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Immunotropic Effects in Cultured Human Blood Mononuclear Cells Pre-exposed to Low-level 1300 Mhz Pulse-Modulated Microwave Field

M. P. Dabrowski, Wanda Stankiewicz, R. Kubacki, Elzbieta Sobiczewska, and S. Szmigielski*

Department of Microwave Safety, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

ABSTRACT

The samples of mononuclear cells isolated from peripheral blood of healthy donors ($N = 16$) were exposed to 1300 MHz pulse-modulated microwaves at 330 pps with 5 μs pulse width. The samples were exposed in an anechoic chamber at the average value of power density of S = 10 W/m^2 (1 mW/cm^2). The average specific absorption rate (SAR) was measured in rectangular waveguide and the value of SAR = 0.18 W/kg was recorded. Subsequently, the exposed and control cells were assessed in the microculture system for several parameters characterizing their proliferative and immunoregulatory properties. Although the irradiation decreased the spontaneous incorporation of 3H-thymidine, the proliferative response of lymphocytes to PHA and to Con A as well as the T-cell suppressive activity (SAT index) and the saturation of IL-2 receptors did not change. Nevertheless, the lymphocyte production of interleukin (IL)-10 increased ($P < .001$) and the concentration of IFNγ remained unchanged or slightly decreased in the culture supernatants. Concomitantly, the microwave irradiation modulated the monokine production by monocytes. The production of IL-1β increased significantly ($P < .01$), the concentration of its antagonist (IL-1ra)

*Correspondence: Stanislaw Szmigielski, Department of Microwave Safety, Military Institute of Hygiene and Epidemiology, Szaserow 128, PL-00-909 Warsaw, Poland; Fax: + 4822 8104391; E-mail: szmigielski@wihe.waw.pl.

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dropped by half ($P < .01$) and the tumor necrosis factor (TNF-α) concentration remained unchanged. These changes of monokine proportion (IL-1β vs. IL-1ra) resulted in significant increase of the value of LM index ($P < .01$), which reflects the activation of monocyte immunogenic function. The results indicate that pulse-modulated microwaves represent the potential of immunotropic influence, stimulating preferentially the immunogenic and proinflammatory activity of monocytes at relatively low levels of exposure.

Key Words: Microwaves; Immune system; Immunotropic effects; Monokines; Lymphocytes.

INTRODUCTION

The undisturbed defensive, tolerogenic, and proregenerative activities of the immune system are commonly recognized as one of the most important homeostatic functions of the human organism (Deschaux and Khan, 1995). There exist a number of reports in the literature that suggest that microwave (MW) radiation at nonthermal levels can affect the immune system of humans and laboratory animals. Whole-body exposure to MW radiation was reported to affect lymphocyte populations (Novoselova et al., 1999; Shao and Chiang, 1989; Veyert et al., 1991) and influence cell-mediated and humoral responses to external antigens (Dabrowski et al., 2001a; Fesenko et al., 1999; Negeswari et al., 1991). However, the results of single experiments depended strongly on schedule and timing of the exposure, as well as on frequency, modulation, and power density of the MW field, applied in particular studies and therefore, both symptoms of stimulation and suppression of certain immune reactions were noted as an outcome of the exposure. For example, many studies indicate that MW radiation increases responsivness of lymphocytes to antigens (Sinotova et al., 2002; Veyert et al., 1991), whereas others indicate depressed responsivness, characteristic of a state of immunosuppression (Dabrowski et al., 2001a; Lyle and Adey, 1983). Still more, the experimental data on immune response to nonthermal MW exposures in animals are rather fragmentaric, report changes of only several selected immune functions (mainly phagocytosis, lymphocyte proliferation or antibody production), are frequently controversial or not replicable (Dabrowski et al., 2001a; Fesenko et al., 1999; Lyle and Adey, 1983; Negeswari et al., 1991; Novoselova et al., 1999; Shao and Chiang, 1989; Sinotova et al., 2002; Veyert et al., 1991). Therefore, interpretation of these data in terms of both mechanisms of effects and assessment of possible health risks in humans is difficult.

