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Reversible Effect of Magnetic Fields on Human Lymphocyte Activation Patterns: Different Sensitivity of Naive and Memory Lymphocyte Subsets

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The aim of this study was to investigate the influence of 50 Hz magnetic or static magnetic fields of 0.5 mT on subsets of human $CD4^+$ T cells in terms of cytokine release/content, cell proliferation and intracellular free calcium concentration. $CD4^+$ T cells can be divided into different subsets on the basis of surface marker expression, such as CD45, and T cells can be divided into naive ($CD45RA^+$) and memory ($CD45RA^-$) cells. In this study, the effects of magnetic fields after 24 and 48 h of cell culture were analyzed. We found that the $CD4^+CD45RA^-$ T subset were more sensitive after 2 h of exposure. Decreases in the release/content of IFN-γ, in cell proliferation and in intracellular free calcium concentrations were observed in exposed $CD4^+CD45RA^-$ T cells compared to $CD4^+CD45RA^+$ T cells. The results suggest that exposure to the magnetic fields induces a delay in the response to stimulants and that modifications are rapidly reversible, at least after a short exposure.

INTRODUCTION

The potential health risk of exposure to extremely low-frequency (ELF) magnetic fields has received considerable attention in recent years, mainly due to the ubiquitous presence of electricity and electrical appliances in the home and workplace, and some findings suggest the occurrence of adverse health effects (*1, 2*).

ELF magnetic fields cover the frequency range of 3 Hz–3 kHz; the most intensively studied frequency is 50/60Hz. Non-ionizing electromagnetic fields (EMFs) and in particular the ELF magnetic field are able to affect many biological functions (*3–6*), but current data do not provide a satisfactory explanation for these effects because of the great diversity of specialized biological models and applications studied and the limited information concerning the underlying mechanisms of interaction.

The guidelines for occupational exposure to power-frequency magnetic fields at 50 Hz are (1) 0.5 mT for the International Committee on Non-ionizing Radiation Protection (ICNIRP) (*7*) and for the International Radiation Protection Association (IRPA) (*8*), (2) 0.1 mT for exposures to the general public, (3) 1 mT for the American Conference of Governmental Industrial Hygienists (ACGI), and (4) 0.5 mT for the World Health Organization's International Agency for Research on Cancer (*1*). While many studies have demonstrated that ELF sinusoidal 50/60 Hz magnetic fields may have harmful effects on human health, other reports have provided inconsistent evidence to support the presence of health hazards due to exposure to EMFs (*9–14*). Medium-intensity static magnetic fields ranging from 1–1000 mT have been shown to influence a wide variety of biological systems. Knowledge of the effects of static magnetic fields is important for human health. It has been reported that exposure to middle-level static magnetic fields affects the morphology, differentiation and/or proliferation of several cell types (*15–18*).

In our previous paper (*11*), we showed that magnetic fields can influence some cellular functions, but these alterations are reversible after 48 h of *in vitro* cell culture. In particular, magnetic fields produce a reversible effect on cell proliferation, IFN-γ production/release and cytosolic free calcium concentration. In the present study we evaluated the effects of a 0.5 mT 50 Hz magnetic field and static magnetic field on subsets of human $CD4^+$ T cells with differing expression of CD45RA. Several isoforms of the CD45 protein exist, including CD45RA and CD45RO. These molecules are involved in various aspects of lymphocyte activation. CD45RA is expressed on naïve T cells, while T cells that have previously encountered this antigen express CD45RO. The number of $CD45RO^+$ cells increases

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with age, and approximately 45% of the T-cell population expresses this isoform. In the present study we investigated the effects of 50 Hz magnetic fields and static magnetic fields on the different subsets of $CD4^+$ T cells in terms of the release/content of IFN-γ, cell proliferation and modification of intracellular cytosolic free calcium content to identify the subsets the were more sensitive to the effects of magnetic fields.

MATERIALS AND METHODS

Subjects

Blood samples were obtained from eight healthy volunteers (four females and four males) with a mean age of 30 ± 3 years (age range 27–33 years); informed consent was obtained from all participants. The analyses were done blindly by two independent research groups.

The internal committee of the Department of Biopathology, Palermo, oversaw the procedures for the collection of the blood samples from 10 ml of heparinized peripheral blood that were used for the generation of anonymized CD4 T-cell lines.

