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Alteration in Cellular Functions in Mouse Macrophages After Exposure to 50 Hz Magnetic Fields

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Abstract The aim of the present study is to investigate whether extremely low frequency electromagnetic fields (ELF-EMF) affect certain cellular functions and immunologic parameters of mouse macrophages. In this study, the influence of 50 Hz magnetic fields (MF) at 1.0 mT was investigated on the phagocytic activity and on the interleukin-1β (IL-1β) production in differentiated macrophages. MF-exposure led to an increased phagocytic activity after 45 min, shown as a 1.6-fold increased uptake of latex beads in MF-exposed cells compared to controls. We also demonstrate an increased IL-1β release in macrophages after 24 h exposure (1.0 mT MF). Time-dependent IL-1β formation was significantly increased already after 4 h and reached a maximum of 12.3-fold increase after 24 h compared to controls. Another aspect of this study was to examine the genotoxic capacity of 1.0 mT MF by analyzing the micronucleus (MN) formation in long-term (12, 24, and 48 h) exposed macrophages. Our data show no significant differences in MN formation or irregular mitotic activities in exposed cells. Furthermore, the effects of different flux densities (ranging from 0.05 up to 1.0 mT for 45 min) of 50 Hz MF was tested on free radical formation as an endpoint of cell activation in mouse macrophage precursor cells. All tested flux densities significantly stimulated the formation of free radicals. Here, we demonstrate the capacity of ELF-EMF to stimulate physiological cell functions in mouse macrophages shown by the significantly elevated phagocytic activity, free radical release, and IL-1β production suggesting the cell activation capacity of ELF-EMF in the absence of any genotoxic effects. J. Cell. Biochem. 99: 168–177, 2006.

Key words: Immune cell activation; phagocytosis; IL-1β; reactive oxygen species; micronuclei; magnetic field

A number of epidemiological studies investigated the connection between occupational or residential exposure to extremely low frequency (ELF) electromagnetic fields (EMF) and the incidence of cancer development [reviewed in IARC, 2002; Skinner et al., 2002; Brain et al., 2003]. Numerous laboratory studies examined the influence of 50/60 Hz EMF on cellular processes resulting in a variety of biological effects. Some in vitro studies described alterations in cell proliferation and signal transduction pathways after EMF-exposure [Nindl et al., 2000; Lange et al., 2002, 2004; Richard et al., 2002] and other authors expressed EMF-induced DNA-damage [Ivancsits et al., 2002, 2005; Wolf et al., 2005]. Several laboratory investigators studied immunological processes after or during EMF-exposure. Investigations in J774.2 macrophages showed decreased cell viability after EMF exposure to certain flux densities [Kawczyk-Krupka et al., 2002]. The authors discussed the biological significance in terms of an anti-tumoricidal effect of EMF. Several other studies examined the secretion of cytokines by immune relevant blood cells affecting specific cellular functions [Cossarizza et al., 1998], such as an increased cytokine production. Walleczek [1992] described that ELF-EMF affects biochemical processes of the immune system and proposed the involvement of the calcium-signaling pathway affecting the cell membrane. Therefore, one potential target for EMF is the immune system.

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The importance of macrophages in immune response is underscored by their phagocytic activity and by the secretion of an assortment of powerful immune regulatory and effector molecules. Resting macrophages have low levels of phagocytic activity and become fully active through binding of pathogens or by local cytokine release. Once activated, macrophages exhibit increased level of phagocytic activity and increased production of reactive oxygen species (ROS) enabling the killing of microbes within their phagosomes. On the other hand, ROS are acting as signaling molecules and targeted to a range of physiological pathways. In addition, activation also causes the secretion of cytokines such as interleukins and TNF-alpha.

In previous studies, we documented the increased formation of free radicals in human monocytes and mouse macrophages and increased phagocytic activity after exposure to 50 Hz magnetic fields (MF) at 1.0 mT [Simkó et al., 2001; Lupke et al., 2004; Rollwitz et al., 2004]. In this study, we investigated the dose-dependent influence of MF (50 Hz, 0.05–1.0 mT) on free radical production and the effects on common macrophage function, such as phagocytic activity, and IL-1β release to strengthen the hypothesis that ELF-EMF induces cell activation. Additionally, the frequency of micronucleus (MN) formation and the number of mitotic cells were analyzed microscopically to test the genotoxic capacity of long-term MF-exposure (12, 24, and 48 h).