On the other hand, immune cells separated from human blood represent an unique and objective model for investigation of possible immunotropic effects of MWs. With the progress in microculture techniques and use of modern methods for measurement of levels of numerous cytokines in the cultures, the basic immunoregulatory activities of immune cells can be observed and precisely quantified in this model. Surprisingly, the techniques of microculture of lymphocytes were used for studies of MW-induced effects only at the very early stage of development of these techniques, mostly throughout the 1970s and 1980s. Stodolnik-Baranska (1974), in her widely discussed article of 1974, reported increased spontaneous transformation of human lymphocytes following single in vitro exposure to 3000 MHz pulse-modulated MWs at 3 mW/cm^2,

but this line of research was rarely continued, because other authors were not able to replicate this finding and no differences were reported in response of MW-exposed lymphoctyes to mitogens (Roberts and Michaelson, 1987). Recently, Vijayalaxmi et al. (2001) exposed human blood lymphocytes to a mobile phone frequency (835.62 MHz) at 86 mW/cm^2 (SAR of 4.4–5 W/kg) and reported no significant differences in respect to mitotic indices in pha-stimulated cultures, incidence of chromatide exchange, aberrations, excess fragments, binucleate cells, and micronuclei. In general, only slight and transient effects, if any at all, were reported in cultured lymphocytes pre-exposed to nonthermal MW exposures. These effects include the suppression of T-lymphocyte–mediated cytotoxicity after exposure to sinusoidally amplitude-modulated (16–100 Hz) 450 MHz MW fields, but not to unmodulated 450 MHz fields (Lyle et al., 1983).

The aim of the present study was to determine potential immunomodulatory influences of pulse-modulated (radar) 1300 MHz MW field on blood mononuclear cells isolated from 16 healthy donors.

MATERIAL AND METHODS

Material

Blood (20 mL) was collected by venous puncture from 16 healthy male blood donors (ages 22–41 years), and peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on Ficol–Paque gradient, washed twice, and resuspended in RPMI 1640 supplemented with 15% autologous inactivated serum. For the present experiments, a specimen of 3 mL of isolated PMBC, placed in sterile tube, was exposed for 1 hr at far field conditions in a large anechoic chamber in pulse-modulated 1300 MHz MW field at 10 W/m^2. The value of specific absorption rate (SAR) of identical sample was measured in a waveguide facility, using a modified method of Guy et al. (Chou et al., 1984; Guy et al., 1979). At incident power density of 10 W/m^2 in the wave guide the absorption of 0.53 mW of energy was recorded by the PBMC sample what gave a SAR value of 0.18 W/kg. The applied method of SAR calculation is described in detail in the following section.

Exposure System

Samples of PBMC were exposed to high-power, pulse-modulated MWs in an anechoic chamber (5.5 m length $\times$ 2.2 m width $\times$ 2.1 m high) with walls covered by pyramid absorbers. The microwave field was generated by radar operating at 1300 MHz (5 μs pulse duration, 330 pps pulse repetition frequency) at average power density of 10 W/m^2 (1 mW/cm^2). This value corresponds to the power density in peak of 6000 W/m^2 (6 kW/m^2). Pulse parameters and waveform of the generated field were measured with oscilloscope and frequency meter coupled to the waveguide transmission line. MW source (military radar generator) was installed in the separate room and the pulse-modulated field was transmitted through rectangular waveguide terminated by gain horn antenna, providing the vertical polarization of the field. The exposed samples

