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Cell Activating Capacity of 50 Hz Magnetic Fields to Release Reactive Oxygen Intermediates in Human Umbilical Cord Blood-derived Monocytes and in Mono Mac 6 Cells

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The aim of this study was to investigate the mechanism of cell activation induced by extremely low frequency magnetic fields (ELF-MF) (50 Hz) in human cells. We examined the production of free radicals in human umbilical cord blood-derived monocytes and in human Mono Mac 6 cells. The release of superoxide radical anions was analyzed using nitroblue tetrazolium chloride and the total of reactive oxygen species (ROS) was detected using dihydrorhodamine 123. Our results show a significant increase of superoxide radical anion production up-to 1.4 fold as well as an increase in ROS release up-to 1.2 fold upon exposure of monocytes to 1 mT ELF-MF (45 min). Mono Mac 6 cells exhibit higher superoxide radical anion and ROS production up-to 1.4 and 1.5 fold, respectively. These results indicate that Mono Mac 6 cells are more sensitive to ELF-MF than monocytes. Using diphenyleneiodonium chloride (DPI) a specific inhibitor for the NADPH oxidase, the MF-effect was not inhibited in Mono Mac 6 cells. Therefore, we suggest that ELF-MF exposure induces the activation of NADH oxidase in these cells. However, the MF-effect was inhibited by DPI in monocytes, indicating the activation of the NADPH oxidase after exposure to ELF-MF.

Keywords: Human monocytes; Mono Mac 6 cells; Cellular activation; Extremely low frequency magnetic fields; Free radicals

Abbreviations: ELF-MF, extremely low frequency magnetic fields; ROS, reactive oxygen species; $O_2^{\cdot-}$, superoxide radical anion; MAPK, mitogen-activated protein kinase; CD, cluster of differentiation; DPI, diphenyleneiodonium chloride; NBT, nitroblue tetrazolium chloride; DHR, dihydrorhodamine 123; AML, acute monocytic leukemia; C.I., confidence interval; NADH, reduced NAD; NADPH, NAD phosphate (reduced); phox, phagocytic oxidase; PKC, protein kinase C; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; RPMI, Roswell Park Memorial Institute; FCS, fetal calf serum; BSA, bovine serum albumin; PBS, phosphate buffered saline; FITC, Fluorescein; SEM, standard error of the mean; HBS, hepes buffered saline; E/C, ratio of exposed cells to control cells

INTRODUCTION

The influence of extremely low frequency electromagnetic fields (ELF-EMF) on the immune system has been investigated in several studies. These studies give evidence that ELF-EMF can cause an increased incidence of leukemia and other tumors, but some studies are not in agreement with these results,[1–8] which lead to a discussion in the scientific community as well as in the general public. Therefore, it is necessary to investigate the influence of ELF-EMF on the molecular level and to obtain knowledge about fundamental interaction mechanisms and to perform risk assessment.

Only few investigations have been made to examine the cell activation in murine macrophages[9] and in human immune cells[10–12] after exposure to ELF-EMF. Monocytes play a major role in the activation of innate and acquired immunity and exert a wide variety of functions including the regulation of the immune response (pro- and anti-inflammatory processes), scavenging senescent cells, phagocytosis of infected or malignant cells, wound healing, repair, and detoxification, but also the generation of free radical killing, e.g. of invading micro organisms are capable.[13–15] Each type

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and source of free radical enhances important physiological processes, e.g. signal transduction of various membrane receptors and further immunological functions. An imbalance between excessive formation of reactive oxygen species (ROS) and the limited antioxidant defense, known as oxidative stress[16] can cause potential damages on nucleic acid leading to mutations, but also on membranes, proteins, lipids and polysaccharides,[17] whereas low levels of ROS are continuously produced during the well controlled basal cellular metabolism.[18]

Superoxide radical anions ($O_{2\cdot}^{-}$) are the precursors of additional ROS. The formation of $O_{2\cdot}^{-}$ is mediated by enzymes such as the NAD(P)H oxidase, xanthine oxidase and the non-enzymatic redox reactive compounds of the mitochondrial respiratory chain constituting the primary defense mechanisms.[18–20] Activated phagocytic cells produce a large amount of $O_{2\cdot}^{-}$ via the NADPH oxidase, whereas non-phagocytic cells produce $O_{2\cdot}^{-}$ through the induction of NADH oxidase leading to a smaller level of free radicals. The activation of NAD(P)H oxidase can also be stimulated by the receptor independent acting agent PMA or by the receptor dependent activator LPS. PMA activates protein kinase C (PKC), which phosphorylates $p47^{phox}$ and activates the small G protein Rac through the mitogen-activated protein kinase (MAPK) pathway.[21] Both $p47^{phox}$ and Rac are part of the cytosolic components of the NAD(P)H oxidase, which is a multicomponent complex additionally consisting of two membrane associated subunits $p22^{phox}$ and $gp91^{phox}$ (resp., Mox for NADH oxidase) and the cytosolic factors $p67^{phox}$ and $p40^{phox}$.[21,22] The activation of Rac can also be induced by the receptor mediated pathway through the LPS activating MAPK pathway. LPS binds to the membrane receptor CD14, which is facilitated by a serum-derived protein.[23]

The activity of NADPH and NADH oxidases can be distinguished by using the specific NADPH oxidase inhibitor diphenyleneiodonium chloride (DPI). Morré[24] reported that the NADH oxidase is not affected by DPI, whereas NADPH oxidase activity was nearly fully inhibited, implicating the different molecular mechanism of the enzyme activating process.

