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Immunotropic effects in cultured human blood mononuclear cells exposed to a 900 MHz pulse-modulated microwave field

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ABSTRACT

The specific biological effect of electromagnetic field (EMF) remains unknown even though devices present in our daily lives, such as smartphones and Wi-Fi antennae increase the environmental level of electromagnetic radiation. It is said that the human immune system is able to react to discrete environmental stimuli like EMF. To investigate the effect of 900 MHz microwave stimulation on the immune system our research aimed to analyze lymphocyte proliferation and observe and assess the basic immunoregulatory activities using a newly developed and improved anechoic chamber. Samples of mononuclear cells (PBMC) isolated from the blood of healthy donors were exposed to 900 MHz pulse-modulated radiofrequency radiation (20 V/m, SAR 0.024 W/kg) twice (15 min each) or left without irradiation (control group). Subsequently, the control and exposed cells were set up to determine several parameters characterizing T cell immunocompetence and monocyte immunogenic activity. Although the microcultures of PBMC exposed to radiofrequency radiation demonstrated higher immunogenic activity of monocytes (LM index) and T-cell response to concanavalin A than control cultures after first exposure, this parameter decreased after a second stimulation. Saturation of the interleukin-2 (IL-2) receptor rose significantly after the second day of exposure. On the other hand, response to mitogen dropped after EMF stimulation. The results suggest that PBMC are able to overcome stress caused by mitogens after stimulation with 900 MHz radiation.

Keywords: RF-EMF; immune system; immunoregulation; monokines; lymphocytes

INTRODUCTION

After decades of research, it is still unknown what the specific biological effect of the electromagnetic field (EMF) on the human organism is. However, the lack of knowledge on this subject did not stop radiocommunication and radiolocation development. The presence of electronic devices such as smartphones, Wi-Fi antennae and Long Term Evolution (LTE) modems in virtually every aspect of our life increases the environmental level of electromagnetic radiation. Therefore, even a limited impact of electromagnetic radiation on human biology could lead to major public health consequences.

In order to investigate the 'weak biological influence', which is how EMF is described by WHO Environmental Health Criteria #137 (WHO, 1993), an appropriate and sensitive enough detector had to be chosen [1]. The immune system is an important part of the homeostatic neuro-endocrine–immune network, designed to provide regenerative support for injured tissue, protect from infections and maintain its activity at the optimal level by identifying 'self' and 'foreign' elements [2, 3]. Since EMF may influence the organism in an extremely subtle way, and the immune system is able to react to discrete environmental stimuli, it is the perfect biological system in which to investigate the effects of the EMF. More precisely, one of the easiest methods of measuring the reaction of the immune system to stimuli is to analyze lymphocyte proliferation, and thus microcultures of immune cells isolated from human blood. This biological model allows for observation and quantification of the basic immunoregulatory activities. It is important to notice that since 2011, EMF is categorized as 'possibly carcinogenic to humans' by IARC (International Agency for Research on Cancer)—group 2B according to Hill's criteria [4]. The National Toxicology Program (NTP) also reported that lifelong but intermittent exposure to a 900 MHz radio-frequency electromagnetic field (RF-EMF) signal with a SAR of 1.5–6 W/kg for 18 h per day increased the risk of glioblastoma and black-heart disease in male rats. Interestingly, there