$CD4^+$ T-cell lines were generated from peripheral blood mononuclear cells (PBMC) isolated from heparinized blood by centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Cells were maintained in RPMI-1640 culture medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated pooled human AB^+ serum, 2 m*M* L-glutamine, 20 m*M* Hepes, 100 U/ml penicillin, 100 µg/ml streptomycin, 5×10^{-5} *M* 2-mercaptoethanol, and 20 IU/ml rIL-2, hereafter referred to as IL-2-supplemented culture medium. $CD4^+$ T lymphocytes were sorted by immunomagnetic beads using an anti-CD4 specific monoclonal antibody (mAb) and were then expanded *in vitro* in IL-2-supplemented culture medium supplemented with purified PHA (Leucoagglutinin, 0.5 µg/ml, Sigma, St. Louis, MO) and irradiated (30 Gy from a cesium source) allogeneic feeder cells (PBL and Epstein Barr Virus B cells at a ratio of 10/1). Cells were maintained for 2 weeks without any stimulation before functional analysis. The purity of the $CD4^+$ T cells was assessed by two-color flow cytometry. PE-conjugated anti-CD3 mAb was used with FITC-conjugated anti-CD4 mAb (both from Becton Dickinson, San Jose, CA). The cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA). This T-cell procedure induces expansion of virtually all $CD4^+$ T cells and does not introduce any bias in the T-cell repertoire (*19*).

Cells were split in two groups of samples, one to be exposed to the magnetic fields and the other to serve as a control to be kept under isothermal conditions for the whole time of the experiment. The experiments were carried out in triplicate for each sample and were repeated four times.

Terms and Conditions of Exposure

Each set of samples included subsets of human $CD4^+$ T cells.

Sham-exposed cells were maintained at 37°C while treated cells were exposed and were maintained under the same conditions as the exposed cells after their exposure to the magnetic fields.

The exposures to the 0.5 mT EMF were performed inside a double cylindrical coil (50 cm and 15 cm inner diameter). The electric current intensity in the coils ranged between 0 and 8 A and the measured magnetic-field values were between 0 and 7.5 mT. A gaussmeter with an axial Hall effect probe was used to measure the magnetic-field distribution at various points across the solenoid. The overall uncertainty of measurement was 2%. The field inside the exposure area showed about 3% homogeneity.

The 0.5 mT static magnetic field was generated by double magnet (35 cm and 10 cm inner diameter). The direct current (d.c.) intensity ranged between 0 and 6 A and the measured magnetic field values were respectively between 0 and 10 mT. The field inside the exposition area showed about 5% in homogeneity.

The temperature was maintained at 37 ± 0.5°C by a water circulation system connected to a Circulator Haake DC10 temperature regulator (Thermo Fisher Scientific, Karlsruhe, Germany) equipped with connector for external circulation.

All the exposed samples were maintained in the magnetic fields for 2 h; after exposure, the samples were maintained at 37°C and then sent to the laboratory for immunological tests.

Immunomagnetic Separation of Exposed and Sham-Exposed Cells

Sham-exposed and exposed cells were sorted by negative selection with immunomagnetic beads using anti-CD45RA and anti-CD45RO specific monoclonal antibodies (mAbs) according to the Miltenyi Biotec protocol. Briefly, after 2 h of exposure, sham-exposed and exposed cells were incubated with the antibodies for 15 min at 4°C. After incubation, the cells were washed with buffer (0.5% PBS/BSA and 2 m*M* EDTA, pH 7.2) and resuspended in 80 µl of buffer. Then the cells were incubated with 20 µl of goat anti-mouse IgG beads for 15 min at 4°C. After incubation, the cell suspension was placed on top of the column separation (MS Miltenyi), the buffer (1 ml) was added three times, and the negative fraction was collected. The negative fraction, represented by cells without magnetic beads, was washed, resuspended in buffer, and checked by flow cytometry, and used for further experiments.

Determination of IFN-γ

Exposed and sham-exposed cells (2×10^6/ml) were cultured in 24-well plates in 1 ml of complete medium in the presence or absence of PHA (0.5 µg/ml) for 24 and 48 h. The PHA was used as polyclonal mitogen that interacts with the T-cell receptor (TCR) or accessory surface structures (indirect TCR crosslinking) used to evaluate the functional activity of T lymphocytes.

The supernatants were harvested and stored at −70°C until they were tested for cytokines; the cytokine levels were determined using an immunoenzymatic method. The supernatants of cells exposed to 50 Hz magnetic fields were analyzed with an enzyme-linked immunosorbent assay (ELISA) performed with a reagent set human IFN-γ (Euroclone, Devon, UK). The limit detection range was from 12.5 pg/ml to 400 pg/ml for IFN-γ concentration; instead the supernatants of cells exposed to static magnetic fields were analyzed with reagent set human IFN-γ (R&D Systems, UK). The limit detection range was from 12.5 pg/ml to 1000 pg/ml for IFN-γ concentration. All tests were performed according to the manufacturer's instructions.

