

Low energy electromagnetic fields and immunity

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SUMMARY

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The reasons are presented for which the interest of many investigators is directed to the possible immunotropic influences of microwave low energy electromagnetic fields, in terms of their potential harmful effects and also for the perspective of therapeutic purposes. The available literature data on the influence of electromagnetic fields (EMF) on the immune system are up to now fragmentary, describing the changes of a few immune functions, mainly phagocytosis, lymphocyte proliferation, or antibody production, and are frequently controversial or not confirmed by the results of repeated experiments. On the grounds of results of the three series of demonstrated experiments the authors indicate which methodological elements, including not only precise dosymetric circumstances but also the timing of exposure in relation to the cell cycle and the initial functional state of exposed cells may be decisive for the final effect of exposition *in vitro*.

Key words: *microwave immunotropic effects, sensitivity of immune cells to EMF, cell cycle, functional state of exposed cells, low frequency magnetic field, clinical application*

Recent rapid development of electromagnetic field (EMF) emitters (mobile phones, microwave broadcast stations, radiolocation, microwave sources in household devices) increased the environmental level of electromagnetic radiation. This, in turn, increased the interest on the possible risk of harmful influence of exposure to EMF. On the other hand, the low energy EMF of both low (homogenous magnetic field) and high frequencies (microwaves 900 and 1300 MHz) appeared to modulate the regulatory functions of immune system and some of them were even administered for therapeutic purposes [1]. There exist

STRESZCZENIE

Niskoenergetyczne pola elektromagnetyczne a odporność

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Praca przedstawia powody rosnącego zainteresowania licznych badaczy możliwością immunotropowego oddziaływania niskoenergetycznych pól elektromagnetycznych (EMF) w odniesieniu do ich potencjalnych szkodliwych wpływów oraz do perspektywy terapeutycznego wykorzystania. Dane dostępnej literatury dotyczące wpływu EMF na układ odpornościowy nadal pozostają fragmentaryczne, opisując niektóre zmiany immunologicznej aktywności, głównie fagocytozy, proliferacji limfocytów lub wytwarzania przeciwciał. Dane te są często kontrowersyjne i nie potwierdzone w powtarzalnych badaniach. Na podstawie wyników trzech serii własnych doświadczeń autorzy wskazują, jakie metodologiczne elementy, dotyczące nie tylko precyzyjnego określenia warunków dozymetrycznych, ale także czasu ekspozycji w odniesieniu do faz cyklu komórkowego i stanu czynnościowego eksponowanych komórek mogą decydować o uzyskanych wynikach.

Słowa kluczowe: *immunotropowe wpływy mikrofal, wrażliwość immunocytów na EMF, cykl komórkowy, stan czynnościowy eksponowanych komórek, kliniczne zastosowanie wolnozmiennego pola magnetycznego*

a number of reports in literature suggesting that microwave (MW) radiation at non-thermal levels can influence the cell-mediated and humoral response to antigens and mitogens in humans and laboratory animals [2-13]. However, the results of single experiments depend on the schedule, the timing of exposure and frequency, modulation and power density of EMF used in particular studies and therefore, the effects of controlled experimental expositions may substantially differ from the accidental and uncontrolled environmental expositions. These unmeasurable casual influences of EMF, according to prof.

Olle Johanson from Karolinska Institute, Stockholm, may be responsible for an increasing incidence of not explained as to the reason, the illnesses (allergy, neoplasms) appearing among the inhabitants of the vicinity of EMF emitting installations.

The confusion in opinions on the biological EMF influence are well reflected by the WHO Environmental Health Criteria #137 [14] description that nonthermal intensities of MW represent a "weak factor of biological influence". If the detector of this "weak influence" would be sensitive enough to receive and to react to the signal, the reaction could be unexpectedly much greater than expected. One of the possible candidates to be a such detector is the immune system. As an important part of homeostatic neuro-endocrine-immune network of the organism, the immune system is responsible for efficient defense against infections, regenerative support for injured tissues and maintenance of immune tolerance toward self or foreign but neutral elements [15-17]. These different reactions of the immune system can be investigated by the tests performed both *in vivo* and *in vitro*, to evaluate the possible influence of external stimuli (eg. drugs or physicochemical influences), including well dosimetrically measured exposures to EMF. The available data on the immunotropic influence of EMF, in contrast to the more than 30 years lasting investigations on the topic, are still fragmentary and not conclusive enough. They describe the changes of a few immune functions, mainly phagocytosis, lymphocyte proliferation, or antibody production, and are frequently controversial or not confirmed by the results of repeated experiments [3-6].