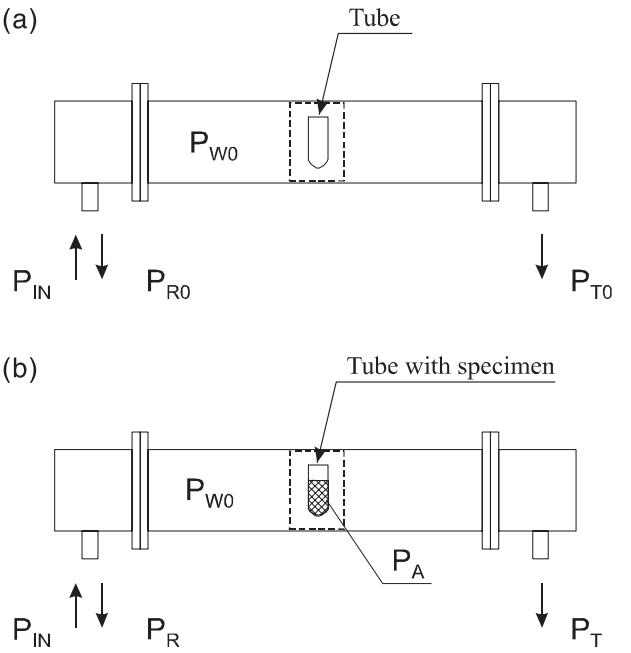

Figure 1. Schematic representation of the system for SAR measurement in rectangular waveguide. (a) With empty tube, (b) with tube filled with specimen.

were located 1.5 m from the antenna. The average value of incident field (10 W/m^2) at the exposure point was measured in absence of the PBMC sample by NARDA meter (Model 8718). AQ3

Ambient temperature in the chamber was kept between 25 and 27°C and the chamber was ventilated by external fan to maintain relative humidity at 55–60%.

SAR Measurements

The value of SAR of the sample (specimen) was measured in rectangular waveguide (WR 650) with the sample positioned in the maximum field of the TE_{10} mode. To improve the accuracy of measurements of the energy absorbed by the sample (P_A) in rectangular waveguide a modified method of Guy et al. (Chou et al., 1984; Guy et al., 1979) has been applied. This method has been developed for rectangular waveguide terminated with waveguide-coaxial junctions. Fractions of power (transmitted and reflected) in the waveguide with empty tube, as well as with tube filled with specimen were measured (Figure 1) with all measurements being realized at the same power incident (P_{IN}). F1

For the waveguide with empty tube, the sum of power (see Figure 1a) can be expressed as follows (calibration measurements):

$$P_{IN} = P_{T0} + P_{R0} + P_{W0} \quad (1)$$

where:

P_{IN}—power incident to the waveguide–coaxial junction

P_{T0}—power transmitted to the terminal (next waveguide–coaxial junction)

P_{R0}—power reflected

P_{W0}—power absorbed by the walls of waveguide and empty tube

When the tube is filled with specimen (see Figure 1b) the sum of power can be expressed according the following formula:

$$P_{IN} = P_T + P_R + P_{W0} + P_A \quad (2)$$

where:

P_T,P_R—power transmitted and reflected with the specimen placed in the waveguide

P_A—power absorbed by the specimen

Taking into consideration that power incident (P_{IN}) has the same value (formula 1 and 2), the power absorbed by specimen (P_A) can be calculated according an equation:

$$P_A = (P_{T0} - P_T) + (P_{R0} - P_R) \quad (3)$$

or in a simplified way:

$$P_A = \Delta P_T + \Delta P_R \quad (4)$$

where:

$\Delta P_T = (P_{T0} - P_T)$

$\Delta P_R = (P_{R0} - P_R)$

In the experiment, the energy absorbed by sample was measured in waveguide WR 650. The value of power incident (P_{IN}) was established to assure value of RMS of electric field strength in the waveguide (at the point of measurement) the same as in the anechoic chamber ($S = 10\ W/m^2 \rightarrow E = 61.4\ V/m$). In such a condition the value of energy absorbed by specimen was received of $P_A = 0.00053$ W (53 mW), and finally, the value of SAR is of $SAR = P_A/m$, where m-mass of sample (in the experiment $m = 0.003$ kg). Thus the apparent average SAR = 0.18 W/kg.