In the present study, we investigated the activating capacity of extremely low frequency magnetic fields (ELF-MF) at 1 mT to induce ROS production in human umbilical cord blood-derived monocytes and in Mono Mac 6 cells. Based on our former studies[9] we assumed that after ELF-MF exposure (1 mT) immune relevant cells posses an inductive capacity of the NADH and/or the NADPH oxidase. To detect which pathway is involved, we used the specific inhibitor for NADPH oxidase DPI, which does not influence the NADH oxidase activity. Treatment with LPS and PMA alone as well as in combination with ELF-MF in the presence or absence of DPI allowed us to distinguish between the inductions of NADPH and the NADH pathways.

MATERIAL AND METHODS

Reagents and Antibodies

RPMI 1640 + 2 mM L-glutamine culture medium, FCS, penicillin/streptomycin, BSA, PBS and Ficoll ($d = 1.077$) were purchased from PAA (Coelbe, Germany). Non-essential amino acid was obtained from Biochrom KG (Berlin, Germany). OPI media supplement, PMA, LPS from *Escheria coli* (Serotype 026:B6), DPI, nitroblue tetrazolium chloride (NBT), EDTA were acquired from Sigma Aldrich (Munich, Germany). Dihydrorhodamine 123 (DHR) was obtained from Molecular Probes (Leiden, Netherlands). Hepes and NaCl were purchased from Roth (Karlsruhe, Germany). For flow cytometry, antibodies (anti-CD14 FITC, anti-CD69 FITC, and anti-CD71 FITC) were acquired from Becton Dickinson (Heidelberg, Germany).

Isolation and Culture of Human Umbilical Cord Blood-derived Monocytes

Human umbilical cord blood was obtained from the maternal end of the severed cord immediately after the birth. The heparinized blood was isolated within 24 h by standard density gradient Ficoll hypaque separation (density, $d = 1.077$). The density gradient was centrifuged at 630g for 20 min at room temperature. The upper layer containing plasma was collected and used as autologous serum.[25] Monocytes were purified from the middle layer of the gradient representing the mononuclear cells. Cells were washed twice in buffer (PBS, 2 mM EDTA, 0.5% BSA pH 7.4) and separated by cell sorting (Epics Altra, Beckman Coulter, Germany) or by using the $CD14^+$ Cell Isolation Kit and the MiniMacs separation unit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturers' protocol. Briefly, cells were incubated with $CD14^+$ coated magnetic microbeads at 4°C for 15 min, washed once and the cell suspension was applied to a MS separation column (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) placed in a static permanent magnet. $CD14^+$ cells are magnetically coated and remain in the column while unlabeled cells pass through. After 3 washes cells were eluted from the magnet by removing the column. Cells were counted using a Neubauer cell count chamber, seeded in a 96 well plate (TPP, Germany) at a density of 0.5×10^6 to 1.0×10^6 cells/ml in RPMI 1640 containing 2 mM L-glutamine, 10% autologous serum and cultured at 37°C in

a humidified atmosphere and 5% CO_2 until the experiments started.

Human Mono Mac 6 Cells

The human acute monocytic leukaemia (AML) cell line Mono Mac 6 was purchased from the German Collection of Micro organisms and Cell Culture GmbH (DSMZ, Heidelberg, Germany) and cultured in RPMI 1640 containing 2 mM L-glutamine, 10% FCS, 200 U/ml penicillin/streptomycin, 2 × non-essential amino acid, 1% OPI media supplement. Mono Mac 6 cells were cultured in a 24 well plate (TPP, Germany) at a density of $0.4–1.0 \times 10^6$ cells/ml at 37°C in a humidified atmosphere and 5% CO_2 and (diluted) at a ratio of 1:2 or 1:3 every 3rd or 4th day.

Flow Cytometric Analysis of the Cells

The percentage of human umbilical cord blood-derived monocytes was evaluated by labeling 0.5×10^6 cells with anti-CD14 FITC. Furthermore, monocytes were also single stained with anti-CD69 FITC, representing monocytes in an early activation stage, and anti-CD71 FITC to detect all activated monocytes/macrophages. A total of 5000 $CD14^+$ monocytes were analyzed per sample by using the flow cytometer Epics Altra (Beckman Coulter, Germany) with a water-cooled 488 nm Argon Laser. Fluorescence values derived from the FITC conjugated antibodies were measured at 525 nm and the percentage of positive cells was calculated.