Some authors [7] conclude that studies of MW-exposed immune cells have shown no damage or change until the cells were heated, while others [8-10] report immunosuppressive or immunostimulatory phenomena in animals with long-term exposure to low-level MW fields. Depending on conditions of exposure, frequency and modulation of the radiation, as well as on animal species used in the experiments, various symptoms of either stimulation or inhibition of certain immune reactions have been reported. Guy et al. [18] in the life-time exposure of rats to MWs (pulsed 2450 MHz, SAR 0.15 - 0.4 W/kg) found lowered response of blood lymphocytes to mitogen phytohaemagglutinin (PHA), while Śmiałowicz [19] after exposure at the same wave frequency, although at higher power intensities (SAR 1 - 5 W/kg) reported increased mitogenic response of lymphocytes. Investigating the humoral immune response in mice exposed to 9.4 GHz at SAR 0.015 W/kg, depending on the carrier wave modulation, Vayert et al. [3] found enhancement or lowering the response.

Even the epidemiological investigations of workers exposed to MW radiation did not confirm the existence of measurable shift in the immune status of the investigated populations, despite some observations on abnormalities in single immune parameters in several individuals (e.g. changed number of blood lymphocytes, lowered level of serum immunoglobulins or weaker response of lymphocytes to mitogens).

At the present state of knowledge it is, therefore, not possible to conclude about the specific immunotropic potencies of MW radiation, as the assessment of the immunotropic potency requires a general insight into the whole complex immune network, taking in advance the determination of immune status of the host or the investigated cellular population prior to the MW exposure.

The final effect of exposition of biological material to MW radiation depends on the physical properties of applied electromagnetic field on the one side, and on the functional state of exposed living target on the other. The EMF used in different experiments may differ in countless dosymetric elements, including wave length and frequency, pulsative modulation, intensity of EMF influencing the degree of specific absorption rate (SAR) and duration of the exposure. The functional characteristics of biological material, e.g. blood mononuclear cells mainly used for *in vitro* studies, is even more complex. The EMF exposure may affect the cell at different levels of its structure: the surface receptors changing their distribution and conformation, the cellular membrane changing its rigidity and permeability, mitochondrial metabolic activity, transcription and translation processes or several of these elements at different intensities.

One of the best methods of evaluation of immunotropic influences of EMF administered *in vitro* is the system of microcultures of mononuclear cells isolated from the blood (PBMC), representing *in vitro* the abilities of the immune system present *in vivo*. The advantages of the method are accessibility of human cells, donor safety, and wide repertoire of immune tests which can be performed.

The mononuclear cells isolated from the vein blood remain in their most stable and inert metabolic state, the G₀ phase of cell cycle, in which the cell represents low sensitivity to external influence [20, 21]. When the cell cultured *in vitro* enter more active phases of cell cycle (G₁, S, G₂, M), its sensitivity to EMF influence may change significantly. In these circumstances the cells exposed to EMF after isolation from the blood, like in the most published studies *in vitro*, and cultured after that, stimulated specifically and tested for their different activities, may not display any significant changes. The exposition to EMF during the culture, of already activated cells, although

methodically much more difficult, may deliver better insight into the potential immunotropic effects of the exposition.

Recently, using these methods, we investigated the behavior of PBMC in a microculture system after exposure to pulsed (5 μ sec pulses) 1300MHz microwaves (10W/m², SAR 0.18W/kg) [11]. The exposure resulted in the increased immunoregulatory activity of T cells, increased production of IL-10, increased IL-1 production by monocytes, and decreased concentration of IL-1ra in culture medium. We concluded that MW may support the inductive phase of immune response, increasing the activity of monocytes and T cells. The special feature of this experiment was that cells were exposed to EMF before the culture, indicating that at the time of exposure they remained metabolically neutral (Go phase of cell cycle), which is normal for lymphocytes freshly isolated from blood.

