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Effects of Exposure to Gradient Magnetic Fields Emitted by Nuclear Magnetic Resonance Devices on Clonogenic Potential and Proliferation of Human Hematopoietic Stem Cells

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This study investigates effects of gradient magnetic fields (GMFs) emitted by magnetic resonance imaging (MRI) devices on hematopoietic stem cells. Field measurements were performed to assess exposure to GMFs of staff working at 1.5 T and 3 T MRI units. Then an exposure system reproducing measured signals was realized to expose in vitro CD34+ cells to GMFs (1.5 T-protocol and 3 T-protocol). CD34+ cells were obtained by Fluorescence Activated Cell Sorting from six blood donors and three MRI-exposed workers. Blood donor CD34+ cells were exposed in vitro for 72 h to 1.5 T or 3 T-protocol and to sham procedure. Cells were then cultured and evaluated in colony forming unit (CFU)-assay up to 4 weeks after exposure. Results showed that in vitro GMF exposure did not affect cell proliferation but instead induced expansion of erythroid and monocytes progenitors soon after exposure and for the subsequent 3 weeks. No decrease of other clonogenic cell output (i.e., CFU-granulocyte/erythroid/macrophage/megakaryocyte and CFU-granulocyte/macrophage) was noticed, nor exposed CD34+ cells underwent the premature exhaustion of their clonogenic potential compared to sham-exposed controls. On the other hand, pilot experiments showed that CD34+ cells exposed in vivo to GMFs (i.e., samples from MRI workers) behaved in

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Luciana Teofili and Claudio Grassi shared leadership of the study and contributed equally to the work reported.

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culture similarly to sham-exposed CD34+ cells, suggesting that other cells and/or microenvironment factors might prevent GMF effects on hematopoietic stem cells in vivo. Accordingly, GMFs did not affect the clonogenic potential of umbilical cord blood CD34+ cells exposed in vitro together with the whole mononuclear cell fraction. Bioelectromagnetics. 37:201–211, 2016.

Key words: CD34+ cells; clonogenic potential; complex magnetic field waveforms; occupational exposure assessment

INTRODUCTION

In recent years, concerns have been raised on possible adverse effects of the numerous electromagnetic sources present in the daily life and work environments [Santini et al., 2005; Desai et al., 2009; Belyaev et al., 2015]. Several lines of evidence indicate that electromagnetic waves markedly impact on cell biology and different effects have been attributed to non-ionizing radiations depending on their frequencies and intensities [Funk et al., 2009]. Extensive literature has focused on effects of electromagnetic fields (EMFs) on stem cells of various types given their self-renewal, multipotency, and proliferative capacity that potentially confer them greater susceptibility to oxidative, biochemical and microenvironmental changes induced by intrinsic or exogenous stimuli. As such, EMFs have been suggested as promising tools to positively influence different steps of neurogenic and osteogenic processes [Boyette and Herrera-Soto, 2012; Bai et al., 2013; Di Lazzaro et al., 2013; Ongaro et al., 2014; Podda et al., 2014; Leone et al., 2014, 2015]. On the other hand, epidemiological reports, although controversial, suggest a possible association between exposure to EMF and frequency of childhood leukemia [IARC, 2001; Calvente et al., 2010] thus prompting studies evaluating effects of EFs on human hematopoietic stem/progenitor cells [Nafziger et al., 1997; Reipert et al., 1997; Monzen et al., 2009].

A relevant source of non-ionizing radiation is represented by diagnostic equipment. Recent advances in technologies using static magnetic fields and, especially, in magnetic resonance imaging (MRI), have increased amplitude of static magnetic fields used for research, diagnostics and medical applications from 1.5 to over 7 Tesla (T) and this has raised relevant health issues for MRI staff [Schenck et al., 1992; de Vocht et al., 2006; Wilén and De Vocht, 2011; Schaap et al., 2014b]. In particular, acute transient symptoms including vertigo, nausea, and metallic taste have been described following exposure to high-intensity static magnetic fields and motion-induced time-varying magnetic field to which workers are exposed when moving through spatial gradients of static magnetic stray field around an MRI scanner. However, cellular mechanisms underlying these acute transient effects have not been elucidated yet. Furthermore, biological effects derived from chronic exposure to static magnetic fields are even far less understood.