MATERIALS AND METHODS

Reagents

RPMI-1640 medium (PAA-Laboratories GmbH, Karlsruhe, Germany) supplemented with 30% conditioned medium from L929-cell line, 6% heat-inactivated fetal calf serum (FCS, Gibco BRL, Karlsruhe, Germany), 0.5% penicillin/streptomycin (10,000 U/10,000 μg/ml), non-essential amino acids (Biochrom KG, Berlin, Germany), and 0.125% 2-mercaptoethanol was used. 12-*O*-tetradecanoylphorbol (TPA), lipopolysaccaride (LPS, from *E.coli*, Serotyp 026:B6), dimethyl sulfoxide (DMSO), and bisbenzimide (Hoechst 33258) were purchased from Sigma-Aldrich (Munich, Germany). For interleukin-1β detection, an immunoassay Kit (OptEIA^{TM} Mouse IL-1β Set) from BD-Pharmingen (San Diego) was used. All other used antibodies were obtained from BD Bioscience (San Diego).

Cell Preparation and Culture

Promonocytes were isolated as bone marrow precursor cells from femur and tibia of adult Shoe-NMRI mice and cultured as described previously with some modifications [Rollwitz et al., 2004]: To eliminate all erythrocytes from cell suspension, directly after cell preparation and centrifugation at 1,000 rpm for 5 min, cell-pellet was treated with H_2O for 10 s. After adding 0.8% NaCl, cells were decanted in medium and centrifuged at room temperature for 5 min. Cells were directly used for investigation or seeded (ca. 3×10^6 cells/ml) in tissue culture dishes (100×20 mm, Biochrom-TPP, Berlin, Germany) in medium for cultivation. Macrophages were obtained as an adherent monolayer after in vitro differentiation of bone marrow precursor cells (within 2–3 days). Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air, using conventional incubator (Binder, Germany). Medium was changed at regular intervals of 3 days.

Electromagnetic Field Exposure

A Helmholtz coil system was used for MF generation, which was described previously [Rollwitz et al., 2004]. For experiments, a horizontally polarized 50 Hz EMF with flux densities of 0.05, 0.1, 0.5, or 1.0 mT was used. Cell culture plates were placed in the center of the Helmholtz system situated in a 37°C humidified incubator (Binder, Germany), containing 5% CO_2 or in a control incubator for the exposure time period (45 min up to 48 h) according to the experimental conditions. The magnetic flux density was monitored by a precision Gauss/Tesla Meter (Model 6010, F.W. Bell, Inc., Orlando).

Phagocytosis Assay

Macrophages cultured for up to 6 days were plated on glass coverslips (10^4 cells/coverslip, Ø 11 mm, Menzel, Braunschweig, Germany) that were placed in 6-well plates (Biochrom-TPP, Berlin, Germany) for at least 8 h prior to use. Non-adherent cells were removed and adherent cells were incubated with carboxylated green latex beads (FluoSphere® carboxylate-modified microspheres, Ø 1 μm, green fluorescent, 2 % solids (Molecular Probes, The Netherlands)) in medium with a cell to bead proportion of 1:100

for 30 min. For experiments, the following condition were used: macrophages were cultured with beads only as control, or in the presence of 1 μM TPA as positive control, or in the presence of MF-exposure conditions. After 30 min, latex beads were removed, macrophages were washed with PBS, and incubated for 15 min in fresh medium. Cells were then fixed in methanol (−20°C, 5 min) and the number of internalized beads in at least 200 cells per cover slip was counted in triplicates by differential interference contrast microscopy (Nikon DIAPHOT 300). Phagocytosis assay was performed in three independent experiments.

Interleukin-1β Assay

Secretion of IL-1β was determined in macrophage supernatants collected after MF exposure (1.0 mT) or control conditions for 45 min up to 24 h by an ELISA Kit (OptEIA^{TM}Mouse IL-1β Kit, BD Pharmingen). Macrophages were cultured in 96-well flat-bottomed microtiter plates (Biochrom-TPP, Berlin, Germany) at a density of 5×10^4 cells per well, at least 24 h prior to experiment start. For IL-1β detection, cell supernatants of controls, MF-exposed cells, and standards were incubated in antibody-coated 96-well plates for 2 h to immobilize IL-1β. According to the manufacturer's instruction, cells were treated with a biotinylated anti-mouse IL-1β monoclonal antibody for 1 h, followed by streptavidin-horseradish peroxidase conjugated reagent for 30 min. Finally, tetramethylbenzidine (TMB) and hydrogen peroxide (TMB Substrate Reagent Set, BD Pharmingen) was added to produce a blue color in direct proportion to the amount of IL-1β. After 30 min, the addition of 1M H_3PO_4 changed the color from blue to yellow and the plates were read at 450 nm (reference 570 nm) with an ELISA Reader (SLT Spectra, TECAN). IL-1β assay was performed in eight replicates for each and in three independent experiments.