Exposure Settings

A Helmholtz coil system (diameter 400 mm, distance between the coils 200 mm, resistance 2.1 Ω, 154 turns of copper wire; Phywe Systeme, Göttingen, Germany)[26] was used as the ELF-MF exposure system, which was located inside a CO_2-incubator (Binder, Germany). The background flux density of both control and exposure incubators was 1–5 μT. Cells were exposed in the central area of the coils to 50 Hz ELF-MF at 1 mT flux density continuously monitored by precision F.W. Bell Model 4048 Gauss/Tesla Meter (Bell, Orlando, USA). Control cells were cultured in an identical incubator without a Helmholtz coil system.

Experimental Design

Monocytes and Mono Mac 6 cells were also treated with 1 μM PMA or with 1 μg/ml LPS and both in co-exposure to ELF-MF. To detect the inhibition of the free radical production, 1 μM DPI was used in combination with PMA, LPS, ELF-MF alone or in different combinations of co-exposure. Cells were incubated at 37°C in a humidified atmosphere and 5% CO_2 for 45 min.

Detection of Free Radicals

Monocytes (1×10^6/well) and Mono Mac 6 cells (0.5×10^6/well) were distributed in 96-well plates with flat bottoms and incubated at 37°C in a humidified atmosphere and 5% CO_2 for 1–4 h in medium until start of the experiments.

Detection of $O_2^{-\cdot}$ was performed by the NBT assay, where NBT is reduced to formazan. Cells in 96-well plate were exposed to 1 mM NBT solution (containing 1 mM $CaCl_2$ in PBS) under indicated conditions. After centrifugation, the supernatant was discarded and DMSO was added to the cells. The release of $O_2^{-\cdot}$ was immediately measured at 550 nm by using a microplate ELISA Reader (BioTek Instruments Inc. Vermont, USA). Measurements were carried out in at least 8 parallel samples.

The production of ROS was detected by using the dye dihydrorhodamine 123 (DHR), which is oxidized by hydrogen peroxide, hypochlorous acid and peroxynitrite anion to the fluorescence dye rhodamine. Cells were seeded in 96-well plates, treated and exposed as indicated. After centrifugation supernatant was discarded, 200 μl of HBS (0.9% NaCl, 14 mM Hepes pH 7.4) and DHR solutions were added to a final concentration of 1 μM for 25 min at 37°C. The rhodamine fluorescence was measured by flow cytometry (Epics Altra, Beckman Coulter, Germany) at 525 nm. Mean fluorescence intensity (X-mean) was determined after gating monocytes by using the software EXPO32 vs. 1.2 analysis (Beckman Coulter, Germany). For each sample 2500 events were acquired. Three parallel samples per exposure and treatment were measured.

Statistical Analysis

All data are expressed as the ratio of exposed to control cells (E/C). Columns show the mean data of E/C ± confidence interval (C.I.) of at least 9, independent, experiments (triplicates for DHR assay and at least 8 replicates for NBT assay). Differences between investigated groups were analyzed by the Student's t test and ANOVA considering values $p < 0.05$ as significant.

RESULTS

Expression of CD Antigens on Human Umbilical Cord Blood-derived Monocytes

Monocytes express special differentiation antigens on their cell surface to differentiate them from other immune cells. CD14 is only expressed on