Concerns regarding occupational exposure in MRI facilities have also become a recent focus in the International Committee of Non-Ionizing Protection (ICNIRP) Guidelines [ICNIRP, 2009, 2010, 2014] and European Union (EU) Directive 2013/35/EC, thus confirming relevance of these issues for occupational and public health.

So far various studies have been carried out to measure and accurately define exposure levels of individual workers at MRI facilities [Jonsson and Barregård, 1996; Decat, 2007; Fuentes et al., 2008; Glover and Bowtell, 2008; Karpowicz and Gryz, 2013; Schaap et al., 2014a; Yamaguchi-Sekino et al., 2014]. Of relevance to our study, it has been reported that in 1 T and 3 T MRI scanners, significant gradient fringe fields exist just out of the bore where healthcare workers may have access. Of note, even if exposures to gradient magnetic fields can exceed ICNIRP [1998] reference levels, they are compliant with ICNIRP [2010] ones [Bradley et al., 2007; Andreuccetti et al., 2013]. In general, fast sequences, as Echo Planar Imaging (EPI), have higher peak dB/dt, thus rarely exceeding the latest ICNIRP reference level outside the bore [McRobbie, 2012].

Within this scenario, the present study was aimed at assessing whether occupational exposure to gradient magnetic fields (GMFs) emitted by MRI scanners causes biological changes in human cells. In particular, as measurable endpoints, cell proliferation, and clonogenic potential of human CD34+ hematopoietic stem cells were evaluated following in vitro exposure to GMFs obtained by using a customized exposure system able to reproduce gradient signals measured during MRI routine diagnostic exams performed in a pediatric hospital with two different MRI scanners (1.5 T and 3 T). Indeed, several kinds of healthcare workers (e.g., radiology technicians, radiologists, anesthetists, surgeons, and anesthesiologist) can be exposed to GMFs, when they stay near

the MRI scanner during image acquisition. In particular, examinations carried out on children requiring constant assistance by medical/paramedical staff significantly expose these health care workers to GMFs generated by MRI.

To provide a proof of principle of validity of our model for future studies investigating biological effects of GMF occupational exposure, we performed pilot experiments to evaluate proliferation and clonogenic potential of CD34+ cells isolated from whole blood units donated by two radiologists and one anesthesiologist working at the selected hospital MRI facilities and therefore exposed to the same GMFs we used for our in vitro experiments.

The study was approved by the Ethics Committee of Bambino Gesù Children's Hospital (Rome, Italy) and of Catholic University and was fully compliant with European and Italian Legislation concerning studies involving human subjects (Good Clinical Practice CPMP/ICH/135/95; Ministerial Decree of July 15, 1997).

MATERIALS AND METHODS

MRI Gradient Signal Acquisition

To investigate the effects of occupational exposure to GMFs emitted by MRI scanners, an exposure system able to reproduce gradient signals actually used during MRI exams was designed and realized. GMFs, produced by gradient coils, were firstly acquired through field measurements [Andreuccetti et al., 2013]; then a procedure to reproduce measured signals by means of a system of coils was set up as in Lodato et al. [2013].

Switched gradient B-fields produced by 1.5 T (Achieva, Royal Philips Amstelplein, Amsterdam, Netherlands) and 3 T (Magnetom Skyra, Siemens, Munich, Germany) whole-body scanners were acquired in measurement surveys aimed at assessing occupational exposure. Procedures followed for measurements and data analysis have been previously described [Andreuccetti et al., 2013]. Measurement set-up consisted of a B-field meter (Narda ELT 400, Narda, Pfullingen, Germany), equipped with a three-axial field probe (bandwidth from 1 Hz to 400 kHz), a data acquisition (DAQ) device (Agilent U2531A, Agilent Technologies, Santa Clara, CA) and a notebook running a purposely developed software (Labview version 9.1, National Instruments, Austin, TX) for data acquisition and storage.

Measurement points were chosen with particular attention to positions occupied by medical staff near the scanner during diagnostic exams. Measurement points were set at three different heights from the ground (70, 120, and 170 cm, respectively) with a distance of 50 cm from the gantry and 67 cm from the center of the couch. In each measurement point, several pulsed B-field signals, produced by gradient coils during implementation of a commonly used clinical sequence, were acquired and analyzed according to "weighted peak method" described in ICNIRP [2010]: a weighted peak index (WPI) was evaluated for each gradient signal, compliance is ensured provided that a WPI not exceeding 1 is achieved.

