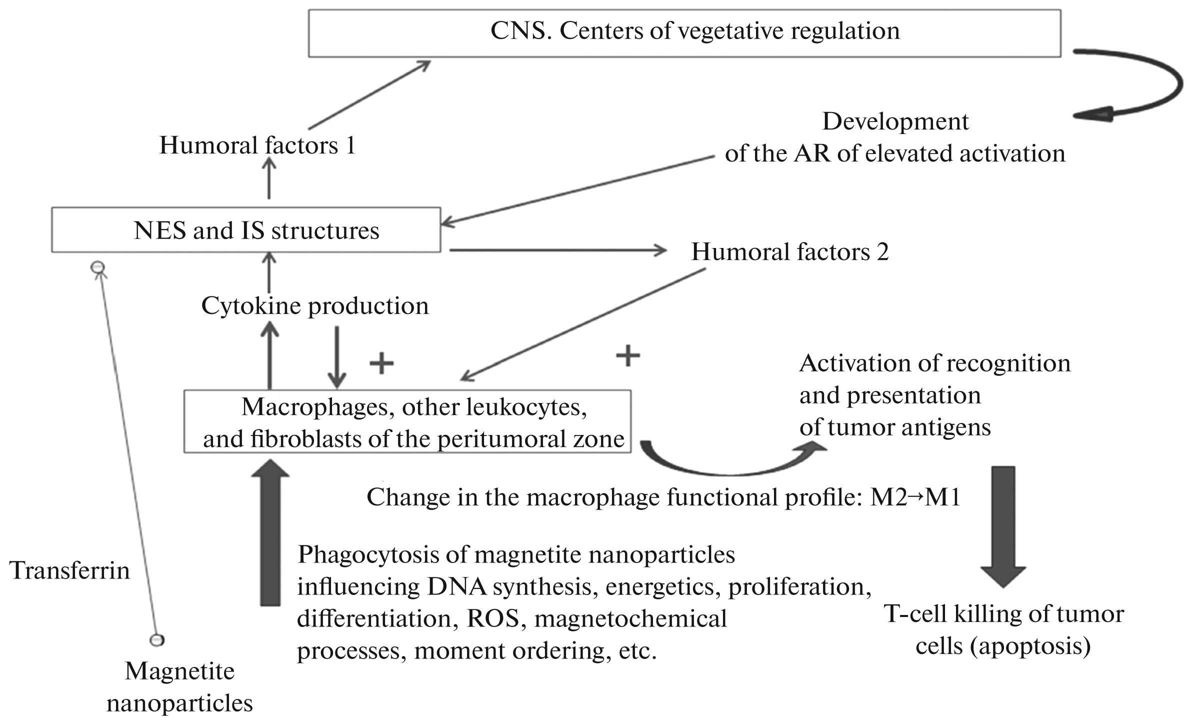

Fig. 11. Putative processes that determine the complete regression of a large Pliss lymphosarcoma (10–20 cm^3 and larger) induced by magnetite nanoparticles. *The regime with exacerbation?* Designations: NES, neuroendocrine system; IS, immune system; and M1 and M2, the known main functional macrophage profiles.