Intracellular FACS Analysis

Cells were exposed to magnetic fields as described. The cells were stained with PECy.5-labeled anti-CD45RA antibody; then they were washed twice in PBS with 1% FCS and fixed with PBS with 4% paraformaldehyde for 30 min at 4°C. Fixation was followed by permeabilization with PBS with 1% FCS, 0. 3% saponin and 0.1% Na-azide for 15 min at 4°C. Alternatively, staining for intracellular antigen was performed by incubating fixed permeabilized cells with FITC-labeled anti-Ki67 antibody and/or with PE-labeled anti-IFN-γ antibody (Becton Dickinson). After two more washes in PBS with 1% FCS, the cells were analyzed by flow cytometry. Viable lymphocytes were gated by forward and side scatter, and analysis was performed on 100,000 acquired events for each sample. Intracellular staining was also used to evaluate the cytosolic free calcium at the single-cell level in both exposed and sham-exposed cell samples after exposure to the 50 Hz magnetic fields and static magnetic fields. Cells were harvested and fixed with 4% (w/v) paraformaldehyde in PBS for 10 min at room temperature. Fixed cells were suspended and washed twice with a

TABLE 1
Levels of IFN-γ Released from CD4⁺ CD45RA⁻ T and CD4⁺ CD45RA⁺ T Cells Exposed to the 0.5 mT Static Magnetic Field or Sham-Exposed Cells after 24 h of Cell Culture

	IFN-γ⁺CD45RA⁻		IFN-γ⁺CD45RA⁺	
	Sham-exposed	Exposed	Sham-exposed	Exposed
RPMI 1640	38 ± 17	33 ± 13	34 ± 15	29 ± 19
RPMI 1640 + PHA	987 ± 98	**707 ± 60**[a]	221 ± 61	163 ± 64

[a] $P < 0.05$.
[b] Results are expressed as mean pg/ml ± SD for eight samples.

permeabilization buffer containing 0.1% saponin (Sigma-Aldrich, Milano, Italy), 1% heat-inactivated FCS, and 0.1% NaN_3 in PBS. The cells were then incubated in the presence of a permeabilization buffer with FITC-conjugated Calcein (Sigma-Aldrich, Milano, Italy) for 1 h at room temperature. After two more washes in PBS with 1% FCS, the cells were analyzed by flow cytometry. Viable lymphocytes were gated by forward and side scatter, and analysis was performed on 100,000 acquired events for each sample. The calcium concentration was monitored by single-excitation flow cytometry. The fluorescence was recorded at 490 nm excitation and 520 nm emission.

Statistics

The IFN-γ concentration was expressed as pg/ml and reported as means ± SD.

The proliferation index was expressed as a percentage of Ki-67-positive cells, and statistical evaluation of the data was performed using the stimulation index (median and 25th and 75th percentiles). The FACS analysis of intracellular IFN-γ was expressed as the percentage of positive cells, median and 25th and 75th percentiles.

Statistical evaluation of the experimental data was performed with a two-tailed Student's *t* test on paired samples with $P < 0.05$ as the minimum level of significance.

The calcein was expressed as mean fluorescence intensity (MFI) and reported as mean ± SD.

RESULTS

Release of IFN-γ from CD4⁺ CD45RA⁻ T and CD4⁺CD45RA⁺ Cells Exposed to 0.5 mT Static Magnetic Fields

We examined the sensitivities of CD4⁺ T-cell subsets to 0.5 mT in the different CD4⁺ T-cell lines used for the experiments.

Table 1 shows the absolute values in pg/ml of IFN-γ release from CD4⁺CD45RA⁻ T-cell subsets and CD4⁺CD45RA⁺ T-cell subsets exposed or sham-exposed to 0.5 mT static magnetic fields after 24 h of *in vitro* culture. A significant difference in the IFN-γ values was found only for the exposed CD4 ⁺ T cells stimulated with PHA compared to sham-exposed cells. The difference in the release of IFN-γ after 24 h was significant for the CD4⁺CD45RA⁻ T-cell subset exposed to the static magnetic fields compared to the sham-exposed cells, while no significant difference in IFN-γ release was found between exposed and nonexposed CD4⁺CD45RA⁺ T cells. Similar results were found in cells exposed to 50 Hz magnetic fields, which showed a 25% inhibition of IFN-γ release (data not shown). It is evident that CD4⁺CD45RA⁻ T cells are more sensitive to static magnetic fields in terms of IFN-γ release.

Release of IFN-γ from CD4⁺ CD45RA⁻ T and CD4⁺CD45RA⁺ T Cells Exposed to 0.5 mT 50 Hz Magnetic Fields

Figure 1 shows a comparison between the levels of IFN-γ released from CD4⁺, CD4⁺ CD45RA⁻ and CD4⁺ CD45RA⁺ T cells exposed to the 0.5 mT 50 Hz magnetic fields and their respective sham-exposed controls after 24 h of *in vitro* culture.

The amount of IFN-γ in CD4⁺ cells cultured in complete medium was produced in equal part by the CD4⁺ CD45RA⁻ and CD4⁺ CD45RA⁺ subsets in sham-exposed and exposed cells, and exposed CD4⁺ cells showed a small decrease in IFN-γ production (14%) compared to sham-exposed cells.