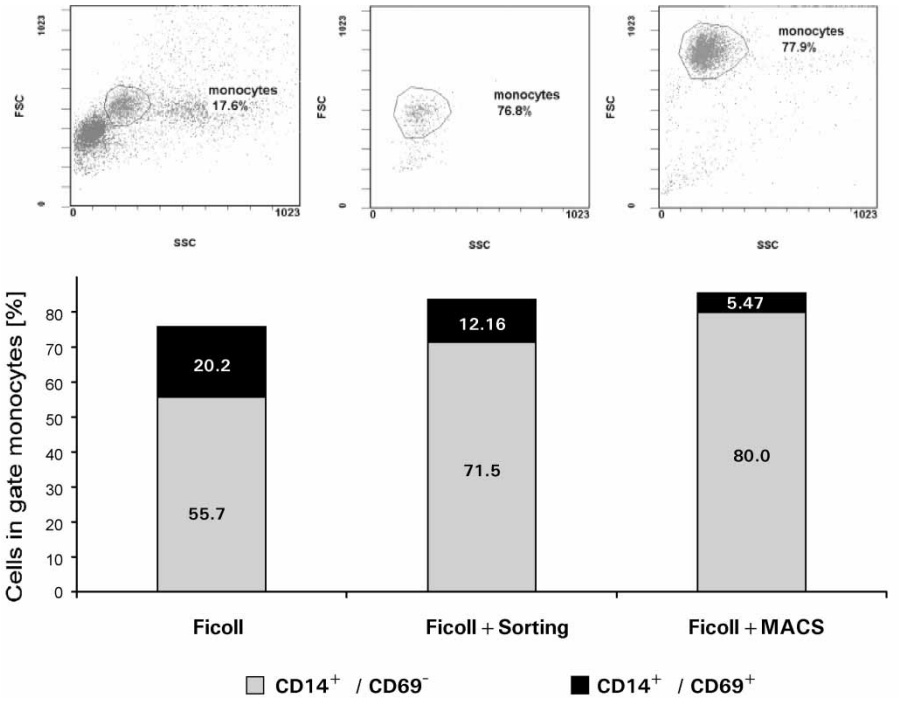

FIGURE 1 Differences in the enrichment of monocytes after standard density gradient Ficoll separation, Ficoll separation followed by cell sorting or MiniMacs unit separation. Monocytes were analyzed by flow cytometry. The histograms (upper) show the distribution of cells expressed by forward scatter (FSC) and side scatter (SSC), whereas gated monocytes are indicated by the circle. The diagram indicates the activation status of monocytes using various separation techniques by measuring the distribution of $CD14^+/69^+$ and $CD14^+/69^-$ cells.

monocytes while CD69 and CD71 represent the activation status of monocytes. We determined the expression level of CD14 and CD69 to characterize the activation status of cells after standard density gradient Ficoll separation, cell sorting, and the enrichment using MiniMacs separation units. The percentage of monocytes was 17.6% after the Ficoll separation and 76.8% after cell sorting. In comparison, the enrichment of monocytes was as high as 77.9% using the MiniMacs separation units (Fig. 1). No differences in the activation status of monocytes could be detected between the MiniMacs separation and cell sorting; therefore, we used the MiniMacs separation for all following investigations. In addition, this procedure was faster and relatively gentle. In regard to ROS production no differences could be observed in control cells after MiniMacs separation (*X*-mean: 5.06 ± 1.7) and cell sorting (*X*-mean: 4.91 ± 1.1) (data not shown). The gated monocytes after MiniMacs separation included at least 90% $CD14^+$ cells, of which 31.8% were early activated monocytes ($CD69^+$ cells) and 0.85% were activated monocytes ($CD71^+$ cells) (Fig. 2).

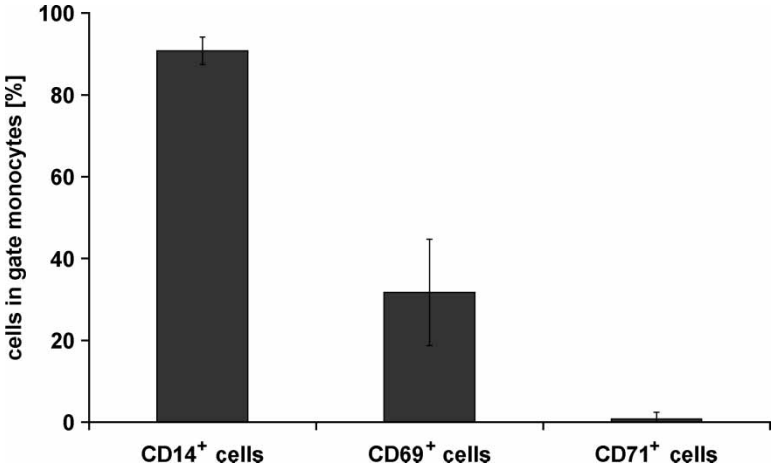

FIGURE 2 Analysis of the activation status of human monocytes after MiniMacs. Monocytes were single stained with anti-CD14 FITC, anti-CD69 FITC and anti-CD71 FITC antibodies and analyzed by flow cytometry. The $CD14^+$ cells represent monocytes generally, $CD69^+$ cells are in an early activation stage and $CD71^+$ cells identify activated monocytes. Columns are mean data ± C.I. ($n = 40$).

Superoxide Radical Anion Production in Human Monocytes and Human Mono Mac 6 Cells

To investigate the activation capacity of ELF-MF, we determined the $O_2^{\cdot-}$ production, which is the precursor of most free radicals, after short-term exposure to 1 mT and/or to different chemicals in human monocytes and in Mono Mac 6 cells.