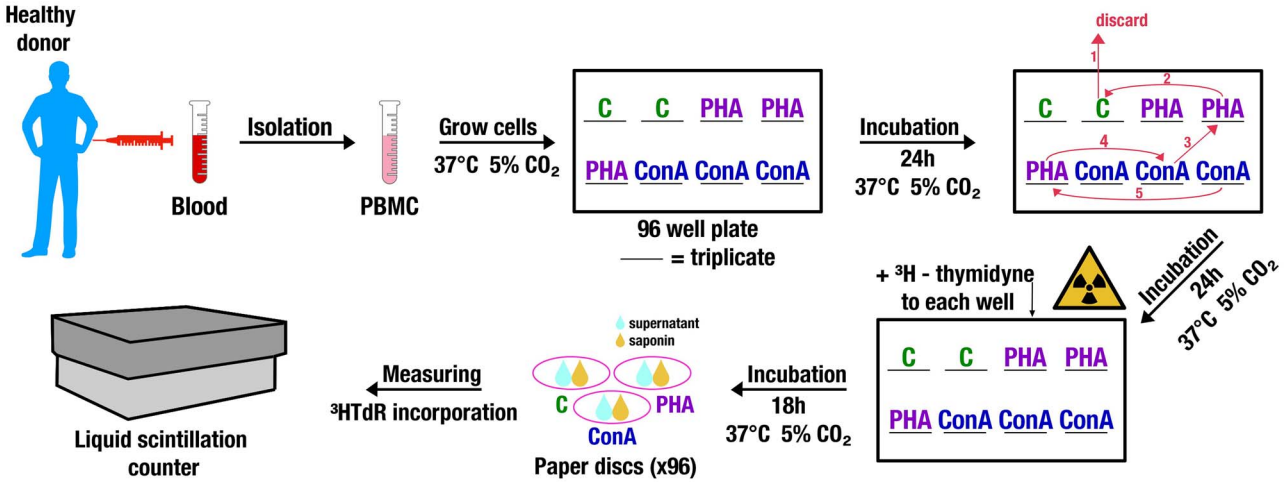


Fig. 1. Schematic representation of performed experiment. PBMC= peripheral blood mononuclear cells, C= control, PHA= phytohemagglutinin, ConA= concanavalin A.

were no similar observations in female rats. Unfortunately, the NTP did not disclose the results for other examined organs, and the details of the study are also missing [5].

The undisturbed activity of the immune system is critical for its defensive, proregenerative and tolerogenic functions. Since the immune system is part of an extremely complicated neuro-endocrine–immune network and numerous experiments have shown that EMF has various biological effects [6–8], it is possible that EMF may indirectly contribute to the emergence and development of various cancers [4]. The effects of EMF exposure depend on various parameters such as frequency, amplitude and duration of exposure along with the characteristics of the cell types subjected to exposure.

Finally, there is an increased interest among WHO member states in the formation and publication of clear guidelines and standards for non-ionizing radiation protection. It has been proposed to form standards for non-ionizing radiation protection using the International Ionizing Radiation Basic Safety Standards (BSS, 2012) as an example [9].

Even though the possible effects of EMF exposure on human health received increased attention from the WHO states, consensus was not reached as a consequence of conflicting results obtained by various experimenters [10–16].

In our previous research, we studied the behavior of peripheral blood mononuclear cells (PBMC) after exposure to 1300 MHz microwaves (5 μs pulse, 10 V/m^2, SAR 0.18 W/kg, 60 min), and immunotropic influence of 900 MHz microwaves (27 V/m^2, Specific Absorbtion Rate (SAR) 0.024 W/kg, 15 min) on *in vitro* activated human blood immune cells [3, 6]. Both studies proved the existence of a significant immunotropic effect of EMF *in vitro*. Therefore, we decided to extend our research to the effects of 900 MHz microwaves on the immune system, using a newly developed and improved anechoic chamber.

MATERIALS AND METHODS

The research was approved by the local Ethics Committee associated with the Military Medical Chamber in Warsaw (decision number 36/06). The material was isolated under prior patient's consent and the data were analyzed anonymously. All research was performed in accordance with the relevant guidelines and regulations.

METHODS OF INVESTIGATION OF PBMC RESPONSE

Samples were collected from 26 healthy donors, and PBMC were isolated from heparinized blood using a Ficol-Paque centrifugation gradient. The viability of cells was checked (≥80% viable cells). In the next step, the cells were resuspended in RPMI 1640 supplemented with 15% autologous inactivated serum. Microcultures were set up in triplicate, using 10^5 cells suspended in 0.2 mL of medium in Nuncoln round-bottomed 96-well microplates. Respective triplicates were stimulated with phytohemagglutinin (PHA 2 μg/ml, HA16, Murex Biotech Ltd Dartford UK) or with concanavalin A (Con A 4 μg/ml, Sigma) or left without stimulation.