In cells stimulated with PHA, the amount of IFN-γ in CD4⁺ T cells was produced almost exclusively by the

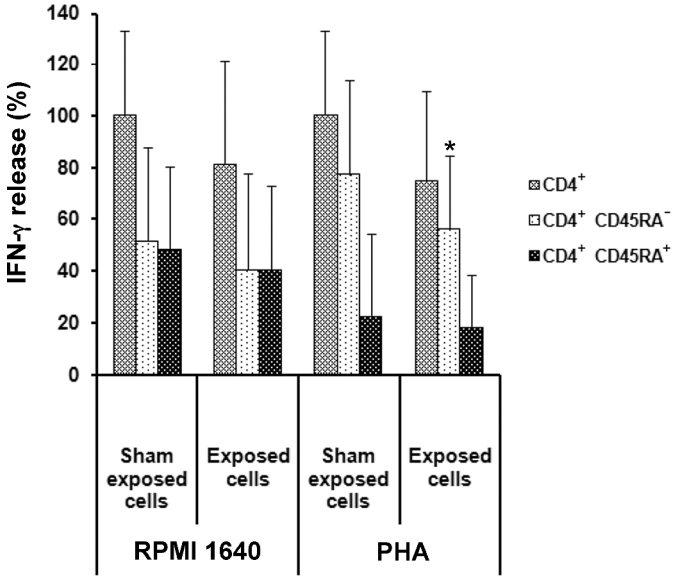

FIG. 1. IFN-γ release from CD4⁺, CD4⁺ CD45RA⁻ and CD4⁺ CD45RA⁺ T cells, sham-exposed or exposed for 2 h to 0.5 mT 50 Hz magnetic fields and stimulated or not with PHA after 24 h of cell culture in complete medium. Data are expressed as the percentage of release of exposed cells compared to controls. * $P < 0.05$. No. of samples: 8.

TABLE 2
Percentage of IFN-γ+CD4+ CD45RA− and IFN-γ+CD4+ CD45RA+ T Cells Exposed to 0.5 mT 50 Hz Magnetic Fields and Static Magnetic Fields and Sham-Exposed Cells after 24 h of Cell Culture

	50 Hz magnetic fields				Static magnetic fields			
	IFN-γ+CD45RA−		IFN-γ+CD45RA+		IFN-γ+CD45RA−		IFN-γ+CD45RA+	
	Sham-exposed	Exposed	Sham-exposed	Exposed	Sham-exposed	Exposed	Sham-exposed	Exposed
RPMI 1640	1.5 ± 1.0	1.0 ± 0.7	1.0 ± 0.8	0.9 ± 0.8	1.1 ± 0.5	1.0 ± 0.5	1.2 ± 0.3	1.2 ± 0.6
RPMI 1640 +PHA	3.4 ± 1.5	**2.1 ± 0.2**[a]	1.3 ± 1.1	1.1 ± 0.8	3.9 ± 1.0	**2.4 ± 0.6**[a]	3.6 ± 1.3	3.1 ± 1.2

[a] $P < 0.05$.
[b] Results expressed as means ± SD of eight samples.

$CD4^+$ $CD45RA^-$ subset; the $CD4^+$ $CD45RA^+$ subset produced only a small amount of IFN-γ. In exposed $CD4^+$ T cells stimulated with PHA, the amount of IFN-γ in the supernatants decreased by 25% compared to sham-exposed cells. In $CD4^+CD45RA^-$ T cells stimulated with PHA, the amount of IFN-γ in the supernatants decreased 21% in exposed cells compared to sham-exposed cells; no significant difference in IFN-γ release was observed in $CD4^+CD45RA^+$ T cells stimulated with PHA.

The results clearly indicate that the $CD4^+CD45RA^-$ subset was more sensitive to the release of IFN-γ upon subsequent exposure to 50 Hz magnetic fields.

To determine whether magnetic fields affect the PHA binding to the cell membrane, the cells were stimulated with PHA during and after exposure, washed to eliminate the unbound PHA, and incubated for 24 h. Exposure to the magnetic fields and static magnetic fields did not modify the ability of PHA to stimulate cells as evaluated by IFN-γ release.

Intracellular IFN-γ in $CD4^+$ $CD45RA^+$ and $CD4^+$ $CD45RA^-$ T Cells Exposed to 0.5 mT 50 Hz Magnetic Fields and Static Magnetic Fields after 24 h of In Vitro Culture

To determine whether the magnetic fields effect on IFN-γ release was dependent on a minor production of this cytokine, the percentage of IFN-γ-positive cells in exposed and sham-exposed cells stimulated or not with PHA was evaluated by flow cytometry. The results are shown in Table 2.

After the 50 Hz magnetic-field exposure, a significant difference in the percentage of IFN-γ+ $CD4^+$ T cells was observed only in the exposed $CD4^+CD4RA^-$ subset stimulated with PHA (Table 2).