Exposure System Design

In vitro exposure was carried out using a device previously described [Lodato et al., 2013]. Briefly, a couple of square coils (side 32 cm) were designed with a mutual distance of 15 cm, in order to achieve a $270 \times 270 \times 136\,mm^3$ nominal volume, suitable for simultaneous in vitro exposure of a large number of samples to a B-field in horizontal direction. Each coil consists of two separate stranded copper wires, wrapped in parallel to realize either a real or a sham exposure, by feeding cables with currents flowing in parallel (real) and in anti-parallel (sham) directions. In this way, sham samples, referred to as "controls," undergo to the same biological treatment as exposed ones, with the exception of the B-field itself. The coil's size allows to place the system inside a standard incubator, to assure suitable environmental conditions (i.e., 37 °C in 5% CO_2 atmosphere) for biological samples during in vitro exposures.

As B-field inside a coil is proportional to current flowing in the windings, the measured voltage signal has to be processed to obtain a new signal, feeding coils, which allows to null the coil impedance effect. To this aim, coils' impedance was measured as function of frequency; a digital filter was implemented to correct distortions due to the same impedance.

Samples

Peripheral blood samples were obtained after informed consent from six blood donors from general population (male subjects aged 40–60 years) and from three blood donors working at selected MRI facilities (i.e., two radiologists and one anesthesiologist; male subjects aged 45–60 years). In all cases, 450 ml of whole blood was drawn into citrate-phosphate-dextrose (CPD)/SAG-Mannitol quadruple-bag blood container system (Fenwal, La Chatre, France). After storage at 2–4 °C for a maximum of 6 h, blood bags were centrifuged at 3200 rpm for 18 min at room temperature. Leucocyte buffy coats were then

recovered using automated equipment (Optipress, Fenwal) and utilized for CD34+ cell sorting. Umbilical cord blood samples ($n = 3$) were obtained at the Unicatt Cord Blood Bank (Catholic University, Rome, Italy) after normal full term deliveries, according to institutional guidelines and after informed consent. Umbilical cord blood samples were collected in CPD and maintained at 2–6 °C until use, for a maximum of 24 h.

Occupational exposure of enrolled workers was assessed using a self-compiled questionnaire containing questions about type of MRI, length of exposure in their career, exposure to particular stressing conditions in the environment and general health. We also considered results of our previous radiometric measurements of healthcare worker exposure to MRI devices.

Based on this information, we considered our workers significantly exposed to GMFs generated by MRI. Particularly pediatric anesthesiologists are subjected to much real exposure, because they assist sedated patients during MRI examination, similar to an interventional MRI investigation.

Information regarding blood donors from general population was obtained from standard questionnaire for blood donation. None of them had occupational exposure to MRI, therefore, they were considered not-exposed subjects in our study.

Peripheral Blood and Umbilical Cord Blood CD34+ Cell Selection

Mononuclear cells (MNCs) were isolated from leukocyte buffy coats (blood donors and MRI-exposed workers) or by cord blood samples by sucrose gradient centrifugation (Histopaque SIGMA-1077, Milan, Italy). MNCs from blood donors and MRI exposed workers were then enriched in the CD34+ fraction by negative depletion of lineage-positive cells (Lineage Cell Depletion Kit, Miltenyi Biotec, Bergisch Gladbach, Germany). The CD34+ cell-enriched population was then purified by Fluorescence Activate Cell Sorting (BD FACS Aria III), according to manufacturer's protocol, thus obtaining a pure population of CD34+ stem cells. Median purity of CD34+ cells so obtained was 90.9% (range 78.8–99.7%).

Umbilical cord blood MNCs were subjected to CD34+ selection by immuno-magnetic method after GMF exposure (see below). The EasySep Human CD34 Positive Selection Kit (Stemcell Technologies, Vancouver, Canada) was used according to manufacturer's instruction, as previously described [Teofili et al., 2015]. Purity of CD34+ cell population so obtained was always above 92%.

CD34+ Cell Exposure to GMFs: 1.5 T-Protocol and 3 T-Protocol

CD34+ cells were exposed to GMFs by using the device described above. Two identical exposure devices were used to run in parallel sham and real GMF experiments. Devices were located in two different standard CO_2 incubators (Sanyo, Electric Biomedical Co., Osaka, Japan and Thermo Electron Corporation, Marietta, OH) and each experimental condition randomly performed in one of the two incubators.