Flow Cytometry

For characterization of surface antigens, freshly prepared cells (0.5×10^6) were incubated in 200 μl PBS supplemented with 2 mM EDTA, 0.1% BSA, and 10 μl FITC-conjugated monoclonal rat anti-mouse CD11b-antibody or FITC-conjugated rat monoclonal IgG_{2b}, κ Isotype control for 10 min at 4°C. Fluorescence intensities were measured at 525 nm using a flow cytometer (Beckmann/Coulter Epics Altra, Germany) equipped with a water-cooled 488 nm Argon Laser. Values were calculated using the EXPO^{TM}32 MultiCOMP software version 1.2 (Beckman Coulter, Germany). For ROS detection, only $CD11b^+$ cells were gated and the fluorescence intensity was measured.

Production of ROS

Freshly isolated bone marrow precursor cells were cultured in 96-well plates for 45 min in medium with or without TPA or LPS in the presence or absence of MF. The MF dose-dependent ROS formation was tested with magnetic flux densities at 0.05, 0.1, 0.5, or 1.0 mT. As positive control macrophages were stimulated with 1 μM TPA or 1 μg/ml LPS. To detect the free radical release after exposure, cells ($CD11b^+$ gated cells only) were loaded with 1 μM dihydrorhodamine 123 (DHR) in HBS-buffer (0.9% NaCl solution with 14 mM HEPES) for 25 min at 37°C in darkness. The mean fluorescence intensity (x-Mean) was measured at 525 nm in triplicates in at least three independent experiments.

Micronucleus Assay

Cells (10^4) were seeded on cover slips (Menzel, Braunschweig, Germany) 8 h prior to starting the experiments and incubated for 12, 24, or 48 h in the presence or absence of 1.0 mT MF. After EMF-exposure, cover slips were washed in PBS and fixed with −20°C methanol (100%) for 2 h. Air-dried slides were washed in PBS and stained for 1 min with the fluorescent DNA dye bisbenzimide (Hoechst 33258; 1 mg/ml). After washing in distilled water, slides were mounted for fluorescence microscopy and evaluated for MN formation and for mitotic cells. For identification of MN, the criteria of Countryman and Heddle [1976] were applied as follows: the MN has to be at least one-third smaller than the main nucleus and not touching the main nucleus. Cells containing one or more micronuclei were scored as MN-positive cells. The numbers of MN were counted on three simultaneously exposed slides in at least 1,000 cells for each time point of measurement. For evaluation, slides were coded. Experiments were repeated three times. Data of exposed cells were analyzed with reference to their own control for every time point. Differences between MN rates and number of

mitotic cells in control and in exposed cells were tested for significance using the Student's t-test ($P > 0.05$).

Statistical Analysis

Data of ROS production were expressed as the ratio of exposed to control cells (E/C), of IL-1β as a concentration (pg/ml) calculated by using a standard and the phagocytic uptake of beads as percent. All data were received from at least triplicate samples and at minimum three independent experiments (±SD). Statistical differences between investigated groups were analyzed by the Student's t-test, considering $P < 0.05$ as significantly different to the respective control.

RESULTS

ROS Production in CD11b$^+$ Cells

The cell surface antigen, CD11b is characteristic for mouse monocytes and macrophages. We identified a homogenous population of CD11b-positive cells with FITC-conjugated anti-CD11b antibody by flow cytometric analysis in mouse bone marrow-derived cells directly after preparation. In this gated population, the production of free radicals after exposure to MF at different flux densities (50 Hz) or to chemical treatment was examined using the DHR assay. Figure 1 demonstrates a significant increase of ROS generation in CD11b-positive mouse promonocytes (up to 1.5-fold ± 0.3) after exposure