After 48 h of culture, the percentages of intracellular IFN-γ+ $CD4^+CD45RA^-$ and $CD4^+CD45RA^+$ T cells in exposed or sham-exposed samples stimulated or not with PHA were not significantly different (data not shown).

The experiment was repeated, exposing the two T-cell subsets ($CD4^+CD4RA^-$ and $CD4^+CD45RA^+$) to a 0.5 mT static magnetic field. A significant difference in IFN-γ+ cells was observed after 24 h of *in vitro* culture only in the $CD4^+CD45RA^-$ subset stimulated with PHA after 2 h of exposure to the static magnetic field (Table 2).

After 48 h of culture, the percentages of intracellular IFN-γ+ cells in both the $CD4^+CD45RA^-$ and $CD4^+CD45RA^+$ subsets remained constant compared to the percentages found in the same subsets of cells exposed to the static magnetic field in either the sample stimulated with PHA or the sample cultured in RPMI 1640 medium as the control (data not shown).

In conclusion, we observed that there was a significant difference in the percentage of IFN-γ+ $CD4^+CD45RA^-$ T cells at 24 h in exposed cells compared to sham-exposed cells stimulated with PHA. Additional experiments are required to explain the enhanced effect of PHA in exposed cells.

Proliferation in $CD4^+$ T-Cell Subsets

Upon mitogenic stimulation after the exposure to the 0.5 mT 50 Hz magnetic field and static magnetic field, the proliferation of $CD4^+CD45RA^-$ and $CD4^+CD45RA^+$ T cells was also investigated by FACS analysis using the anti-Ki67 antibody.

The Ki67 protein is accumulated from G_1 phase to mitosis, where it reaches the highest levels (48 h after PHA stimulation). Cell proliferation was evaluated as the stimulation index and was expressed as the median percentage of cells expressing this protein.

The percentages of $Ki67^+$ $CD4^+$ $CD45RA^+$ and $CD4^+$ $CD45RA^-$ T cells in exposed samples and samples sham-exposed to the 50 Hz magnetic field or static magnetic field after 48 h of cell culture are shown in Table 3.

The results of the FACS analysis demonstrated that the stimulation index of exposed $Ki67^+CD4^+CD45RA^-$ T cells compared to sham-exposed cells regardless of the magnetic field applied. No significant differences in proliferation were observed in $CD4^+CD45RA^+$ T cells stimulated with PHA after exposure to the 0.5 mT 50 Hz ELF and static magnetic field (Table 3).

TABLE 3
Stimulation Index of Ki67$^+$CD4$^+$ CD45RA$^-$ and Ki67$^+$CD4$^+$ CD45RA$^+$ T Cells Exposed to 0.5 mT 50 Hz Magnetic Fields or Static Magnetic Fields and Sham-Exposed Cells after 48 h of Cell Culture

	50 Hz magnetic fields				Static magnetic fields			
	Ki67$^+$CD45RA$^-$		Ki67$^+$CD45RA$^+$		Ki67$^+$CD45RA$^-$		Ki67$^+$CD45RA$^+$	
	Sham-exposed	Exposed	Sham-exposed	Exposed	Sham-exposed	Exposed	Sham-exposed	Exposed
RPMI 1640	5.0 ± 2.2	4.1 ± 1.9	3.1 ± 1.1	1.8 ± 1.2	2.6 ± 0.9	1.4 ± 0.1	2.2 ± 2.0	1.4 ± 1.1
RPMI 1640 + PHA	11.5 ± 4.3	**8.3 ± 3.6**[a]	6.2 ± 2.2	4.5 ± 1.9	12.9 ± 6.8	**6.6 ± 3.2**[a]	11.5 ± 5.0	8.0 ± 3.0

[a] $P < 0.05$.
[b] Data are expressed as means ± SD of eight samples.

Intracellular Calcein in CD4$^+$ CD45RA$^-$ T and CD4$^+$ CD45RA$^+$ T Cells

To understand the mechanisms contributing to the differences in the rates of proliferation, the content of intracellular free calcium was evaluated as a biological indicator of viability/activation of cells, using anti-calcein antibody in CD4$^+$CD45RA$^-$ and CD4$^+$-CD45RA$^+$ T lymphocytes stimulated or not with PHA after 24 h of culture after exposure to magnetic fields. Eight samples were analyzed for each kind of magnetic field, and values are reported as the mean fluorescence intensity for each sample.