The $O_2^{\cdot-}$ production was significantly increased in both cell types up-to 1.4 fold comparing to control after exposure to ELF-MF for 45 min (Table I). Using PMA as an intracellular activator for $O_2^{\cdot-}$ production, a 4.5 fold increase was detected in monocytes, whereas Mono Mac 6 cells showed a 3 fold increase. Additional effects after co-exposure to ELF-MF/PMA could not be detected in monocytes. However, Mono Mac 6 cells showed a significant increase in $O_2^{\cdot-}$ production (3.8 fold) after co-exposure to ELF-MF/PMA compared to PMA alone (3 fold). A significant release of $O_2^{\cdot-}$ of up-to 2.4 fold in monocytes and up-to 1.4 fold in Mono Mac 6 cells appeared after exposure to LPS, which is a receptor dependent inducer. Co-exposure to ELF-MF and LPS did not induce additional release of $O_2^{\cdot-}$ in both cell types (Table I).

ROS Production in Human Monocytes and Human Mono Mac 6 Cells

The generation of $O_2^{\cdot-}$ and its conversion to other ROS that can cause pathological and physiological responses as an inflammatory process were also measured to obtain information about the cell activation after ELF-MF exposure.

Our studies show that ELF-MF exposure induces a significant increase in ROS production up-to 1.2 fold in monocytes and up-to 1.5 fold in Mono Mac 6 cells (Table I). PMA-treatment showed a 10-fold increase in monocytes, whereas only a 3-fold increase was noticed in Mono Mac 6 cells. Co-exposure to ELF-MF and PMA was also investigated but no additional influence could be detected in both cell types, when compared to PMA exposure alone. Using LPS monocytes and Mono Mac 6, there was a significant increase in ROS production of 4.1 fold and 2 fold, respectively; however, no additional effects were found in either cell type after co-exposure to ELF-MF and LPS (Table I).

Inhibition of Free Radical Production by DPI

Experiments were performed to assess the generation of free radical production by blocking the NADPH oxidase activity using DPI as a flavoprotein inhibitor without influencing the NADH oxidase activity.

The ELF-MF induced $O_2^{\cdot-}$ production was completely inhibited by DPI in monocytes (Fig. 3a), whereas PMA induced $O_2^{\cdot-}$ release was not totally reduced to the control level (1.7 fold). However, a complete inhibition of $O_2^{\cdot-}$ production could be detected after co-exposure to ELF-MF/PMA. The difference between both PMA treated and co-exposed groups is significant. LPS as well as ELF-MF/LPS treated monocytes showed an inhibited $O_2^{\cdot-}$ production in the presence of DPI to the control level.

The total inhibition of ROS release by DPI was also determined in ELF-MF exposed monocytes (Fig. 4a). ROS production was significantly diminished by DPI after exposure to PMA and ELF-MF/PMA (7.1 and 6.4 fold) although decreases did not reach the control level. Inhibition of ROS release could be detected in LPS and ELF-MF/LPS treated cells to 1.3 and 1.4 fold. Again, DPI did not down regulate the ROS production to the control level.

The significant increase (1.3 fold) in $O_2^{\cdot-}$ production after ELF-MF exposure was not inhibited by DPI in Mono Mac 6 cells (Fig. 3b). PMA-treated Mono Mac 6 cells and co-exposed cells to ELF-MF/PMA showed a depression of $O_2^{\cdot-}$ production after DPI treatment from 3.0 to 1.6 fold and from 3.8 to 1.9 fold; however, this did not reach the control level. The inability to inhibit $O_2^{\cdot-}$ production using DPI was also detected in LPS and in ELF-MF/LPS treated Mono Mac 6 cells. The induced ROS production in ELF-MF-exposed Mono Mac 6 cells could be minimized from 1.5 to 1.2 fold by DPI and in PMA and ELF-MF/PMA-treated Mono Mac 6 cells from 3.0 to 1.4 fold and from 3.3 to 1.5 fold, respectively (Fig. 4b). LPS induced ROS production is declined from 2.0 to

TABLE I Free radical production in human monocytes and in human Mono Mac 6 cells after exposure to ELF-MF (1 mT), 1 μM PMA, 1 μg/ml LPS and co-exposure to ELF-MF/PMA or ELF-MF/LPS for 45 min. (mean ± SEM)

	Human monocytes		Mono Mac 6 cells	
Treatment	$O_2^{\cdot-}$ (±SEM)	ROS (±SEM)	$O_2^{\cdot-}$ (±SEM)	ROS (±SEM)
Control	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
ELF-MF	1.4 ± 0.1*	1.2 ± 0.04*	1.4 ± 0.2*	1.5 ± 0.1*
PMA	4.5 ± 0.5*	10.0 ± 1.2*	3.0 ± 0.3*	3.0 ± 0.3*
PMA + ELF-MF	4.9 ± 0.6$^{n.s.}$	10.8 ± 1.6$^{n.s.}$	3.8 ± 0.1*	3.3 ± 0.3$^{n.s.}$
LPS	2.4 ± 0.3*	4.1 ± 1.0*	1.4 ± 0.1*	2.0 ± 0.2*
LPS + ELF-MF	2.7 ± 0.5$^{n.s.}$	4.5 ± 1.1$^{n.s.}$	1.4 ± 0.2$^{n.s.}$	2.3 ± 0.3$^{n.s.}$

*Significant difference vs. respective control (Student's t test) $p < 0.05$. n.s., non significant.