Methods for Investigation of PBMC Response

The samples of mononuclear cells isolated from heparinised blood by density gradient centrifugation were suspended in RPMI 1640 supplemented with 15% autologous inactivated serum (3×10^6 cells/3 mL) and exposed for 1 hr to pulse modulated electromagnetic field. Before and after exposure the viability of cells was estimated

and microcultures were set up in triplicates (10^5 cells/0.2 mL RPMI + 15% autologous inactivated serum). Respective triplicates of micrcultures were left without stimulation or stimulated with phytohemagglutinin (PHA, HA16, 0.4 μg/culture, optimal dose, Murex Biotech Ltd, Dartford, UK) or with concanavalin A (Con A, Sigma, 8 μg/culture, optimal dose) and incubated in ASSAB incubator (CO_2 5%) at 37°C for 72 hr. At 24 hr of incubation, the rearrangements of the cultures were performed as described elswhere (Dabrowski et al., 1987, 2001b). For the last 18 hours of incubation the microcultures were added with 3H-thymidine (3HTdR, Amersham, UK, spec. act. 5 Ci/mM) in a dose of 0.4 μC/culture. At 72 hr the cultures were harvested and 3HTdR incorporation was measured in Packard Tri-carb 2100 TR liquid scintillation counter. The results were calculated as a mean value of dpm ± standard deviation (SD) for each triplicate of the cultures. The following parameters of T-lymphocytes and monocytes were measured: 1) spontaneous 3HTdR incorporation, 2) T-cell response to PHA, 3) T-cell response to Con A, 4) ratio of PHA to Con A response (P/C index), 5) suppressive activity of T cells (SAT index), 6) saturation of inteleukin (IL)-2 receptors (IL-2 index) and 7) monokine influence on T cell proliferative response (LM index) (Dabrowski et al., 1987, 2001b). Concomitantly, the samples of cell-free medium removed at 24 hr from nonstimulated cultures were frozen and subsequently assessed quantitatively by ELISA for IL-1β, IL-1ra, tumor necrosis factor (TNF-α), IFN-γ, and IL-10 content. The assessments were carried out in the universal microplate analyzer Elx800 (Biokom, Poland) with application of respective Quantikine kits (R&D Systems, Abingdon, UK). The experiments were repeated 16 times, one for the each of the blood donors. The results are presented as a mean values ± SD for each tested parameter with calculated significance of differences between the MW-exposed and control groups of cultures. The analysis of individual differences between exposed and control samples as well as statistical significance of differences (Wilcoxon ''twin pair'' analysis) also are presented in the tables.

RESULTS

One-hour exposure of isolated PMBC from healthy male donors in relatively weak (SAR 0.18 W/kg) pulse-modulated 1300 MHz MW fields resulted in appearance of detectable functional changes of the cells, which could be recorded at specific microculture conditions. The exposure conditions did not cause lethal effects in PBMC and viability of cells tested at the end of cultures remained at the level of 80% of initial number of viable cells set in the culture. The cultures of cells exposed to MW displayed discrete, but significant ($P < .01$) decrease of spontaneous incorporation of 3HTdR (1.25×10^3 vs. 1.91×10^3 dpm in control cultures). The proliferative responses of lymphocytes to PHA and to Con A remained at the level of normal response and were similar in the exposed and the control cultures (Table 1). Also Con A–induced T cell suppressive activity (SAT index) and the saturation of lymphocyte IL-2 receptors were at the normal level and did not differ between the MW-exposed and control cultures (Table 2). In contrast to that, the concentration of IL-10 in the cultures exposed to MW was significantly ($P < .01$) higher (631.5 ± 193.9 pg/mL) than in control cultures (311.3 ± 122.7 pg/mL) and the contents of IFN-γ remained unchanged or was slightly diminished in the supernatants of some exposed cultures. The MW

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T1.1 ***Table 1.*** Influence of 1-hr exposure in 1300 MHz pulsed microwave fields at 10 W/m^2 (SAR 0.18 W/kg) on mitogenic response of human blood mononuclear cells in microculture.