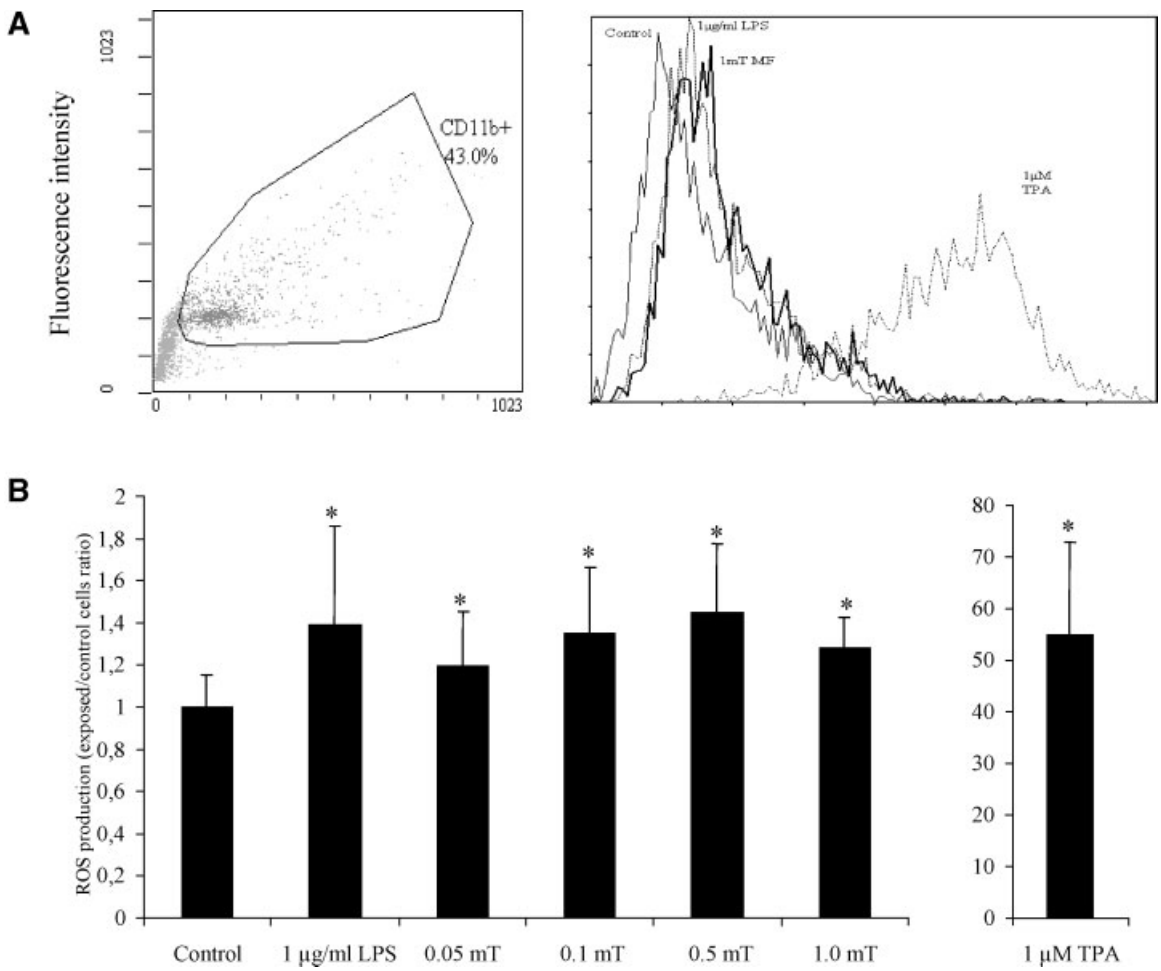

Fig. 1. Generation of ROS in mouse promonocytes detected by DHR assay using flow cytometry **A**: Fluorescence intensity of CD11b$^+$ cells detected by antibody staining in control cell cultures (**left**) and changes in rhodamine fluorescence intensity showing the transformation of DHR in cell cultures exposed to 1.0 mT MF, LPS, or TPA for 45 min (**right**). **B**: ROS release in CD11b$^+$ cells after exposure to 0.05, 0.1, 0.5, and 1.0 mT MF, 1 µg/ml LPS or 1 µM TPA for 45 min. Columns show the mean values of triplicate samples from at least three independent experiments ± SD; *$P < 0.01$ (Student's t-test, compared to control).