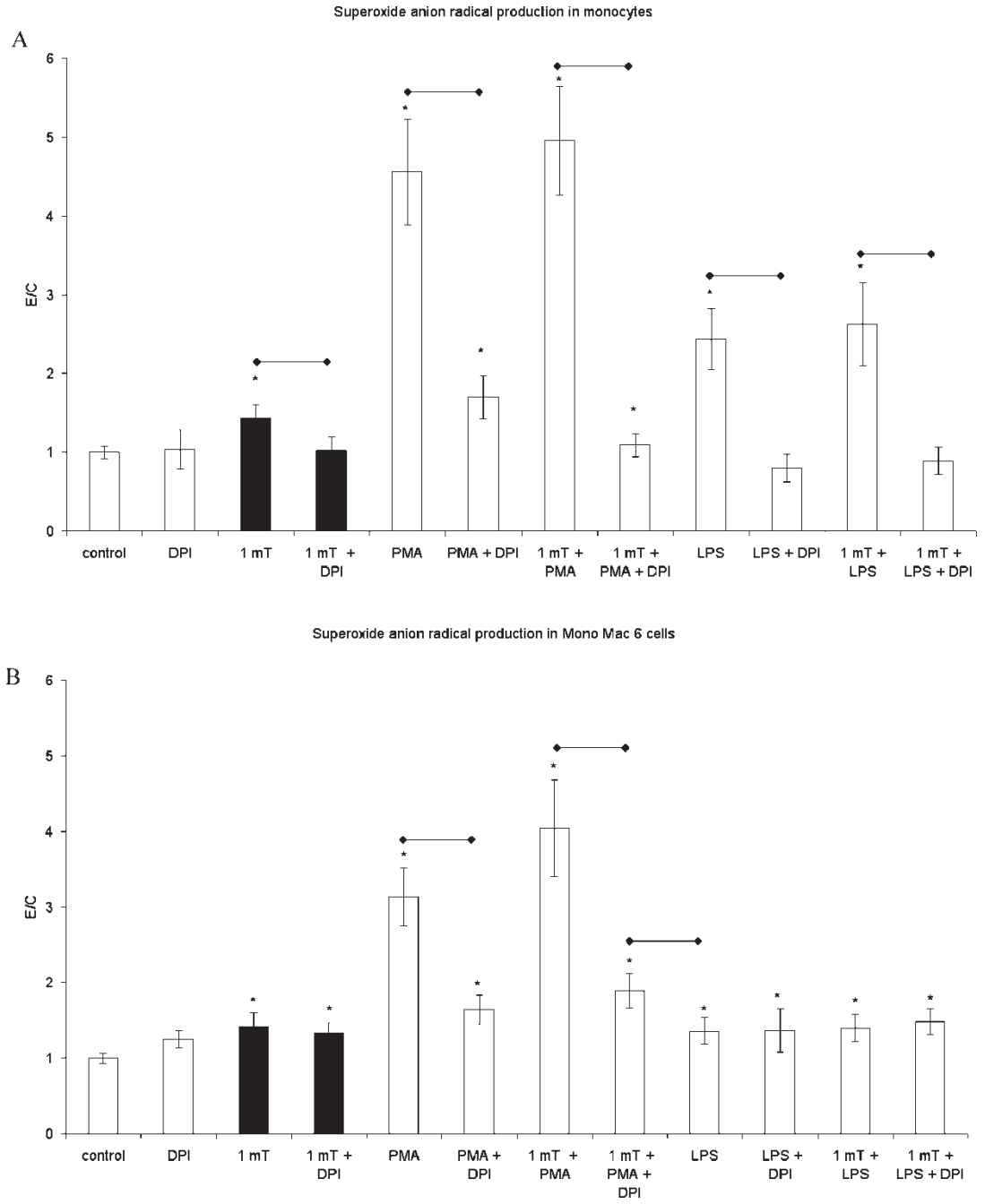

FIGURE 3 Superoxide radical anion and production in human monocytes (A) and in Mono Mac 6 cells (B) in the presence or absence of DPI as an NADPH oxidase inhibitor after exposure to ELF-MF (1 mT), 1 μM PMA, 1 μg/ml LPS or co-exposure to ELF-MF/PMA or ELF-MF/LPS for 45 min detected by the NBT assay. To inhibit the O_2^- production DPI was added to a final concentration of 1 μM. The bars represent the mean value of absorption as a ratio of exposed to control cells (E/C ± C.I.). Asterisks marks significant differences (Student's *t* test, $p < 0.05$) between control and treated cells whereas the horizontal bars (◆—◆) indicate significant differences conditions in the indicated pairs.