T1.2			Spontaneous 3HTdR incorporation (DPM × 10^3/10^5 cells) (N = 16)		PHA-induced response of PBMC (DPM × 10^3/10^5 cells) (N = 16)		Con-A-induced response of PBMC (DPM × 10^3 /10^5 cells) (N = 16)		P/C ratio (ratio of PHA/ ConaA response) (N = 16)	
T1.3	Immune test performed in microculture		MW-exposed	Control	MW-exposed	Control	MW-exposed	Control	MW-exposed	Control
T1.4	Mean value (x)		1.25	1.91	81.27	68.29	37.95	35.19	2.15	2.06
T1.5	Standard deviation (SD)		0.31	0.65	11.60	9.21	2.47	9.19	0.36	0.65
T1.6	Significance of difference between groups (P)		$P < .01$		Not significant		Not significant		Not significant	
T1.7	Analysis of individual	Increased (>2 SD)	0		2		4		3	
T1.8	differences between	Unchanged	11		14		10		10	
T1.9	exposed and control samples	Decreased (>2 SD)	5		0		2		3	
T1.10	Differences between exposed and control samples (mean ± SD)		−0.66 ± 0.63		12.16 ± 7.57		2.59 ± 10.07		0.08 ± 0.74	
T1.11	Statistical significance of differences between exposed and control samples (Wilcoxon ''twin pair'' analysis)		$P < .05$		Not significant		Not significant		Not significant	

T2.1 ***Table 2.*** Influence of 1-hr exposure in 1300 MHz pulsed microwave fields at 10 W/m^2 (SAR 0.18 W/kg) on immunoregulatory properties of human blood mononuclear cells in microculture.

T2.2			SAT index (Con-A-induced suppressive activity of T lymphocytes) (N = 16)		Saturation of IL-2 (interleukin-2) receptors on T lympcytes (%) (N = 16)		IFNγ concentration in culture medium (pg/mL) (N = 16)		IL-10 (interleukin-10) concentration in culture medium (pg/mL) (N = 16)	
T2.3	Immune test performed in microculture		MW-exposed	Control	MW-exposed	Control	MW-exposed	Control	MW-exposed	Control
T2.4	Mean value (x)		22.73	29.71	91.13	96.67	510.5	630.4	631.5	311.3
T2.5	Standard deviation (SD)		9.25	3.95	9.16	5.74	118.0	92.4	193.9	122.7
T2.6	Significance of difference between groups (P)		Not significant		Not significant		$P < .05$		$P < .001$	
T2.7	Analysis of individual	Increased (>2 SD)	0		1		1		10	
T2.8	differences between	Unchanged	14		14		12		6	
T2.9	exposed and control samples	Decreased (>2 SD)	2		1		3		0	
T2.10	Differences between exposed and control samples (mean ± SD)		−6.56 ± 11.21		− 5.19 ± 7.25		−151.81 ± 186.23		358.56 ± 238.88	
T2.11	Statistical significance of differences between exposed and control samples (Wilcoxon ''twin pair'' analysis)		Not significant		Not significant		$P < .05$		$P < .01$	

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T3.1 ***Table 3.*** Influence of 1-hr exposure in 1300 MHz pulsed microwave fields at 10 W/m^2 (SAR 0.18 W/kg) on production of cytokines by human blood mononuclear cells in microcultures.