The plates prepared in this way were placed in the anechoic chamber at 37°C and 5% CO_2. An identical set of plates with microcultures was set as a control and also placed in the incubator. After 24 h of incubation, rearrangement of the cultures was performed as described by Dąbrowski *et al.* [17–19] and presented in the form of a scheme in Fig. 1. This method involves the exchange of 0.1 mL of cell free medium (CFM) between the appropriate cultures. ^{3}H-Thymidine (3HTdR, Amersham, UK, specific activity 5 Ci/mM) was added into the cultures in a dose of 2 μCi/culture for the last 18 h of incubation. Incorporation of ^{3}HTdR (cpm) was measured, at the 72nd hour of culture, using Packard Tri-carb 2100 TR liquid scintillation counter.

The results were calculated as a mean value of dpm (disintegrations per minute) ±SEM (standard error of the mean). For each parameter, more than 23 samples were analyzed. Results obtained from the EMF-exposed cultures were compared with the control cultures. Data distribution was assessed using the Shapiro-Wilk test. Statistical evaluation of the results was performed using unpaired, two-tailed T test for data with normal distribution and unpaired, two-tailed Mann-Whitney test for the nonparametric data. Results at $P < 0.05$ were considered

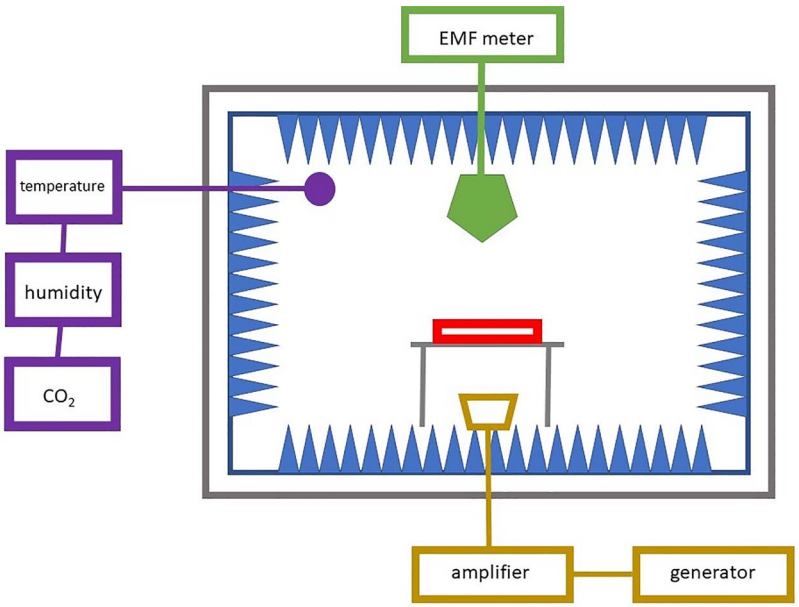


Fig. 2. Schematic diagram of a minature anechoic climate chamber for cell culture exposure to electromagentic field.

statistically significant. GraphPad Prism ver 7 software was used to perform the statistical calculations (La Jolla, CA, USA).

Similarly to our previous research the following parameters were studied:

- spontaneous ^{3}HTdR incorporation
- T-cell response to PHA
- T-cell response to Con A
- suppressive activity of T cells (SAT index)
- saturation of the interleukin-2 receptors (IL-2 index)—which is described by the ratio of ^{3}HTdR incorporation into the second and the first triplicate of microcultures
- monokine influence on the T cell proliferative response (LM index).