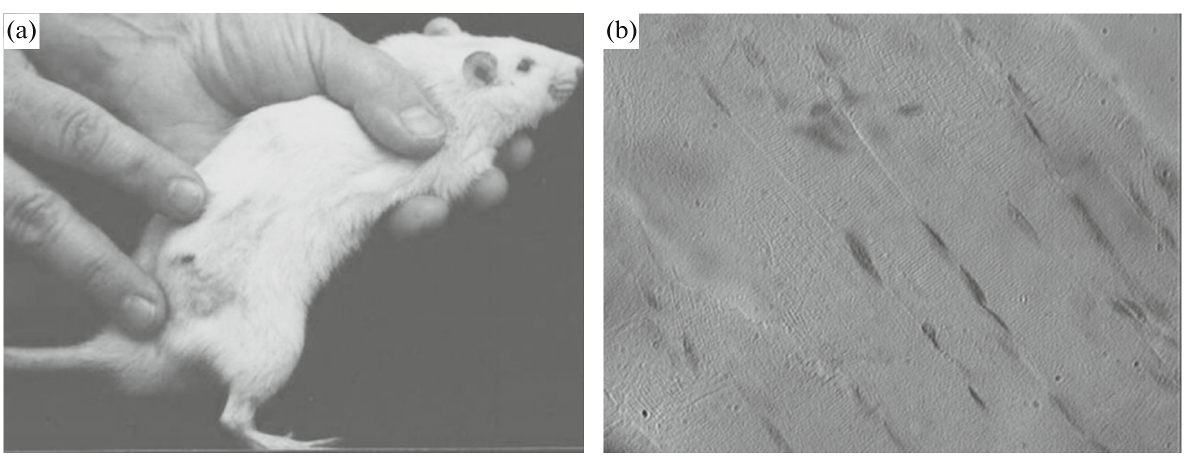

Fig. 12. Regression of a large Pliss sarcoma induced by modulated EHF EMR applied before tumor transplantation. *The regime with exacerbation?* (a) Examination of an animal with a complete tumor regression at the end of the experiment. (b) Micro image of the tissue from the site of a regressed tumor 2 weeks after the completion of the experiments. Any sign of the tumor is absent; only muscle cells are seen (staining according to Brachet).

According to the theory of ARs, states of areactivity develop when a particular AR of a single or several similar reactivity levels is maintained in the body [42]. Exceptional stability is characteristic of these states; while they display the pattern of changes that are similar to the corresponding AR, they differ from it by more balanced and, consequently, less energy-consuming metabolic processes and more favorable states of various regulatory structures, including a higher level of the body's nonspecific resistance. From the standpoint of nonlinear dynamics, the phase portrait of such states can be characterized as a limit cycle [12, 41].

The ratio of the calorigenic thyroid hormone triiodothyronine (T3) in the blood to thyroxine (T4) was two times lower in rats with sarcoma 45 regression

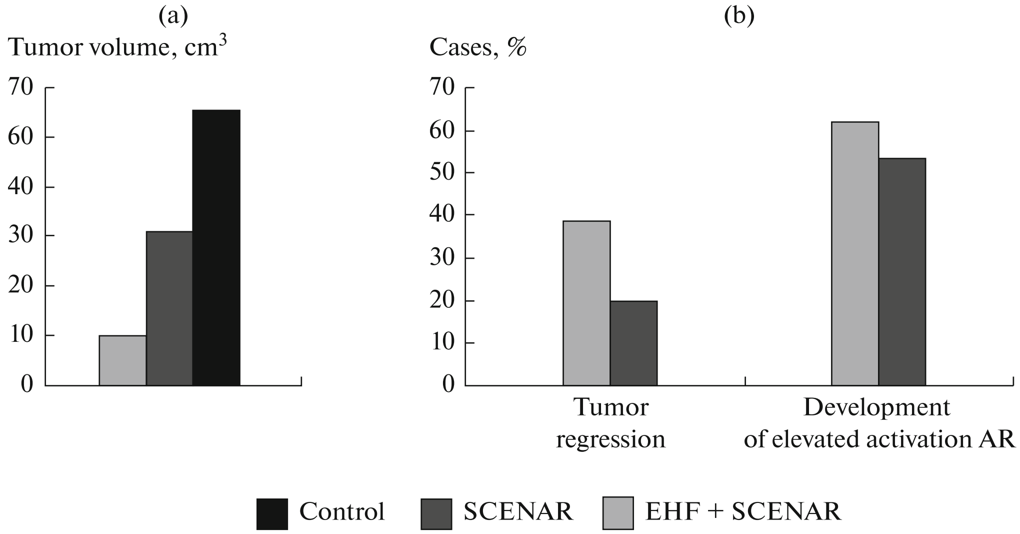

Fig. 13. Antitumor and antistress effects induced by combined application of EHF EMR and SCENAR therapy: (a) the size of the sarcoma 45 at the end of the experiment and (b) the rates of tumor regression and elevated activation AR development.