Sorted CD34+ cells were suspended in Iscove Modified Dulbecco's Medium (IMDM) 10% Fetal Bovine Serum (FBS) at 5×10^4/well in 96 well-plates. Cell samples were divided in two aliquots: one of them was incubated for 72 h at 37 °C in a 5% CO_2 humidified atmosphere incubator containing the device generating GMF; control samples underwent sham stimulation under the same conditions. Effects of two different GMF exposure protocols were investigated, mimicking exposure of healthcare workers, and patients at two different MRI scanners (1.5 T and 3 T MRI scanners), thereafter named "1.5 T-protocol" and "3 T-protocol."

Regarding umbilical cord blood samples, the whole MNC fraction was exposed to GMFs and CD34+ cells were isolated after GMF exposure. In this case, cells were suspended in IMDM 10% FBS at 10×10^6/ml in 75 cm^2 tissue culture flask.

Cell Cultures

CD34+ cells were counted and seeded in 48-well-plates at 2×10^4 cells/well in Stem Span serum free medium (Stemcell Technologies Ottawa, Canada) containing Stem Cell factor 100 ng/ml, GM-CSF 100 ng/ml, Flt3-ligand 100 ng/ml, Thrombopoietin 50 ng/ml (all purchased from Miltenyi Biotec, Bergisch Gladbach Germany) to maintain the CD34+ cells in their undifferentiated status.

In the 1.5 T-protocol cells were maintained in culture for 1 week and CFU assay was performed soon after the exposure and after 1 week of culture. In the 3 T-protocol CD34+ cells were maintained in culture for 4 weeks. Once a week cells were collected and counted using trypan blue dye exclusion method (Sigma–Aldrich, Milan, Italy) to assess cell proliferation. Cells were then suspended again in fresh medium at 2×10^4 cells/well and aliquots were plated in methylcellulose-based medium (1×10^3/ml MethoCult, Stemcell Technologies Ottawa, Canada) for CFU assay. After 2 weeks, colonies were enumerated according to their morphology as burst forming unit-erythroid (BFU-E), colony forming unit-macrophage

(CFU-M), CFU-granulocyte/macrophage (CFU-GM), and CFU-granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM).

CD34+ cells obtained from MRI workers (in vivo GMF exposure) were treated and studied as those that underwent 3 T-protocol. In particular, after CD34+ cell sorting and before starting the clonogenic assay, cells were suspended in IMDM, 10% FBS medium and maintained for 72 h in 5% CO_2 humidified atmosphere [Grassi et al., 1994].

On the whole, three blood donors were evaluated in the 1.5 T-protocol (each experiment was performed in triplicate) and three blood donors were evaluated in the 3 T-protocol (two experiments were performed in duplicate and one in triplicate). All experiments on MRI workers were performed in triplicate.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA). Statistical comparison of continuous variables was performed with the Mann–Whitney U test or Student's t-test, as appropriate. Data are expressed as means ± standard errors of the means (SEM). Level of significance was set at 0.05.

RESULTS

GMF Exposure Sequences

Gradient signals with maximum WPIs were chosen among different signals, measured near 1.5 T and 3 T scanners, to be reproduced by the exposure system we used for in vitro experiments. Specifically, the chosen sequences are two EPI signals with different rise times: 200 μs for the EPI signal measured near the 1.5 T scanner, and 270 μs for EPI signal measured near the 3 T scanner. Maximum amplitude of B-field, measured in positions occupied by medical staff, was about 50 μT for both EPI signals; corresponding maximum computed WPI was about 0.14, compliant with international safety guidelines. The time behavior (a) of a spatial component of the EPI signal, measured near the 3 T scanner, and the frequency spectrum (b) are shown in Figure 1.

To check accuracy of the gradient B-field signal reproduced by our exposure system, with respect to the measured one, cross correlation between measured and reproduced signals was evaluated: this was equal to 0.99, indicating excellent reproduction of gradient B-field inside exposure setup. Maximum value of the B-field reproduced by the exposure system was about 100 μT for both EPI signals, corresponding to maximum B-field amplitude measured in close proximity of bores of both scanners (1.5 T and 3 T) during surveys, thus representing a worst case exposure scenario for in vitro experiments. For sham exposures, a residual B-field of about 0.4 μT (RMS) was measured within coils, which is comparable to the background B-field due to incubator electronics.