SAT and LM values were calculated as described by Dąbrowski *et al.* [18, 19] and Stankiewicz *et al.* [3].

$$LM\ index = \frac{\text{mean value of dpm in rearranged cultures}}{\text{mean value of dpm in non} - \text{stimulated cultures}}$$

$$\text{SAT\%} = 100 - \left[\frac{\text{mean value of cpm in PHA microclutures with addition of half—volume of Con A cell suspension}}{\text{mean value of cpm in PHA microcultures with added Con A—CFM} + \text{half of value of the cpm in ConA microcultures with added PHA—CFM}} \times 100 \right]$$

These equations seemed to be the most appropriate to use since sparse cells in PBMC population, which spontaneously incorporated ^{3}HTdR, were not involved in the response of other cells to the stimulation with mitogen as they already entered the S phase of cell cycle. Furthermore, the simple subtraction of the values of radioactivity incorporated by the cells stimulated in wholly different ways did not appear to be well-founded. The most precise information on how many cells contributed to the observed total value of incorporated radioactivity most accurately provided the ratio of mitogen stimulated to spontaneous incorporation.

The possible mutual influences of 'mixed' microculture supernatants on cellular proliferation were omitted because the values of these influences if they exist, were equally expressed in the numerator and denominator of the equation used for calculation of SAT.

ANECHOIC CHAMBER AND EXPOSURE CONDITIONS

A miniature anechoic climate chamber for cell culture exposure to EMF was designed and produced in our department (Fig. 2). This anechoic chamber assures the homogeneity of the EMF in the exposure area and proper cell culture conditions (Fig. 3). The chamber is a modified T305 GF CO_2 incubator, with internal dimensions of 25 × 25 × 25 cm. The area of the homogeneous EMF exposure is 12.5 × 8.5 cm.

The EMF was produced by radar operating at 900 MHz, with average power density E = 20 V/m, pulse duration Ti = 570 μs and repetition period Tp = 1.14 ms (Fig. 4). The value of SAR was of 0.024 W/kg—the applied method of SAR calculation was described in detail by Dąbrowski *et al.* [19].

The cell cultures placed in the anechoic chamber were exposed to the EMF for 15 min immediately after set-up of microcultures and 24 h after the first exposure. Control cultures were not exposed to the EMF.

RESULTS AND DISCUSSION

These experiments were conducted as an extension of our previous research [3]. The viability of cells tested at the beginning and the end of cultures remained at the level of 80%. The cells were exposed