T3.2			LM index (lymphocyte-monocyte cooperation index) (N = 16)		IL-1β concentration in culture medium (pg/mL) (N = 16)		IL-1ra concentration in culture medium (pg/mL) (N = 16)		TNFα concentration in culture medium (pg/mL) (N = 16)	
T3.3	Immune test performed in microculture		MW-exposed	Control	MW-exposed	Control	MW-exposed	Control	MW-exposed	Control
T3.4	Mean value (x)		17.15	4.83	740.9	287.8	670.4	1312.5	2421.1	1986.6
T3.5	Standard deviation (SD)		5.96	1.18	129.2	131.6	256.9	691.5	478.9	985.8
	Significance of difference between groups (P)		$P < .001$		$P < .001$		$P < .01$		Not significant ($P = .12$)	
T3.6										
T3.7	Analysis of individual	Increased (>2 SD)	12		15		1		5	
T3.8	differences between	Unchanged	4		1		7		10	
T3.9	exposed and control samples	Decreased (>2 SD)	0		0		8		1	
T3.10	Differences between exposed and control samples (mean ± SD)		11.56 ± 6.79		453.25 ± 169.44		−643.87 ± 840.34		806.94 ± 1188.76	
T3.11	Statistical significance of differences between exposed and control samples (Wilcoxon ''twin pair'' analysis)		$P < .01$		$P < .01$		$P < .05$		$P < .05$	

exposure increased significantly the LM index from the control value of 4.83 ± 1.18 to the value of 17.15 ± 5.96 ($P < .01$) (Table 3). The concentrations of respective monokines which determine the LM value also have changed. The concentration of IL-1β increased from the control level of 287.8 ± 131.6 to 740.9 ± 129.2 pg/mL ($P < .01$). The reverse has been observed for the concentration of IL-1 receptor antagonist (IL-1ra) whose value decreased from the control level of 1312.5 ± 691.5 to 670.4 ± 256.9 pg/mL in the supernatants of cultures exposed to MW ($P < .01$). The exposure did not change in a significant manner the concentration of TNF-α in the supernatant.

DISCUSSION

In the present experiments, suspension of PBMC, which represents a mixture of lymphocytes and monocytes, was exposed for 1 hr in pulse-modulated 1300 MHz (radar) MW field at power density of 10 W/m^2. SAR measurements performed for an identical sample placed in a waveguide facility revealed the energy absorption of 53 mW, which allowed the calculation the SAR value of 0.18 W/kg. The modified method of Guy et al. (Chou et al., 1984; Guy et al., 1979), applied for SAR calculations, allowed measurement of power absorption both in empty tube, tube with medium and tube with suspension of PBMC in medium. The formula 4 allows to calculate the power absorbed by the sample as a function of power differences measured at the same ports. This guarantee much better accuracy of measurements than in the original method proposed by Guy et al. (Chou et al., 1984; Guy et al., 1979)—subtraction is realized between comparable values of power, which yields to minimization of the uncertainty of measurements. In addition, the modified method enables to calculate the power absorbed directly by specimen because the energy absorbed by waveguide walls and empty tube is measured in the calibration procedure (formula 1) and is considered in formula 2. In case of rectangular waveguide, especially when frequency of operation is closed to the cut-off frequency, the energy absorbed by waveguide walls can reach higher values (Chou et al., 1984), which cannot be neglected in the method of calculations.

Isolated PBMC are a mixture of monocytes and various subtypes of lymphocytes, including T, B, and natural killer cells. Monocytes, which belong to the group of antigen-presenting cells (APC) and diverse T lymphocytes (e.g., TCD4 helper-inducer and CD4, CD25 T-regulatory cells) are the main cellular elements that determine initiation and development of immune response. The way of cooperation of APC and T cells greatly depends on the repertoires of produced monokines and lymphokines and is responsible for the kind of induced response (defensive humoral and cellular, tolerogenic that blocks the destructive effects, or supportive for processes of tissue regeneration). Both these groups of cells, which participate in immune response (monocytes and T lymphocytes), are present in natural proportions in the population of PBMC and their functional state can be characterized in culture by quantitative determination of produced cytokines (e.g., IL-1β, IL-1ra, TNF-α, IL-2, IL-10, IFN-γ) and by estimation of respective indices of cellular cooperation (LM index, SAT index, IL-2 receptor saturation index). Therefore, observation of changes in these parameters, evoked by microwave irradiation of cultured cells, may provide a sensitive tool estimating the potential and the mechanism of immunotropic influence of the tested factor (Dabrowski et al., 2001b).