to 0.05, 0.1, 0.5, and 1.0 mT MF compared to controls. The slightest increase of ROS formation was detected in 0.05 mT (1.2-fold ± 0.3) and the highest in 0.5 mT-exposed cells (1.5-fold ± 0.3). LPS was used as a positive control for cell activation inducing a receptor-dependent pathway, leading to the production of free radicals. We detected a significant increase of ROS production (1.4-fold ± 0.5) in promonocytes after 45 min exposure to 1 μg/ml LPS. The level of ROS production in MF exposed cells is comparable to the level of LPS-stimulated cells, whereas significantly lower levels were obtained in TPA-treated cells, which were used as a second positive control. The phorbol ester TPA is known to stimulate the NAD(P)H-oxidase by activating the PKC and subsequently the MAPK pathway leading to an oxidative burst [Laskin et al., 1980; Phaire-Washington et al., 1980]. Our data show a rise in ROS formation, up to 55-fold (±18) after 45 min exposure to 1 μM TPA in $CD11b^+$ cells (Fig. 1B), indicating the induction of an oxidative burst.

Bead Internalization During MF-Exposure

The influence of MF on the internalization activity of differentiated mouse bone marrow-derived (MBM) macrophages was investigated by determining the phagocytic uptake of carboxylated latex beads in the presence or absence of 1.0 mT MF (50 Hz). The phagocytic activity was measured by counting the number of internalized beads per cell, in at least 200 cells per cover slip in triplicates, in three independent experiments, using DIC-microscopy (Fig. 2). Our results show a significant increase in bead internalization (1.6-fold ± 0.2) after exposure to 1.0 mT MF for 45 min, in comparison to the control. To stimulate the phagocytic activity of MBM-macrophages, TPA was used as positive control. Figure 2B shows that 1 μM TPA results in a significant increase of bead internalization up to 1.6-fold ± 0.1, similar to the activation potential of MF.

Interleukin-1β Release

The influence of 1.0 mT MF on IL-1β generation was examined in mouse macrophages. For quantitative analysis, the concentration of IL-1β was measured in the cell supernatants after MF exposure for different time periods (45 min, 90 min, 4, 8, 12, 16, 20, or 24 h). Exposure to 1.0 mT MF results in an increase of IL-1β generation after 4, 16, 20, and 24 h of exposure (Fig. 3). The maximum increase of IL-1β production was measured after 24 h, namely 71.5 pg/ml ± 36 pg/ml, which is a 12.8 ± 0.5-fold increase compared to control.

Genotoxic Effects After MF Exposure

To examine the possible genotoxic effect of EMFs, we analyzed the formation of micronuclei in mouse macrophages after exposure to 1.0 mT MF in a time-dependent manner (Fig. 4). Since the mechanism by which EMF induce cellular responses is not known yet, interactions between EMF and microfilaments cannot be excluded. It is known that cytochalasin-B inhibits the actin polymerization, but also

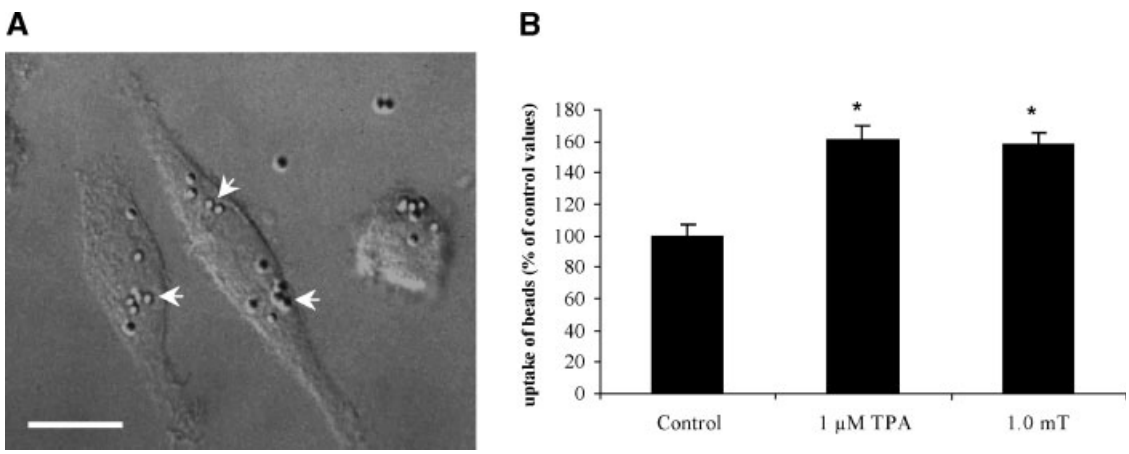

Fig. 2. **A**: DIC-video microscopic image of internalized latex beads (arrows) in mouse macrophages (bar = 10 μm). **B**: Bead internalization by mouse macrophages exposed to 1 μM TPA or 1.0 mT MF for 45 min. Columns show the mean values of triplicate samples from at least three independent experiments ± SD; *$P < 0.01$ (Student's *t*-test, compared to control).