In the *in vivo* situation, the accidental or deliberate exposure of the individual to MW may influence neutral or active immune cells, both normally present in the body. On the way to find the answer how the active cells, e.g., stimulated *in vitro* with mitogens and entering G1 and S phases, will react to the subsequent exposure to MW, we have introduced special device into our experiments. It was an anechoic chamber constructed and technically tested in the Department of Microwave Safety, Military Institute of Hygiene and Epidemiology in Warsaw, Poland [12]. The chamber containing the microplate with cultured cells and MW-emitting antenna, was installed inside the ASSAB CO₂ incubator, so the PBMC could be exposed to MW at different periods of culturing without removing them from the incubator.

The examples of three different experiments performed in our laboratory will show how the range and kind of immunotropic effects of EMF may depend on the wave length and frequency (microwaves or low frequency magnetic field), on the time of exposure in relation to the cell cycle of exposed lymphoid cells and, finally, on the differences in functional state of immune system between healthy donors of PBMC or patients suffering from chronic virus B hepatitis.

EXPERIMENT I

Immunotropic influence of 900 MHz microwave GSM signal on human blood immune cells activated *in vitro* [12]

Methods. Blood samples were collected from healthy donors and mononuclear cells (PBMC) were isolated on Ficol-Paque gradient. The microcultures of PBMC were set up in triplicates (105 cells/0.2 ml RPMI + 15% autologous inactivated serum) in Nunclon microplates. Respective triplicates were left

without stimulation or stimulated with phytohemagglutinin (PHA, HA16, Murex Biotech Ltd Dartford U.K., 0.4 μ g/cult.) or with concanavalin A (Con A, Sigma, 8 μ g/cult.). The plates were placed inside the anechoic chamber in the ASSAB incubator at 37°C and 5% CO₂. An identical plate of control cultures was also set up and placed in the ASSAB incubator beyond the chamber. At 24h of incubation, rearrangements of the cultures were performed as described elsewhere [11, 22, 23].

As a result of rearrangements of cultures, the following parameters of T cell and monocyte activities were measured at the end of cultures: T lymphocyte response to PHA and to Con A, saturation of IL-2 receptors, T cell suppressive activity (SAT index), and the index of monocyte immunogenic activity (LM) related to the ratio of produced monokines (IL-1 β versus IL-1ra) [23].

For the last 18h of incubation, 3H-thymidine (3HTdR, Amersham, U.K., spec act. 5Ci/mM) was added into the cultures in a dose of 0.4 μ Ci/cult.

At the beginning of each of the three consecutive days of incubation, the cultures which were placed in the anechoic chamber were exposed to MW (900MHz, 20V/m, SAR 0.024W/kg) for 15 min. Control cultures were not exposed to MW.

At 72h the cultures were harvested and incorporation of 3HTdR was measured in Packard Tri carb 2100 TR scintillation counter. The results were calculated as a mean value of dpm (desintegrations per minute) per triplicate of cultures \pm SD. The experiments were repeated 10 times, and the results observed in the exposed cultures were compared with those obtained in the control cultures.

The data were analyzed with STATGRAPHICS PLUS 4.0 version (Nr. 471000349). The differences between the mean values were assumed statistically significant if the p values, calculated with the use of U Mann-Whitney's test, were lower than 0.05.

Results. The relatively short time of exposure of cultured cells to MW (15 min, administered repeatedly at the beginning of each of the three consecutive days of culturing) was chosen deliberately. First, our intention was to check the effects of exposure similar in duration to the average use of a mobile phone. Second, the cells, stimulated with mitogens, were exposed immediately after entering the G1 phase of cell cycle (first day exposure), again when the majority of cells responding to mitogen entered the S phase (second day exposure), and finally when the responding cells, after replication of DNA, reached stage G2 and mitosis (third day exposure). In this way the repeated exposures to MW covered the main periods of metabolic activity during the cell cycle of cultured cells [20, 21].

The summarized results of 10 experiments indicate that activity of lymphocytes and monocytes tested *in vitro* increased significantly under the influence of MW administered during the culture. The proliferative response of T lymphocytes exposed to MW increased from the value of 60.7 to 82.8 dpm in response to PHA ($p < 0.001$) and from the value of 55.9 to 73.8 dpm in response to Con A ($p < 0.001$). The exposure to MW also increased the immunogenic activity of monocytes. The value of LM index, which depends on the ratio of IL-1 β to IL-1ra [23], (both monokines produced by monocytes), increased from the value 8.0 to the value 18.0 ($p < 0.001$). In contrast to these immunostimulatory effects, the suppressive activity index (SAT), which represents regulatory function of T cells, and the saturation of T lymphocyte receptors with interleukin 2 remained at normal level after the exposure to 900 MHz microwaves.