In Vitro Exposure to 1.5 T-Protocol Promotes Expansion of Erythroid and Monocyte Progenitor Cells

We first investigated the effect of 1.5 T-protocol on proliferation and clonogenic cell output of peripheral blood CD34+ cells obtained from general population (blood donors). GMFs had no effects on cell proliferation as evaluated after 72 h exposure (23 ± 8 and $21 \pm 6 \times 10^3$ cells/ml in controls and GMF samples, respectively, $P = 0.711$) and after 7 days of culture (372 ± 126 and $391 \pm 69 \times 10^3$ cells/ml control and GMF samples, respectively, $P = 0.842$; Fig. 2a). Nevertheless, GMF samples displayed a significant higher output of clonogenic cells (total CFU) than controls (30 ± 4 vs. 19 ± 2 CFU/1000 cells, respectively, $P = 0.004$; Fig. 2b). These figures resulted from the selective expansion of monocytes and erythroid progenitor cells after GMF exposure, as demonstrated by the greater yield of BFU-E and CFU-M in GMF-exposed samples (GMF samples: 71 ± 5 BFU-E and 30 ± 5 CFU-M; sham-exposed controls: 39 ± 4 BFU-E and 16 ± 2 CFU-M; $P = 0.001$ and 0.004 vs. BFU-E and CFU-M in controls, respectively; Fig. 2c and d). GMF effects were no more detectable after 7 days of culture (131 ± 25 vs. 116 ± 17 control BFU-E/1000 cells and 19 ± 3 vs. 21 ± 2 control CFU-M/1000 cells; Fig. 2c and d). GMF effect was not accompanied by any alteration in CFU-GEMM or CFU-GM progenitors, which were produced at comparable levels in sham-exposed control and GMF samples, both at baseline and after 7 days of culture (Fig. 2e and f).

In Vitro Exposure to 3 T-Protocol Causes Long-Lasting Expansion of Clonogenic Progenitors Without Affecting Proliferative Potential of CD34+ Cells

We then investigated effects of a different GMF sequence, that is, 3 T-protocol. As for the 1.5 T-protocol, cells were cultured and exposed to GMFs for 72 h. In this set of experiments, we checked whether expansion of clonogenic potential produced by GMF exposure resulted in premature exhaustion of clonogenic potential of CD34+ cells. For this purpose, CD34+ cells were maintained in culture up to 4 weeks and clonogenic cells were weekly evaluated. As shown in Figure 3a, we found that also the 3 T-protocol had no significant effect on proliferative