1.6 fold and in ELF-MF/LPS co-treated cultures from 2.3 to 1.7 fold after DPI-treatment in Mono Mac 6 cells. However, these differences are statistically not significant.

DISCUSSION

In this study, we investigated the activating ability of ELF-MF in primary human umbilical cord blood-derived monocytes. Additionally, a human AML cell like Mono Mac 6 cells were studied in parallel to compare the effectiveness of ELF-MF. Our results show a significantly increased production of free radicals after exposure to ELF-MF (1 mT) in both human monocytes and human Mono Mac 6 cells. To determine the cell activating capability, we also examined free radical release using PMA as an independent receptor[21] and LPS as CD14

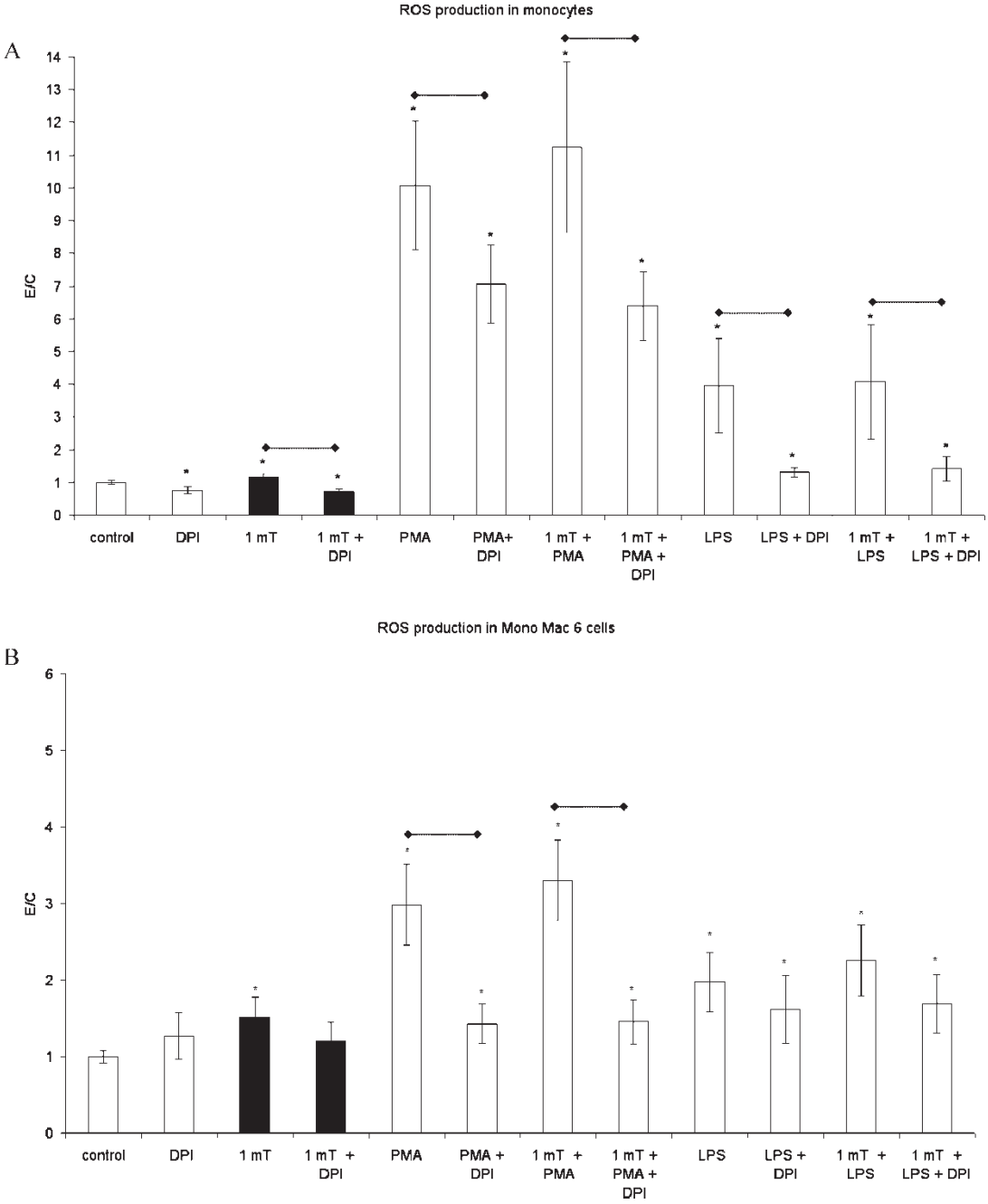

FIGURE 4 ROS production in human monocytes (A) and Mono Mac 6 cells (B) in the presence or absence of DPI as an NADPH oxidase inhibitor after exposure to ELF-MF (1 mT), 1 μM PMA, 1 μg/ml LPS or co-exposure to ELF-MF/PMA or ELF-MF/LPS for 45 min using the DHR assay. To inhibit O_2^- production DPI was added to a final concentration of 1 μM. The bars represent the mean of fluorescence intensity (*X*-mean) as a ratio of exposed to control cells (E/C ± C.I.). Asterisks marks significant differences (Student's *t* test, $p < 0.05$) between control and treated cells whereas the horizontal bars (◆—◆) indicate significant differences conditions in the indicated pairs.