The free calcium concentrations after 24 h in subpopulations of CD4$^+$ T cells exposed or sham-exposed to the 0.5 mT 50 Hz magnetic field or static magnetic field are shown in Fig. 2. Exposure to the static magnetic field induced a significant decrease of cytosolic free-calcium in exposed CD4$^+$CD45RA$^-$ T cells compared to cells that were sham-exposed and stimulated with PHA (Fig. 2A). Similar results were obtained after exposure to 50 Hz magnetic fields; there was a slight decrease of cytosolic free calcium only in exposed CD4$^+$CD45RA$^-$ T cells stimulated with PHA compared to sham-exposed cells (Fig. 2B). These results indicate that the rate of cytosolic free calcium was decreased only in CD4$^+$CD45RA$^-$ T cells exposed to magnetic fields.

DISCUSSION

In this study, we focused on the effects of 50 Hz magnetic fields and static magnetic fields (0.5 mT) on the release/content of IFN-γ, on cell proliferation and on the modifications of intracellular calcium in subsets of human CD4$^+$ T cells divided on the basis of surface marker expression and their functional properties.

In the last 30 years, there has been an increasing public concern about the possible harmful effects of magnetic fields generated by power lines and domestic appliances. The omnipresence of the 50 Hz magnetic fields from domestic electricity sources in modern society makes it important to investigate possible deleterious health effects from exposure. In our previous investigations (*11*), we found evidence that 50 Hz magnetic fields affected the expression of surface markers, the release/content of IFN-γ, cell proliferation and intracellular calcium in CD4$^+$ T cells. The mechanism by which the magnetic fields affect the lymphocyte response has not been fully elucidated. However, the release/content of cytokines and the proliferative activity of lymphocyte can be affected by the magnetic fields at several levels of signal transduction (*15, 20*). Such processes involve activation of numerous enzymes associated with different signaling pathways (*21*) that may be affected by the magnetic

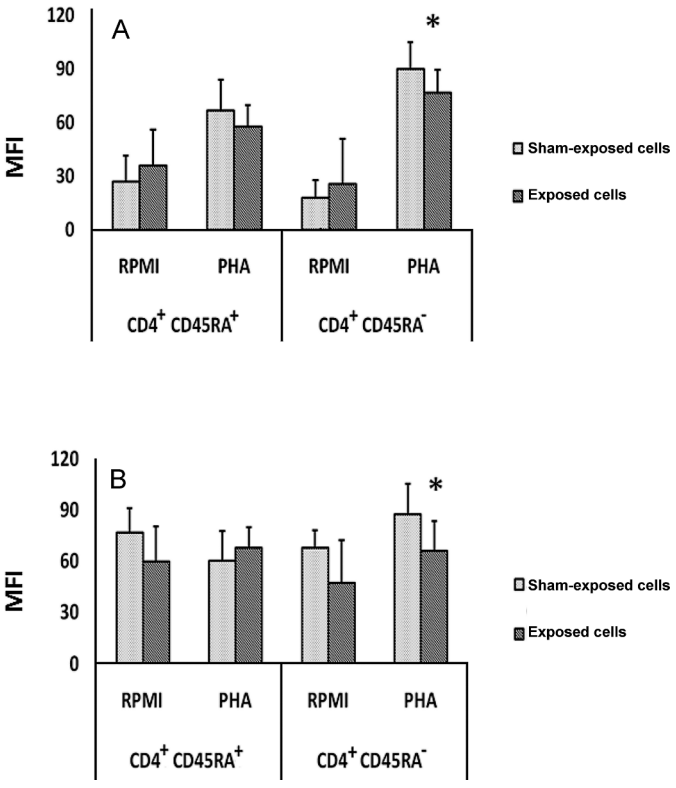

FIG. 2. Mean fluorescence intensity (MFI) of calcein in CD4$^+$CD45RA$^-$ and CD4$^+$CD45RA$^+$ T cells exposed to static magnetic fields (panel A) or 50 Hz magnetic fields (panel B) and sham-exposed after 24 h of cell culture. Data are expressed as the mean fluorescence intensity of exposed cells compared to controls. * $P < 0.05$. No. of samples: 8.

fields. Bersani *et al.* (*22*) reported that magnetic fields induced the clustering of large intramembrane proteins, for example, receptors, enzymes and ion channels.

Static magnetic fields are time-independent fields of constant strength. As opposed to electrostatic fields, static magnetic fields are difficult to shield and can penetrate biological tissues freely. They interact directly with moving charges (ions, proteins, etc.) and magnetic materials found in tissue through several physical mechanisms (*23*). Static magnetic fields are one of the key components of magnetic resonance imaging (MRI), which has been introduced in many hospitals as a useful diagnostic tool because it does not cause any injury or fatigue.

We observed that 0.5 mT 50 Hz magnetic fields or static magnetic fields induced a decrease in the release and content of IFN-γ in $CD4^+CD45RA^-$ cell subsets. This alteration was more evident at 24 h for both magnetic fields. The release of IFN-γ from exposed $CD4^+CD45RA^-$ T cells was significantly decreased compared to that released by $CD4^+CD45RA^-$ T cells that were sham-exposed and stimulated with PHA.