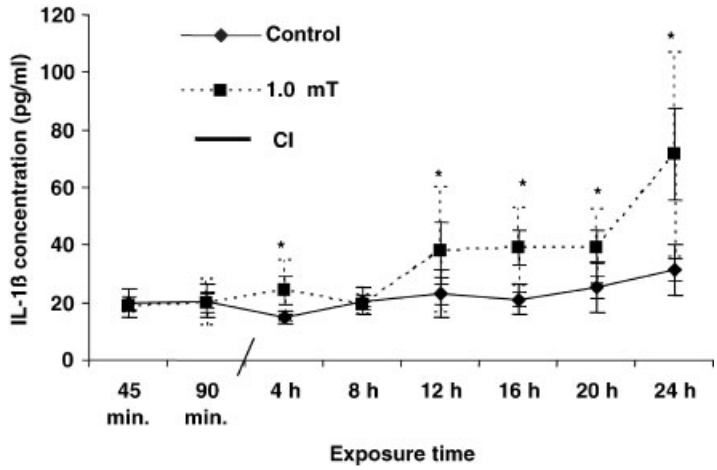

Fig. 3. Time-dependent IL-1β [pg/ml] production in macrophages after exposure to 1.0 mT. Graph indicates the data of three independent experiments (eight parallel measurements each) presented as mean value ± SD [dotted line] and ± confidence intervals CI [thick line]; $*P < 0.05$ (Student's *t*-test, compared to control).

interferes with mitotic cells [Lindholm et al., 1991] and induces MN by itself [Kirsch-Volders et al., 2000]. Since these circumstances can influence the results of possible genotoxic potential of EMF, the MN assay was carried out without using cytochalasin-B. Table I shows no significant differences between control and MF-exposed cells. Interestingly, an increase in MN formation was detected during the investigated time period both among the control and the MF-exposed cells. We also determined the numbers of mitotic cells, finding no differences between controls and exposed cells.

DISCUSSION

In this study, we utilized mouse bone marrow-derived cells to investigate the activating potential of ELF-EMF in immune relevant cells, using different biological endpoints. Previously, we described the activating capacity of 1.0 mT MF to induce the production of free radicals in mouse macrophages and in their precursor cells [Rollwitz et al., 2004], in human umbilical cord blood-derived monocytes, and in human Mono Mac 6 cells [Lupke et al., 2004]. Here, we investigated the dose-dependent effects of MF affecting the induction of free radical formation in mouse promonocytes. We detected a significant increase in ROS production after 0.05, 0.1, 0.5, and 1.0 mT MF exposure for 45 min in mouse promonocytes (Fig. 1). Data show a slight dose-dependent increase from 0.05 to 0.5 mT exposure, however, there are no significant differences between the tested flux densities. The level of ROS production after 1.0 mT is

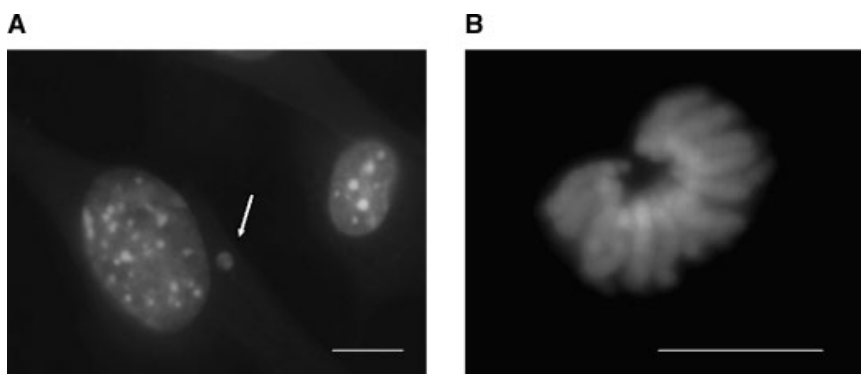

Fig. 4. Representative fluorescence micrograph of mouse macrophages stained with 1 μg/ml bisbenzimide (Hoechst 55258). **A**: Micronucleus formation (arrow). **B**: Mitotic macrophage (cell division at metaphase, bar = 10 μm).