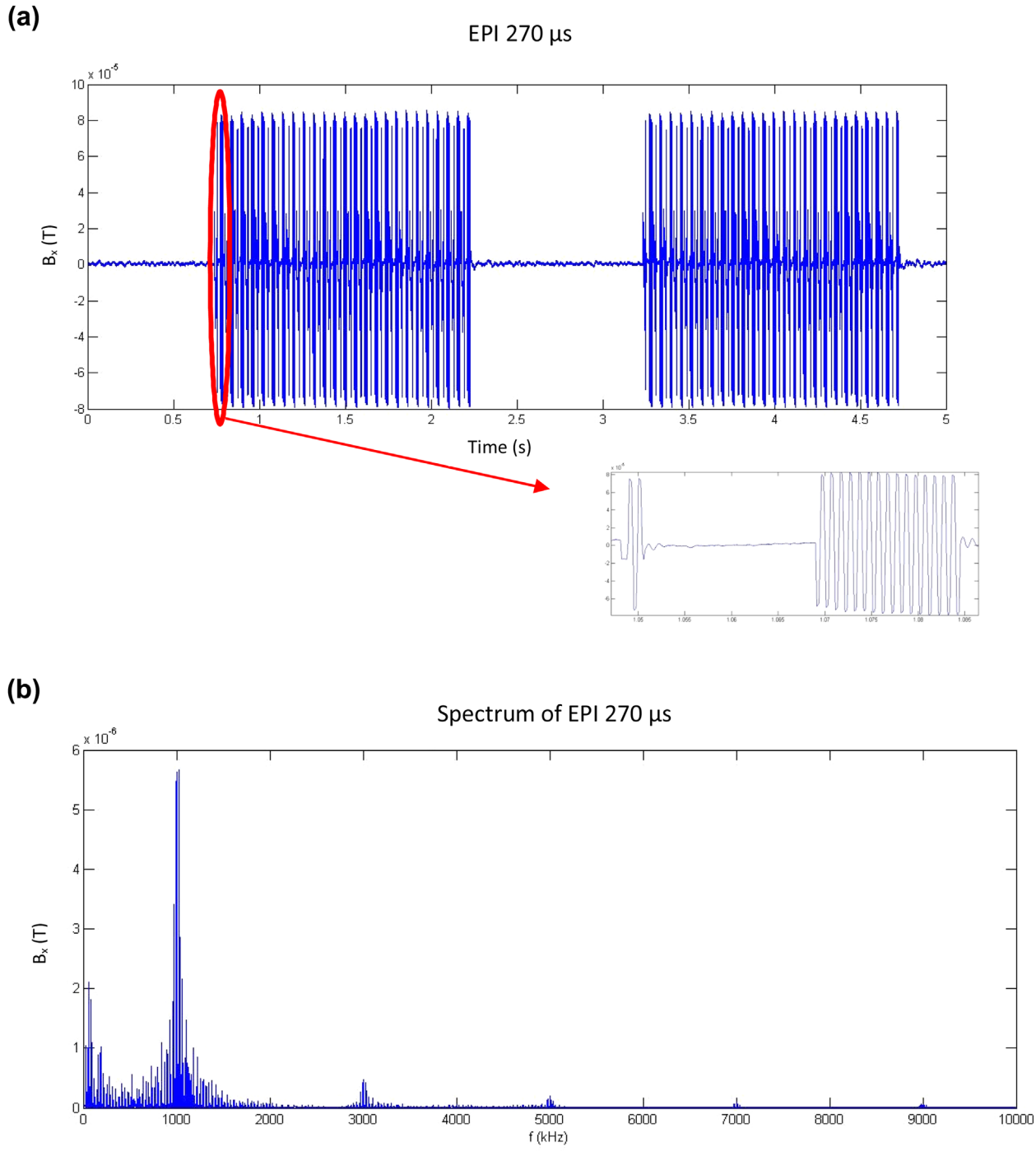

Fig. 1. Spatial component along x direction (B_x) of EPI signal, measured near 3 T scanner: time behavior (**a**) and frequency spectrum (**b**).

activity of CD34+ cells. Moreover, we confirmed that GMF exposure increased clonogenic potential of CD34+ cells. In fact, total CFU output per 1,000 cells in GMF-exposed samples was from 1.4- to 3-folds higher than in controls (Fig. 3b). This finding was mainly due to increased commitment of CD34+ cells towards the erythroid and monocytes lineages (Fig. 3c and d). We also found that effects of GMF 3 T-protocol persisted over time, being detectable up to 2 weeks after exposure. In fact, both BFU-E and