FACS analysis showed that the $CD4^+CD45RA^-$ T-cell population is the most sensitive to the magnetic fields (50 Hz or static magnetic fields). The content of IFN-γ was significantly decreased in this subset after 24 h of exposure to the magnetic fields. However, this decrease was reversed after 48 h of cell culture, when the difference in the percentage of IFN-γ^+ cells in exposed and sham-exposed cells was not significant.

We also observed a decrease in cell proliferation from exposure to both magnetic fields in the $CD4^+CD45RA^-$ subset after 48 h. Several approaches have been developed to study cell proliferation, including the labeling index, measurement of the S-phase fraction, the cytosolic TK assay, and the Ki-67 marker. In our study, cell proliferation was evaluated using an anti-Ki-67 monoclonal antibody (*24*). The Ki-67 molecule is the most widely used marker for proliferation in clinical practice; it is expressed in all active phases of the cell cycle and is absent in quiescent cells. The analysis of intracellular calcium showed that the $CD4^+CD45RA^-$ T-cell subset was more sensitive to the magnetic fields after 24 h of cell culture. In this cell subset, the free calcium concentration was higher in sham-exposed cells than in exposed cells.

The calcium ion is an important intracellular signal that is involved in many cellular pathways. It is associated with lymphocyte activation (*10, 25*), and many studies have reported the biological effects of static or pulsed magnetic fields on membrane ion transport. The influence of magnetic fields on the movement of calcium ion in the context of membranes could explain the diversity and variety of cellular responses observed in cell proliferation, cell differentiation and release of cytokines.

The results reported here, show that the subset most sensitive to the effects of the 50 Hz magnetic fields and static magnetic fields is $CD4^+CD45RA^-$ and that magnetic fields have a significant influence on cells stimulated with PHA.

We found that 0.5 mT magnetic fields produced a significant effect only in activated cells, probably because the PHA stimulus amplifies the influence of magnetic fields on $CD4^+CD45RA^-$ T cells. However, the effect of magnetic fields is transient and is completely reversed after 48 h in the case of the 50 Hz magnetic fields and static magnetic fields.

We do not believe that the magnetic fields affect any particular pathway but rather that they influence all cell activities and that these effects are amplified in cells stimulated with PHA. This peculiarity could suggest the use of magnetic fields to support the traditional therapies for chronic degenerative pathologies in which subsets of the immune system are involved. We hypothesize that the use of magnetic fields for modulation of the role of $CD4^+$ T cells and cytokine release in immune response could be used to develop new therapeutic strategies in medicine.

Our results do not explain why both magnetic fields influence only one subset of the $CD4^+$ T-cell population; only the $CD4^+$ $CD45RA^-$ subpopulation appears to produce the bulk of IFN-γ after PHA stimulation (Fig. 1). This interpretation is not fully supported by data on the number of IFN-γ^+ cells in the two subpopulations. The results suggest that 50 Hz magnetic fields and static magnetic fields delay IFN-γ release by the $CD4^+$ $CD45RA^-$ subpopulation, which appears to be more sensitive to the magnetic fields effects in terms of cell proliferation rate. The reduced number of IFN-γ-positive cells in the $CD45RA^-$ subpopulation supports this hypothesis. Further studies are required to determine the influence of different kinds of magnetic fields or electromagnetic fields like very high-frequency (VHF) magnetic fields on the immune system and to evaluate the biological effects of electric fields in combination with magnetic fields.

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REFERENCES

1. IARC, *Non-Ionizing Radiation, Part 1: Static and Extremely Low-Frequency (ELF) Electric and Magnetic Fields. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 80, IARC, Lyon, 2002.