TABLE I. The Number of Micronuclei and of Mitotic Cells in Mouse Macrophages After 12, 24, and 48 h Exposure to 1.0 mT MF

Time of exposure	Micronuclei		Mitotic cells	
	Control	1.0 mT	Control	1.0 mT
12 h	19.3 ± 10.3	22.2 ± 7.5	7.3 ± 2.7	6.2 ± 4.2
24 h	34.5 ± 7.6	33.8 ± 13.5	11.8 ± 3.3	10.3 ± 3.7
48 h	51 ± 15.4	64.3 ± 12.9	13.5 ± 3.7	11.7 ± 3.1

Micronuclei and mitotic cells were analyzed in 1,000 cells/slide in triplicate samples and in three independent experiments (±SD) and no statistical differences were observed between the analyzed cells.

barely smaller than after 0.5 mT MF exposure, but this difference is not significant. Because of the smaller variance in ROS production at 1.0 mT exposure, we decided to use 1.0 mT for all further investigations. Furthermore, the 1.0 mT data confirm our previous results, showing an increased ROS formation in mouse promonocytes after short-term EMF exposure [Rollwitz et al., 2004].

Reactive oxygen molecules are considered to be involved in general cellular activation processes and in the induction of a multiplicity of cellular pathways, for example, signal transduction, regulation of gene expression, differentiation, proliferation, and cell growth. Some studies discussed the correlation between free radicals and the raised incidence of DNA-damage under the influence of EMFs. Lai and Singh [2004] reported that exposure of rats to ELF-EMF for 24 h caused DNA breaks in brain cells. Furthermore, the authors showed that the induction of DNA-damage could be blocked by antioxidants, indicating, that ELF-EMF caused the accumulation of the oxidative free radicals. A report from Yokus et al. [2005] described oxidative DNA-damage and lipid peroxidation in rats after long-term exposure (50–100 days) to ELF-EMF (50 Hz, 0.97 mT). Wolf et al. [2005] examined the dose-dependent effects of ELF-EMF on DNA-damage in various cell lines and documented a significant increase in DNA-strand breaks after 24 and 72 h. Furthermore, it is discussed that EMF is responsible for the lifetime extension of free radical species [Harkins and Grissom, 1994; Roy et al., 1995] leading to an accumulation of ROS, resulting in an oxidative damage of DNA and other biological molecules such as lipids and proteins. The interaction between free radical production and DNA damage due ELF-EMF is discussed in a hypothesis by Simkó and Mattsson [2004] and Simkó et al. [2004].

To determine the influence of long-term exposure (up to 24 h) to ELF-MF on cell division interferences or genotoxic effects in mouse macrophages, we used the MN assay. Micronuclei are formed during the metaphase/anaphase transition of cell division and can give information about aneugenic or clastogenic damage. No significant differences were detected in MN formation or in mitotic activity in mouse macrophages after exposure to 1.0 mT MF for 12, 24, or 48 h compared to the controls. A number of studies reported different genotoxic effects of ELF-EMF [Nordenson et al., 1984; Khalil and Quassam, 1991; Lai and Singh, 1997]. Simkó et al. [1998b] documented a significant increase of MN frequency in a transformed cell line (SCLII) after exposure to 50 Hz EMF at 0.8 and 1.0 mT for 48 h and 72 h. Interestingly, they have also reported a significant increase in MN formation in non-transformed cells (human amnion fluid cells) after exposure to horizontally applied MFs, but not after exposure to vertically generated MFs [Simkó et al., 1998a]. Similarly, Cho and Chung [2003] documented that EMF at an intensity of 0.8 mT have mutagenic effects in human lymphocytes and could show a significant increase of MN frequency in these cells. Studies from Ivancsits et al. [2002, 2005] indicate a genotoxic potential of ELF-EMF in primary human fibroblasts and in different cell lines. The authors used the alkaline and the neutral comet assays to evaluate DNA single- and double-strand breaks and described effects only after intermittent but not after continuous exposure to EMF. The working group of Nordenson et al. [1994] used intermittent ELF-EMF exposure and found significant differences in exposed human amnion cells. On the other hand, there are various reports describing no MN-formations or DNA strand breaks in vitro [Scarfi et al., 1993; Stronati et al., 2004; Testa et al., 2004]. Simkó [2004] suggested that the reason for the controversial reports regarding genotoxicity can be due the differences in the used cell types and exposure conditions (flux densities, the orientation of electric- and MFs and intermittent or continuous exposure). The existence of an "EMF-sensitive cell fraction" of certain cell types is discussed by Ivancsits et al. [2002],

which could be responsible for the varying condition-dependent cellular responses.