receptor dependent activator.[23] Furthermore, co-exposure to ELF-MF/PMA and ELF-MF/LPS was performed to detect synergistic effects and identify possible target molecules for ELF-MF interaction. It is remarkable that in general monocytes are more responsive to generate ROS than Mono Mac 6 cells after treatment with PMA or LPS. Our results show the activating ability of ELF-MF to induce free radical production in both cell types, but the magnitude of free radical production is below that of PMA and LPS stimulation. However, ELF-MF mainly induced O_2^- generation at the same level in both cell types (1.4 fold), whereas ROS production was increased 1.2 fold in monocytes and 1.5 fold in Mono Mac 6 cells. These results suggest that Mono Mac 6 cells have stronger antioxidant defenses, e.g. superoxide dismutase, glutathione peroxidase[19] compared to monocytes after ELF-MF exposure. Indeed, Kato *et al.*[27] showed that the total SOD activity is significantly greater in AML

cells than in monocytes, which might predispose cells to oxidative stress. It is known that low doses of stress stimuli is sufficient to activate the so-called stress-activated protein kinases (JNK/p38) in transformed cells leading to elevated ROS release.[20,28] One can suggest, therefore, that the higher response to ELF-MF of Mono Mac 6 cells can be explained by an already elevated metabolic activity of stress kinase proteins resulting in an increased response.

On the other hand, PMA treatment induced a relatively higher ROS production in monocytes than in Mono Mac 6 cells, compared to the controls, indicating a higher responsiveness through the PKC mediated pathway. It should be mentioned that the ratio measurements give information in the differences between exposed and non-exposed cells only and does not permit quantification of the data. We detected a lower rate of ROS production in LPS treated Mono Mac 6 cells (2.0 fold) than in monocytes (4.0 fold) demonstrating that expression of the LPS receptor CD14 is lower in Mono Mac 6 cells, which was also reported by Abrink and coworkers.[29]

Our investigations showed the capacity of ELF-MF to induce free radical production in the presence of the flavoprotein inhibitor DPI in Mono Mac 6 cells leading to the conclusion that NADH oxidase is activated after the exposure to ELF-MF. The release of free radicals can also be induced through the mitochondrial respiratory pathway. In this case, the inhibitory effect of DPI does not influence NADH dehydrogenase activity as shown in HL-60 cells.[30] In contrast, the effect of ELF-MF in monocytes was inhibited by DPI, which is an indication for activation of the NADPH oxidase. We suggest, therefore, that the NADPH oxidase mediated pathway is activated in monocytes, whereas the NADH oxidase mediated pathway is activated in Mono Mac 6 cells to produce free radicals after ELF-MF exposure. We also detected an increased production of $O_2^{\cdot-}$ and ROS in both monocytes and in Mono Mac 6 cells, after LPS exposure. LPS treatment activates the cytosolic protein Rac through the MAPK pathway, which is necessary for the activation of the NAD(P)H oxidase.[23] The DPI inhibited free radical production in LPS treated monocytes give evidence for the repression of the NADPH oxidase, because of the lacking activation of the membrane associated subunits $p22^{phox}$ and $gp91^{phox}$. However, the receptor dependent activation by LPS in Mono Mac 6 cells was not inhibited by DPI leading to the assumption that the cytosolic complex activates the Mox protein a subunit of NADH oxidase.[22]

PMA treated monocytes and Mono Mac 6 cells also produced an increased amount of free radicals. Using DPI, a down-regulated free radical production was noticed without reaching the control level. This indicates that PMA stimulation induces both NADPH and NADH oxidase mediated pathways. Therefore, we suggest that the activation of NADPH and NADH oxidase takes place through two different and independently acting pathways.

Summarizing, in this study we showed the activating capacity of ELF-MF to produce free radicals in human monocytes and Mono Mac 6 cells. We also found that Mono Mac 6 cells are more sensitive for cellular activation by ELF-MF than monocytes, which can be explained by the different homeostatic capacity of these cells. We suggest that ELF-MF exposure induced the activation of the NADH oxidase leading to increased free radical release in Mono Mac 6 cells, because DPI did not inhibit the ELF-MF-effect. In monocytes the ELF-MF-effect was inhibited by DPI indicating the activation of the NADPH oxidase mediated pathway after exposure to ELF-MF. Using PMA and LPS in the presence of DPI we showed that both the NADPH and the NADH oxidase activating pathways are acting, independently, in monocytes and in Mono Mac 6 cells.

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