2. *Possible Health Effects from Terrestrial Trunked Radio (TETRA): Report of an Advisory Group on Non-ionising Radiation*. Documents of the NRPB, Vol. 12, No. 2, NRPB, Chilton, UK, 2001.
3. A. Cossarizza, D. Monti, F. Bersani, R. Paganelli, G. Montagnani, R. Cadessi, M. Cantini and C. Franceschi, Extremely low frequency pulsed electromagnetic fields increase interleukin-2 (IL-2) utilization and IL-2 receptor expression in mitogen-stimulated human lymphocytes from old subjects. *FEBS Lett*. **248**, 141–144 (1989).
4. B. Yokus, M. Z. Akdag, S. Dasdag, D. U. Cakir and M. Kizil, Extremely low frequency magnetic fields cause oxidative DNA damage in rats. *Int. J. Radiat. Biol*. **84**, 789–795 (2008).
5. Y. Wei, H. Xiaolin and S. Tao, Effects of extremely low-frequency-pulsed electromagnetic field on different-derived osteoblast-like cells. *Electromagn. Biol. Med*. **27**, 298–311 (2008).
6. M. T. Santini, A. Ferrante, G. Rainaldi, P. Indovina and P. L. Indovina, Extremely low frequency (ELF) magnetic fields and apoptosis: a review. *Int. J. Radiat. Biol*. **81**, 1–11 (2005).
7. International Commission on Non-Ionizing Radiation Protection, Guidelines on limits of exposure to static magnetic fields. *Health Phys*. **66**, 100–106 (1994).
8. International Non-ionizing Radiation Committee of the International Radiation Protection Association, Interim guidelines on limits on exposure to 5060 Hz electric and magnetic fields. *Health Phys*. **58**, 113122 (1990).
9. C. Aldinucci, J. Garcia Blanco, M. Palmi, G. Sgaragli, A. Benocci, A. Meini, F. Pessina, C. Rossi, C. Bonechi and G. P. Pessina, The effect of exposure to high flux density static and pulsed magnetic fields on lymphocyte function. *Bioelectromagnetics* **24**, 373–379 (2003).
10. E. M. Goodman, B. Greenebaum and M. T. Marron, Effects of electromagnetic fields on molecules and cells. *Int. Rev. Cytol*. **158**, 279–338 (1995).
11. S. Salerno, C. La Mendola, A. Lo Casto, G. Mamone, N. Caccamo, A. E. Cardinale and A. Salerno, Reversible effect of MR and ELF magnetic fields (0.5 T and 0.5 mT) on human lymphocyte activation patterns. *Int. J. Radiat. Biol*. **82**, 77–85 (2006).
12. M. Simkó and M. O. Mattsson, Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: possible immune cell activation. *J. Cell Biochem*. **93**, 83–92 (2004).
13. C. A. Morehouse and R. D. Owen, Exposure of Daudi cells to low-frequency magnetic fields does not elevate MYC steady-state mRNA levels. *Radiat. Res*. **153**, 663–669 (2000).
14. K. R. Foster, Mechanisms of interaction of extremely low frequency electric fields and biological systems. *Radiat. Prot. Dosimetry* **106**, 301–310 (2003).
15. C. Aldinucci, J. Garcia Blanco, M. Palmi, G. Sgaragli, A. Benocci, A. Meini, F. Pessina, C. Rossi, C. Bonechi and G. P. Pessina, The effect of strong static magnetic fields on lymphocytes. *Bioelectromagnetics* **24**, 109–117 (2003).
16. M. Ruggiero, D. P. Bottaro, G. Liguri, M. Gulisano, B. Peruzzi and S. Pacini, 0.2 T magnetic field inhibits angiogenesis in chick embryo chorioallantoic membrane. *Bioelectromagnetics* **25**, 390–396 (2004).
17. H. Okano, Effects of static magnetic fields in biology: role of free radicals. *Front. Biosci*. **13**, 6106–6125 (2004).
18. H. Okano, N. Tomita and Y. Ikada, Effects of 120 mT static magnetic field on TGF-beta1-inhibited endothelial tubular formation in vitro. *Bioelectromagnetics* **28**, 497–499 (2007).
19. X. Saulquin, C. Ibisch, M. A. Peyrat, E. Scotet, M. Hourmant, H. Vie, M. Bonneville and E. Houssaint, A global appraisal of immunodominant $CD8^+$ T cell responses to Epstein-Barr virus and cytomegalovirus by bulk screening. *Eur. J. Immunol*. **30**, 2531–2539 (2000).
20. P. Boscolo, A. Bergamaschi, M. B. Di Sciascio, F. Benvenuti, M. Reale, F. Di Stefano, P. Conti and M. Di Gioacchino, Effects of low frequency electromagnetic fields on expression of lymphocyte subsets and production of cytokines of men and women employed in a museum. *Sci. Total Environ*. **270**, 13–20 (2001).
21. R. B. Frankel and R. P. Liburdy, Biological effects of static magnetic fields. In *Handbook of Biological Effects of Electromagnetic Fields*, 2nd ed. (C. Polk and E. Postow, Eds.), pp. 149–183. CRC Press, Boca Raton, FL, 1996.
22. F. Bersani, F. Marinelli, A. Ognibene, A. Matteucci, S. Cecchi, S. Santi, S. Squarzoni and N. M. Maraldi, Intramembrane protein distribution in cell cultures is affected by 50 Hz pulsed magnetic fields. *Bioelectromagnetics* **18**, 463–469 (1997).
23. *Magnetic Fields Health and Safety Guide*. Health and Safety Guide No. 27, WHO, Geneva, 1989.
24. A. Roman, T. Zyss and I. Nalepa, Magnetic field inhibits isolated lymphocytes, proliferative response to mitogen stimulation. *Bioelectromagnetics* **26**, 201–206 (2005).
25. J. J. L. Carson, F. S. Prato, D. J. Drost, L. D. Diesbourg and S. J. Dixon, Time varying magnetic fields increase cytosolic free Ca^{+2} in HL-40 cells. *Am. J. Physiol*. **259**, C687–C692 (1990).