The experiments presented here show for the first time that human lymphocytes and monocytes, induced in culture into active phases of their cell cycle (G1 in terms of monocytes and G1 and S in terms of T cells), further accelerate their metabolic activity under additional stimulus created by the exposure to 900 MHz GSM signal. The observations suggest that 900 MHz GSM signal is immunostimulatory and may increase the immune reaction of lymphocytes and monocytes already participating in the immune response.

Testing possible immunotropic influences of 900 MHz GSM signal on human blood lymphocytes Scarfi et al. [24] did not find any changes in proliferative rate of cells exposed for 24 hour before setting up the cultures. Similar timing of exposure (irradiation before the culturing) was applied for human lymphocytes by Tuschi et al. [25]. They found no changes in several cytokine production and cytotoxic potential of lymphocytes exposed to 1950 MHz, SAR 1 mW/g. The both groups of authors conclude that tested radiofrequencies did not evoke any adverse influences on human immune cells. Nevertheless, in the light of cited above results of our experiments, the improper timing of irradiation could be responsible for observed negative results.

EXPERIMENT II

Immunotropic influence of 1300 MHz MW on cultures of blood mononuclear cells derived from normal donors or patients suffering from chronic virus B hepatitis [13].

Methods. The effect of irradiation may also be dependent on the initial immune state of the donor of blood lymphocytes. Two groups of blood donors,

one of healthy individuals (HD) (N = 16) and the other of patients suffering from chronic virus B hepatitis (HV) (N = 12) were enrolled into our experiments in which blood lymphocytes were exposed to 1300 MHz pulse modulated microwaves at 330 pps with 5 μ s pulse width, or left without irradiation [13]. The specific absorption rate (SAR) was measured and the value of SAR = 0.18 W/kg was recorded. The microcultures of PBMC were subsequently set up to determine several parameters characterizing the T cell immunocompetence and monocyte immunogenic activity, including: proliferative response to mitogens (PHA, Con A), saturation of IL-2 receptors, T cell suppressive activity (SAT index), monocyte immunogenic activity (LM index) and production of chosen cytokines.

Results. The same absorbed dose of 1 mW/cm² reduced response to PHA in HD cultures (from 67.1 \pm 8.7 to 45.8 \pm 13.7 dpm \times 10³/cult) and significantly increased this response in HV cultures (from 75.8 \pm 9.8 to 98.2 \pm 13.7 dpm \times 10³/cult). The response to Con A did not change in the both kind of cultures, but immunoregulatory activity of T cells (SAT values) increased after MW exposition from 11.7 \pm 9.4 to 29.7 \pm 7.3 %, and from 19.8 \pm 11.4 to 28.9 \pm 11.8 %, respectively). Similarly, the saturation of T lymphocyte IL-2 receptors increased in the both HD and HV cultures. Significant increase of production of interferon gamma (IFN γ) and tumor necrosis factor alpha (TNF α) after exposition to MW was observed in the HV cultures but not in the HD cultures. The results suggest that microwave irradiation (1300 MHz, pulse modulated) may exert distinct immunotropic influence and may enhance the effector immune response in patients with chronic virus B hepatitis, including considerable stimulation of the production of IFN γ by immune cells.

EXPERIMENT III

Clinical and immunological effects of magnetostimulation in children with recurrent infections of respiratory tracts [1]

Methods. 40 children (age 4 - 10 years) with frequent respiratory infections (no less than 4 episodes during 6 months) was selected for the study. 20 of them, in addition to the routine antiinfective, antiinflammatory and antipyretic treatment were also treated with magnetostimulation. They received daily expositions (10 during the 14 days, 15 min each), according to M1P2 programme of Viofor JPS low frequency magnetic field generator, with the use of a large ring applicator around the chest. The induced homogenous magnetic field represented basic pulses frequency of 180 - 190 Hz and magnetic induction B

= 3,2 mT (mean) and = 40mT (at the peak of pulse). Our patients were tested clinically and immunologically before the treatment, after the magnetostimulation was finished and 6 months later.