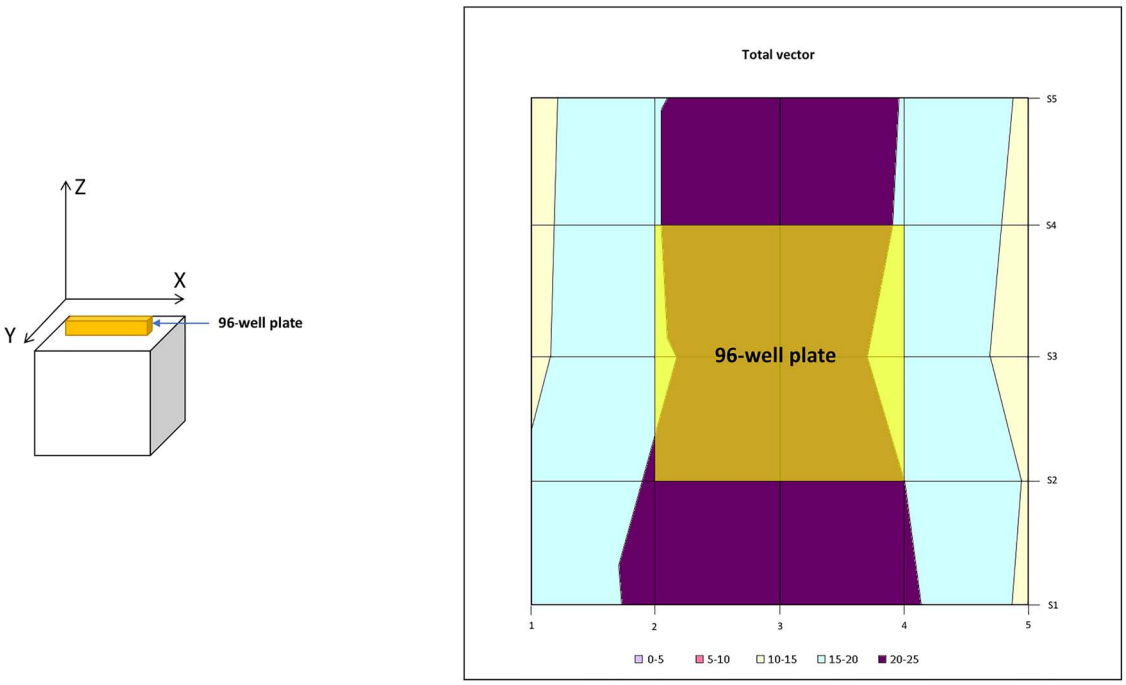


Fig. 3. The distribution of the electric field strength in the measuring microchamber—900 MHz.

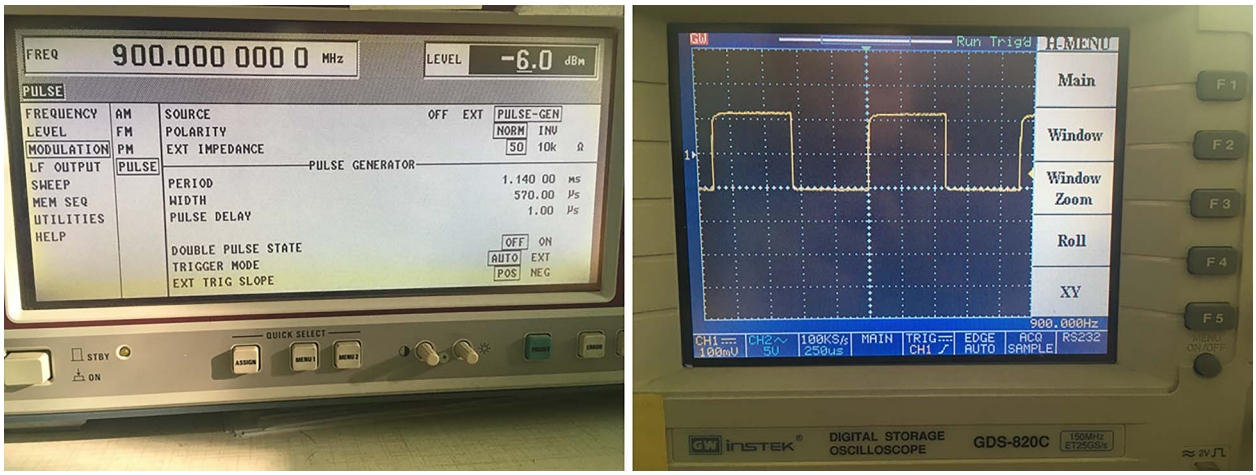
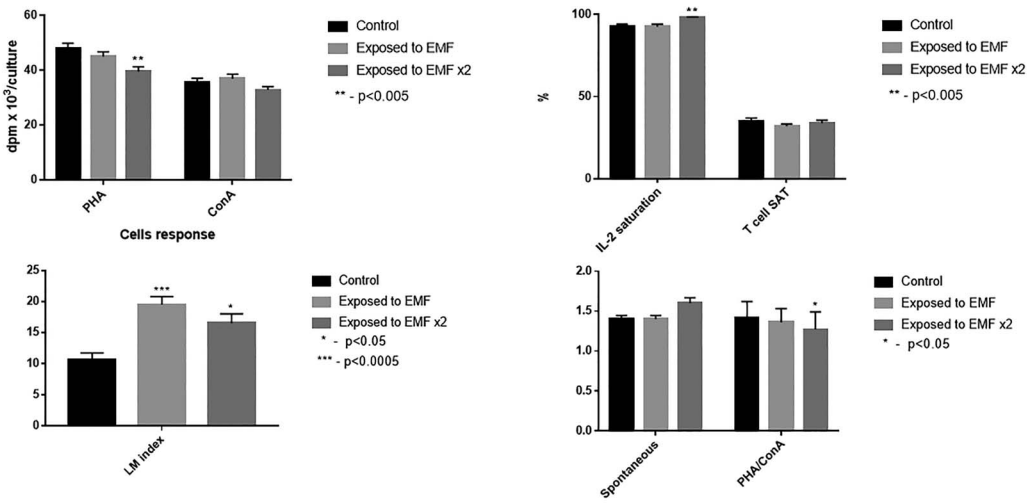