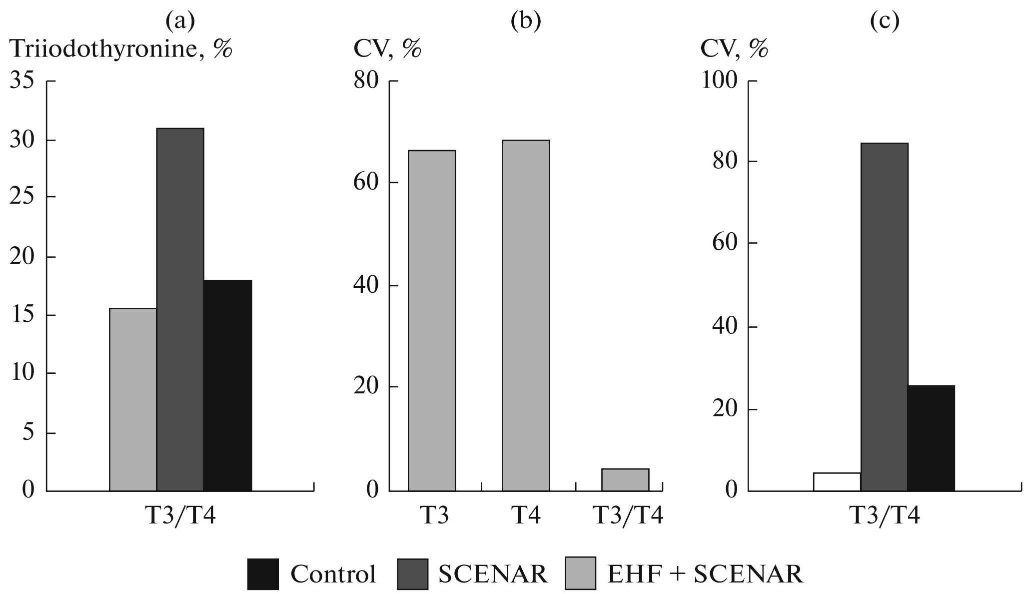

Fig. 14. The ratio of thyroid hormones in the blood after effective combined application of EHF EMR and SCENAR therapy. *Order parameter 2?* Designations: T3, triiodothyronine; T4, thyroxine; and CV, coefficient of variation. (a) A decrease in the triiodothyronine content relative to the thyroxine content after combined electromagnetic exposure; (b) variation of the levels of thyroid hormones and their ratio after electromagnetic exposure; and (c) low variation of the ratio of thyroid hormones after combined electromagnetic exposure.

induced by combined exposure as compared with the animals that were subjected to effective SCENAR therapy (Fig. 14a). Note that this ratio almost did not change: the variation coefficient was extremely low (4%) as compared with the variation for each hormone (Fig. 14b), as well as with the corresponding variation characteristics of this ratio for the control group and (especially) in the case of effective SCENAR therapy (Fig. 14). According to our opinion, a higher antitumor efficiency of the combined impact and concurrent extremely low variation in the ratio of thyroid hormones in the blood are associated with development of the activation arectivity state in the rats that was caused by the combination of EHF EMR and SCENAR therapy. Moreover, the almost complete stability of the ratio of T3 and T4 levels (T3/T4) suggests that this may be a new order parameter that is an integral characteristic for the areactivity state [43].

If this hypothesis is true, the former order parameter, viz., the relative (percentage) lymphocyte count in the peripheral blood, is uninformative. In fact, it is possible to distinguish a subgroup of rats among the