The immunological tests comprised: 1. in microcultures of mononuclear cells separated from the blood (PBMC) estimations of response to PHA and to Con A, saturation of IL-2 receptors, T-cell suppressive activity (SAT index), monocyte activity in IL-1 β and IL-1ra monokine production (LM index), and 2. in microculture supernatants quantitative determination of chosen cytokines (IL-1 β , IL-1ra, IL-10,) (ELISA Quantikine kits). The detailed description of the methods was published earlier [1].

Results. In the group which received magnetostimulation, but not in the group treated routinely, the number, severity and duration of infective episodes diminished significantly (the number dropped from $5,3 \pm 1,3$ to $0,25 \pm 0,4$ and the duration shortened from $10,0 \pm 2,4$ to $1,2 \pm 2,2$ days) at the end of observation.

Before the treatment all our patients demonstrated immune deficits of T cell competence (low mitogenic response, low saturation of IL-2 receptors), deficient regulatory T cell abilities (low values of SAT index and IL-10 concentration in culture supernatants) and elevated immunogenic activities of monocytes (high value of LM index and IL-1 β concentration). In the group of children which received routine treatment only, the immune characteristics remained not changed after the treatment. In contrast to that the patients of the group exposed to magnetostimulation, represented improved values of immunocompetent (T cell features) and immunogenic (monocyte activities) parameters. The response to Con A increased from 47.0 ± 19.7 to 62.2 ± 14.3 dpm $\times 10^3$ /cult, the value of SAT index increased from 15.8 ± 11.2 to 31.2 ± 14.4 % and saturation of T lymphocyte IL-2 receptors increased from 76.3 ± 12.4 to 89.9 ± 11.3 %.

DISCUSSION

The results of two experiments presented above suggest, that exposition *in vitro* of human blood mononuclear cells to different radiofrequencies of low energy MW (e.g. 900 and 1300 MHz) is potent to modulate the immune activity of lymphocytes and monocytes. The range of affected immune parameters depend not only on the wave length, frequency and intensity of EMF but also on the timing of exposures (before or during the culture) and on the initial immune status of the donor of immune cells. The results of the second experiment indicate also that the initial functional state of immune cells is decisive for the effect of exposition to EMF. In the cultures of

PBMC derived from patients suffering from chronic virus B hepatitis significant increase of proliferative response of lymphocytes to PHA and increased production of interferon gamma and tumor necrosis factor were observed in contrast to the PBMC cultures of healthy donors where these parameters remained not changed.

The results of the third experiment show that immunotropic influences of EMF are not the exclusive feature of high frequency microwaves but are also observed after the clinical application of Viofor JPS generating very low frequency homogenous magnetic field.

The use of large ring applicator of Viofor JPS situated around the chest of the patient creates the possibility of direct influence of homogenous magnetic field on the thymus. The thymus is a lymphopoietic organ responsible for delivery of matured T lymphocytes to the peripheral immune system. Their presence in peripheral blood may be detected in the microcultures of PBMC by the observation of a proper ability to respond the mitogenic stimulation (PHA, ConA), proper values of T lymphocyte suppressive activity (SAT index), full saturation of IL-2 receptors and efficient production of IL-10 [22, 23]. These properties of T cells improved significantly in the group of children after magnetostimulation. Concomitantly, the previously excessive immunogenic functions of monocytes (high values of LM index, elevated production of IL-1 β), responsible for pro-inflammatory activities of immune system, considerably diminished. These thymic-dependent, magneto-stimulation-induced changes in immune cell population resulted in increased defensive properties of immune system, reflected by reduced number of infective episodes in our patients. It is, therefore, logic to expect, that low frequency, homogenous magnetic field, administered in the way described above, is able to exert immunocorrective influence on the process of thymic-dependent process of T lymphocyte maturation.

CONCLUSIONS

The low energy electromagnetic fields of both high and low frequencies possess ability to modulate different functions of immune cells. The kind and range of immunomodulation depend on the time of exposure to EMF, its frequency, pulse modulation, the value of specific absorption rate (SAR) and on the initial functional state of exposed immune cells. Several kinds of strictly dosimetrically controlled EMF may be employed as a valuable immunotherapeutic agents. Nevertheless, its safety, similarly to the pharmaceutical drugs, depends on the proper dosage and administration in accordance with therapeutic

indications. The causal, uncontrolled expositions may cause unexpected harmful effects. The problem of safety and therapeutic usage of different EMF needs further extensive studies.

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