Fig. 4. Oscilloscope parameters used during examination and envelope excursion of tested signal.

twice, immediately after the culture set-up (G1 phase of the cell cycle) and after 24 h of stimulation with a mitogen (S phase of the cell cycle).

The results obtained from the experiment indicate that the activity of monocytes and lymphocytes tested *in vitro* decreased significantly under the EMF influence. T lymphocytes proliferative response, when exposed to EMF, decreased from a value of 48.1 ± 1.7 to 39.5 ± 1.8 (EMF × 2 vs control: $P = 0.001$) in response to PHA. The decrease in the response to PHA in cells exposed to EMF may be caused by the specific experimental design. The dose and the short time of exposure may be aiding the cells to overcome stress caused by mitogens [20, 21]. This hypothesis is enforced by our observation of the same phenomenon in our other experiments [22].

What is more, the saturation of the IL-2 receptors increased from 92.65% ± 1.4 to 97.91% ± 0.5 (EMF x2 vs control: $P = 0.004$) on the second day of the experiment. The role of IL-2 receptor signaling in the induction of Foxp3 expression and regulatory T lymphocytes (Treg) differentiation has been well established [23]. Tregs are known for downregulating the immune response, so this might explain why the response to PHA is reduced. Moreover, T cell suppressive activity—SAT index, which represents the regulatory function of T cells, did not change significantly.



MEAN ± SEM	Spontaneous n=26	Response to PHA (dpm x 10^3/culture) n=21	Response to Con A (dpm x 10^3/culture) n=26	Saturation of IL-2 receptors (%) n=23	T cell suppressive activity SAT (%) n=26	LM index n=23	Mitogen response ratio, PHA/Con A n=26
Control	1.4 ± 0.045	48.1 ± 1.7	35.6 ± 1.4	92.61 ± 1.4	35.3 ± 1.6	10.55 ± 1.17	1.42 ± 0.2
Exposed to EMF	1.4 ± 0.045	45 ± 1.7	36.9 ± 1.6	92.65 ± 1.4	32 ± 1.3	19.5 ± 1.3	1.36 ± 0.17
Exposed to EMF x 2	1.6 ± 0.065	39.5 ± 1.8	32.8 ± 1.2	97.91 ± 0.5	33.9 ± 1.7	16.5 ± 1.5	1.27 ± 0.22
Statistical significance	EMF vs. Control: p=0.779 EMF x2 vs. Control: p=0.127	EMF vs. Control: p=0.204 **EMF x 2 vs. Control: p=0.001**	EMF vs. Control: p=0.543 EMF x2 vs. Control: p=0.147	EMF vs. Control: p=0.89 **EMF x2 vs. Control: p=0.004**	EMF vs. Control: p=0.126 EMF x2 vs. Control: p=0.552	**EMF vs. Control: p=0.0002** **EMF x 2vs. Control: p=0.009**	EMF vs. Control: p=0.213 **EMF x2 vs. Control: p=0.01**

Fig. 5. Influence of the EMF on the activity of T lymphocytes and monocytes in microcultures. Results are presented as mean dpm ± SEM.

The LM index, which depends on the ratio of IL-1beta to IL-1ra monokines, increased from 10.55 ± 1.17 in the control group to 19.5 ± 1.3 (EMF vs control: $P = 0.0002$) in cells exposed once to EMF and settled to 16.5 ± 1.5 (EMF x2 vs control: $P = 0.009$) in cells exposed twice. These results can be also associated with the saturation of the IL-2. It is possible that cells might treat the first exposure as mitogen comparing to control.