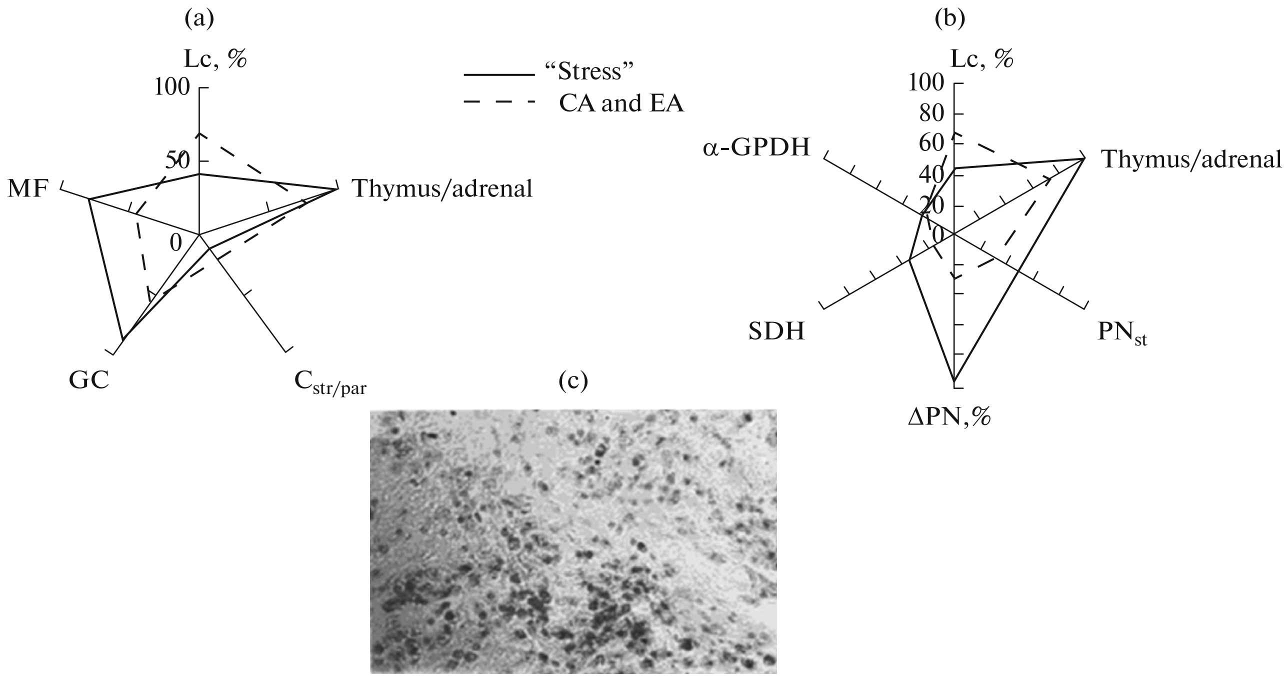

Fig. 15. The parameters of antistress areactivity after effective combined application of EHF EMR and SCENAR therapy at different relative lymphocyte (Lc) counts that correspond to stress AR ("stress") or calm (CA) and elevated (EA) activation ARs. (a) Lymphocyte proliferation activity in the thymus and spleen ($C_{str/par}$, stroma/parenchyma coefficient in the thymus; GC, number of germinal centers in the spleen; and MF, the number of mitotic figures in spleen GCs). (b) Blood leukocyte activity (PN_{st}, phagocytic number of neutrophils after zymosan stimulation; ΔPN, relative change in phagocytic number of neutrophils caused by zymosan stimulation as compared with spontaneous phagocytosis; and SDH, succinate dehydrogenase, and α-GPDH, α-glycerophosphate dehydrogenase, are the key Krebs-cycle enzyme activities in lymphocytes, the former in mitochondria and the latter in the cytoplasm). (c) Abundant infiltration of a regressing tumor with plasma cells with the "stress" leukogram (staining according to Brachet).

animals that displayed a pronounced effect of the combined exposure that had a specific ratio of AR parameters. In these animals, the relative lymphocyte counts in the blood by the end of experiment corresponded to the upper boundary of the range that is characteristic of a stress AR. In contrast, the state of the immune organs, thymus and spleen, corresponded to elevated activation AR. Moreover, the quantitative characteristics of lymphocyte proliferation in the thymus and spleen of these animals, as well as the characteristics of the phagocytic activity of macrophages and the mitochondrial succinate dehydrogenase activity in blood lymphocytes, were even higher as compared with the rats that displayed a hemogram that was characteristic of the calm and elevated activation ARs (Figs. 15a, 15b). Analysis of the overall pattern of the observed changes suggests that the physiological meaning of the decrease in the relative lymphocyte count in the peripheral blood during the development of activation areactivity state is associated with the migration of these cells from the bloodstream and an increase in their activity in tissues. Interestingly, the most pronounced infiltration of the lymphoid element of the regressing tumor tissues was particularly observable in the animals with a decreased lymphocyte percentage. (Fig. 15c).

Thus, integrated application of the factors of an electromagnetic nature according to the algorithms of activation therapy enhanced the optimization of regulatory interactions in the body, which led to the development of specific energy-saving states with a high level of nonspecific antitumor resistance.

CONCLUSIONS

The theory of a periodic system of general nonspecific adaptational reactions of the body can be regarded as a synergetic paradigm for the main regulatory phenomena at the systemic and organism levels. The applied implications of this theory include several principles for the formation of effective impacts that enhance the activation of the mechanisms that underlie nonspecific resistance as the background for a treatment technology, viz., activation therapy. The algorithms of activation therapy make it possible to optimize the biomedical effects of physical and chemical factors, as well as to increase the nonspecific antitumor resistance of the body by restoring the disturbed regulatory interactions at different structural and functional levels.

These results also allow one to define certain promising directions in boosting the antitumor effects of ferromagnetic nanoparticles. An antistress component that is present in the effects of these factors in the body during the development of a malignant process, as well as the involvement of multilevel processes in the formation of a particular AR, suggests that it is appropriate to combine the effects of ferromagnetic nanoparticles with various systemic impacts in activation therapy modes.

Maintenance of antistress ARs of calm and elevated activation in the body is a necessary condition for development of the states that in several cases makes it possible to escape the mechanisms defending malignant tumors from the immune surveillance and to activate efficient elimination of large tumors avoiding toxic responses. Stable antistress ARs are a necessary condition for the development of antistress areactivity state, main characteristic of which is the formation of effective multilevel regulation while optimizing energy processes.

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