The most evident immunotropic influence of 1300 MHz pulsed microwaves observed in the present study was directed toward the monocyte activity. Phagocytosis, digestive processing, and presentation of antigen supported by production of a family of monokines collectively determine the participation of monocytes in the induction of specific immune response. Concomitantly, the immunoregulatory (e.g., IL-1 and IL-1ra) and effector (e.g., TNF-α) monokines contribute to development and maintenance of immunogenic tissue inflammation. One of the most effective immunostimulatory and proinflammatory monokine is IL-1 (Kaye and Janeway, 1984; Oppenheim et al., 1986). To exert its influence, IL-1 has to compete for access to its cellular receptor with the other monokine, the IL-1ra. IL-1ra, in contrast to IL-1, is unable to transduce the stimulatory signal. Thus, when binding to the receptor, IL-1ra prevents its activation (Dripps et al., 1991; Eisenberg et al., 1990; Granowitz et al., 1991).

Assessments of alterations in the IL-1/IL-1ra concentration within the humoral environment of cultured immune cells and determination of the LM index, whose value is dependent on the ratio of IL-1/IL-1ra concentration, may provide important information on the stage of ongoing immune response and on the potential progression of the inflammatory process (Dabrowski et al., 2001b). In general, the higher values of the LM index and IL-1/IL-1ra ratio characterise the activity of developmental phase, whereas decreased values of the both parameters mark the termination of immune inflammation (Dana et al., 1997; Dinarello, 2000; Donati et al., 1997; Highuchi et al., 1997).

In our in vitro experiments irradiation of cultured blood mononuclear cells with 1300 MHz MW resulted in significant increase of the value of LM index, concomitant increase of IL-1β production by monocytes and in decrease of IL-1ra concentration. As the lymphocyte motigenic proliferation activity remained uchanged and the increase of IL-10 production has been observed, the conclusion may be drown that 1300 MHz microwaves preferentially affects the immunogenic monocyte activity, contributing to the development of an inflammatory state and the increase of IL-10 production is a secondary reaction of T cells to control the activated monocytes (Shevach, 2000). Such a profile of immunotropic activity of 1300 MHz MW could be beneficial in some clinical situations, for example, in stimulation of anti-tumor or anti-infective response, but harmful and undesired in chronic inflammatory states in cases of autoaggressive or allergic diseases. There is a need of further investigations to find out whether or not our in vitro observations also are valid for the clinical in vivo situations and if the immunotropic effects of 1300 MHz MW could be applied for immunotherapeutic purposes.

CONCLUSIONS

1. The results of our in vitro experiments performed with mononuclear cells isolated from the blood of healthy donors ($N = 16$) indicate that single 1-hr exposure of the cells to 1300 MHz pulse-modulated MWs at SAR of 0.18 W/kg influenced the monocyte-dependent immunoregulatory mechanisms responsible for the initiation of immune response.
2. The exposure significantly increased production of IL-1 by monocytes and decreased their production of IL-1ra. The changed ratio of IL-1/IL-1ra resulted in an increased value of LM index, which reflects, in turn, the activation of immunogenic and proinflammatory functions of monocytes.

3. The exposure did not change the proliferative response of T lymphocytes to mitogens (PHA, Con A) but significantly increased the production of IL-10, the immunoregulatory lymphokine that is involved in the T cell-monocyte interaction.
4. The immunostimulatory effects of 1300 MHz pulsed MWs seem to affect preferentially the immunogenic functions of monocytes. The increased production of IL-10 seems to be a secondary reaction of T cells in response to activated monocytes. The observed immunotropic potentials of 1300 MHz pulsed MWs needs further investigations to assess whether or not such effects may be also exerted in vivo and applied for possible future immunotherapeutic applications.

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