In our study, it was also shown that the mitogen response ratio (PHA:Con A) shifted from 1.42 ± 0.2 to 1.27 ± 0.22 (EMF x2 vs control: $P = 0.01$) in cells exposed to EMF, which can be explained by the decreased percentage of helper T cells. It may be also connected with downregulation of the immune response. Results are presented in Fig. 5.

In contrast, Tuschl *et al.*'s experiments showed no statistically significant effects of GSM (Global System for Mobile Communications) signal on PBMCs. They evaluated numerous immune parameters, such as the intracellular production of IL-2, but there were no indications that this kind of stimulation is associated with adverse effects of the EMF on the human immune system [24]. What is more, Waldmann *et al.* proved that RF EMF had no genotoxic effects on human peripheral lymphocytes [25].

Unfortunately, there are few new reports that can bring new insight to the immunotropic effects of an RF EMF on PBMCs and the human immune response. Most of the new reports focus on the anti-inflammatory effect of low EMF. In 2010 Augner *et al.* tried to evaluate if 900 MHz stimulation had an effect on psychological stress markers such as IgA, alfa-amylase and cortisol. Even though they hypothesized that a GSM signal of 900 MHz may influence two of these stress markers, they did not find any relationship between RF-EMF exposure and IgA concentration [26]. Ohtani *et al.* in their studies on rats using the RF-EMF field with a frequency of 2.1 GHz and SAR = 0.2 W/kg, did not observe statistically significant changes in the T lymphocyte subpopulations. They also did not note an effect of the RF-EMF field on changes in IL-4 levels [27].

These differences between the mentioned reports might be caused by differences in exposure conditions and methods used in each study.

However, some researchers do not report the positive biological effects caused by RF-EMF. A number of studies conducted by Dasdag *et al.*, among others, showed that exposure to RF-EMF leads to deregulation of cell apoptosis, biochemical changes in the blood of pregnant women, increased oxidative stress and lipid peroxidation [28–30].

Unfortunately, the difference between the results of tests carried out in laboratory conditions and the results of epidemiological studies is significant. The reason for this is that human populations are exposed to a mixture of different frequencies and signals of varying intensity while laboratory tests use one RF-EMF field frequency. In addition, exposure time has a significant impact here—people are exposed to RF-EMF permanently and laboratory exposure time is limited due to high costs.

Due to the lack of a clearly defined hypothesis and the difficulties in choosing the specific RF-EMF parameters to study, it was decided to adopt a pragmatic approach using two or a maximum of three fields that dominate the environment. The combination of all fields is, as mentioned earlier, extremely expensive and time consuming [5].

The fact that telecommunications devices use a wide range of signal modulation methods, often involving various combinations, should be

taken into account. GSM cell phones emit pulses of 577 ms, repeating with a frequency of 217 Hz, resulting from the time division channel access method (TDMA), whereas telephones of the third generation already use CDMA (code-division multiple access) signals, which are more continuous, but not as regularly pulsating as the TDMA signal. Moreover base stations have a more continuous signal caused by communication with many phone units. Therefore the GSM carrier wave can cause different biological effects than base station waves and those used in laboratory conditions [31].

This study, as well as previous experiments on the influence of 900 MHz [3] and 1300 MHz [19] EMF, showed the immunotropic potential of the EMF, however in this study exposure settings were different than in previous research. For future research, the experimental design should include the evaluation of cytokine and chemokine secretion as well as different RF-EMF models.

CONCLUSION

Lymphocytes and monocytes isolated from human blood are sensitive to 900 MHz EMF immunomodulatory influence. Finally, the results indicate that EMF exposure may help mitogen-activated cells to overcome cellular stress, however further studies are needed to define the exact molecular mechanism of this phenomenon.

AUTHORS' CONTRIBUTIONS

Ł.S., conceptualization, writing and formal analysis; E.S., methodology and investigation; A.C., writing and formal analysis; P.S., resources; M.C., writing; W.S., project administration and funding acquisition

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CONFLICT OF INTEREST

None declared.

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