The activation of phagocytosis and the formation of free radicals in macrophages are correlated to each other and to other multiple cellular processes such as cytokine production. In this study, we examined the influence of MF on macrophage cell functions cohesively, and analyzed the phagocytic activity after short-term exposure (45 min) to 1.0 mT MF. Our results show a significant uptake of carboxylated latex beads in macrophages, indicating the effectiveness of EMFs to stimulate the phagocytic activity. TPA was used as positive control to prove the activating capacity of cells. TPA is known to activate the PKC and induce cellular activation processes like pinocytosis and phagocytosis [Laskin et al., 1980; Phaire-Washington et al., 1980]. Figure 2 shows comparable amounts of internalized beads in MF-exposed (1.6-fold) and TPA-treated cells (1.6-fold). These results confirmed a previous study of Simkó et al. [2001] describing the stimulation of phagocytic activity in mouse macrophages after 45 min exposure to 50 Hz MF. Nakajima and Nishimura [1998] reported that 14 mT 50 Hz MFs had no significant effect on the phagocytic activity in pre-stimulated peritoneal mouse macrophages. The authors used pre-stimulated cells and different experimental conditions, such as larger particles (2.67 μm), a stronger MF flux density (14 mT), and a longer exposure time (1–2 h). Flipo et al. [1998] detected a decreased phagocytic activity in pre-stimulated peritoneal macrophages exposed to static MFs (24 h) with flux densities ranging from 25 to 106 mT. These controversial data can be explained by differences in exposure conditions and, by the fact that the used cells were pre-stimulated.

ROS have been implicated as second messengers that activate protein kinase cascades, although the means by which ROS regulate signal transduction remains unclear. ROS release and cytokine production, such as IL-1β, are common cell activation markers in immune relevant cells. In a recent publication, Li and Engelhardt [2005] revealed that ROS is involved in the activation of IL-1β signal transduction pathway. To detect, whether ROS release and IL-1β production are influenced by MF, we investigated the time-dependent IL-1β release after 1.0 mT exposure. Our result shows a significant increase in extracellular IL-1β release of 1.0 mT MF exposed cells (Fig. 3) already after 4 h of exposure, which was continuously increased after 12–24 h of exposure. These data suggest the activating capacity of the fields to release cytokines in murine macrophages. There are only a few studies investigating cytokine formation in immune cells after exposure to EMFs. Cossarizza et al. [1998] described the increased release of IL-2, IL-1, and IL-6 in peritoneal lymphocytes after long-term exposure to ELF-EMF. On the other hand, investigation on cytokine production by Pessina and Aldunucci [1998] showed no effects after EMF on peritoneal blood cells. The authors examined the influences of long-term EMF exposure (24, 48 h) in non-stimulated and in pre-stimulated blood cells. Only in pre-stimulated cells, significant increase in cytokine production was detected after exposure to EMF for 24 or 48 h. In our investigations, non-stimulated in vitro differentiated macrophages were used, demonstrating the induction of IL-1β production after MF exposure.

We have shown in a number of studies (as discussed above) that 50 Hz ELF-MF exposure is able to activate primary monocytes and macrophages from different species and also cell lines. This activation potential is comparable to the activation by certain chemicals resulting in physiologically relevant cellular responses. Our data reported in this study show that ELF-MF (50 Hz at different flux densities) exposure contribute to a general activation of macrophages, resulting in changes of numerous immunological reactions, such as in increased ROS-formation, in an enhanced phagocytic activity, and in an increased IL-1β release. No evidence was detected for genotoxic effects in mouse macrophages, or any anomaly in mitotic events. Therefore, we suggest that MFs activate physiological functions of immune relevant cells. However, the underlying mechanisms of interaction between MF and biological structures are still unknown and needs further studies on the molecular level.

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