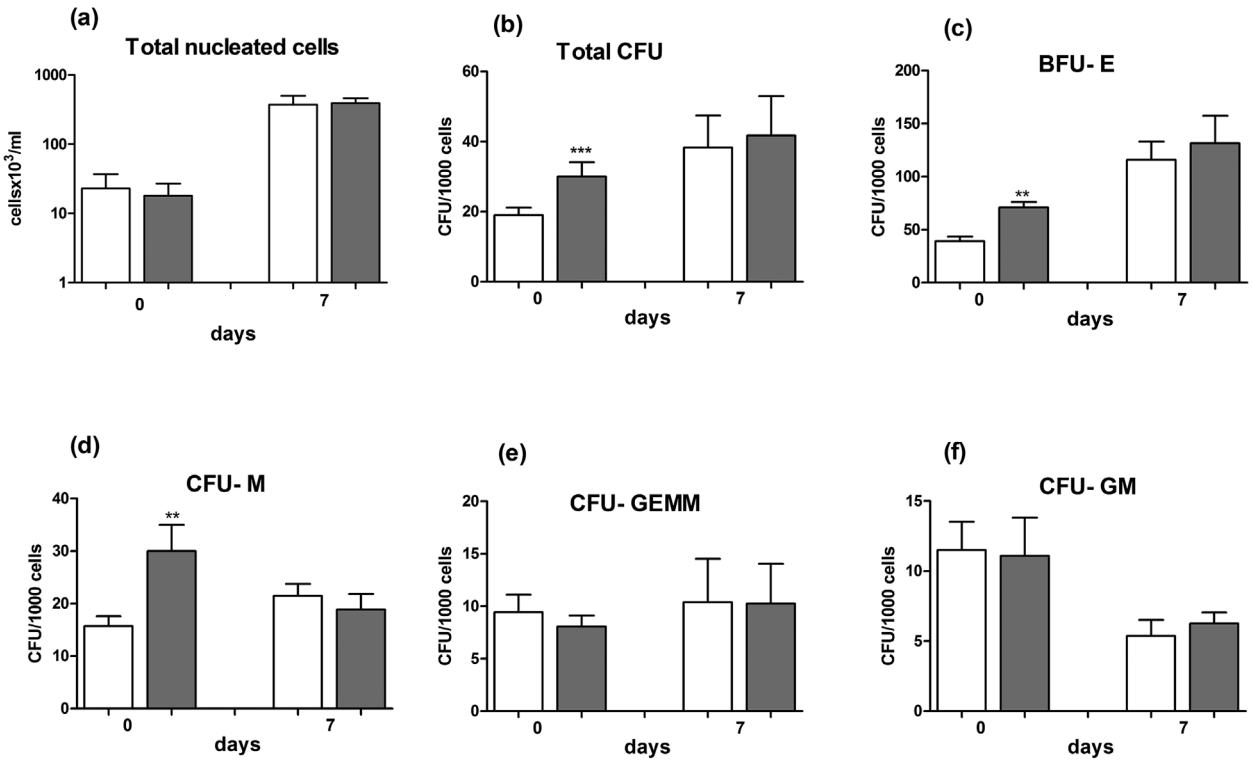

Fig. 2. Effects of GMF exposure according to 1.5 T-protocol on proliferation and clonogenic potential of human CD34+ cells. Results represent mean ± SEM values of three different experiments carried out in triplicate, gathered at baseline (day 0; i.e., soon after 72 h exposure to GMF or sham stimulation) and at day 7. $^{**}P < 0.01$ and $^{***}P < 0.001$ at Student's *t*-test between GMF exposed (grey bars) and sham-exposed controls (white bars).

CFU-M outputs of GMF-exposed CD34+ cells were significantly higher than those of sham-exposed controls at baseline ($P = 0.015$ vs. controls for both BFU-E and CFU-M), 7 days ($P = 0.015$ vs. controls for both BFU-E and CFU-M) and 14 days ($P = 0.009$ vs. controls for both BFU-E and CFU-M; Fig. 3c and d). In addition, GMF-exposed CD34+ cells, but not sham-exposed cells, generated BFU-E also after 4 weeks of culture. As observed for the 1.5 T-protocol, no differences were found regarding the CFU-GEMM and CFU-GM output (Fig. 3e and f). Indeed, these findings strongly suggest that GMFs influence in a lineage-specific way the mechanisms regulating hematopoietic cell differentiation, without affecting cell proliferation.

CD34+ Cells From Subjects Exposed to GMFs Show Similar Clonogenic Potential as Sham-Exposed CD34+ Cells

We also ran pilot experiments to check whether in vivo exposure to GMFs had any effect on hematopoietic stem/progenitor cells. We therefore studied proliferative and clonogenic potential of CD34+ cells obtained from healthcare workers exposed to GMFs from 1.5 T and 3 T MRI scanners. So far we obtained samples from two radiologists and one anesthesiologist, working at selected hospital MRI facilities.

Results were compared to those of samples exposed in vitro to GMFs and of sham-exposed controls. No significant differences regarding cell proliferation were observed among different samples. Furthermore, no different output of clonogenic progenitors was detectable between CD34+ cells obtained from healthcare givers and CD34+ cells not exposed to GMFs. In particular, amounts of erythroid and monocytes colonies produced by CD34+ cells obtained from MRI exposed personnel were comparable to those of in vitro sham-exposed CD34+ cells obtained from blood donors who can be regarded as "not-exposed subjects" since they had no occupational exposure to MRI.

To explain different behaviors displayed by cells exposed to GMFs in vitro or in vivo, we exposed umbilical cord blood mononuclear cells to GMFs according to 3 T-protocol. CD34+ cells were selected

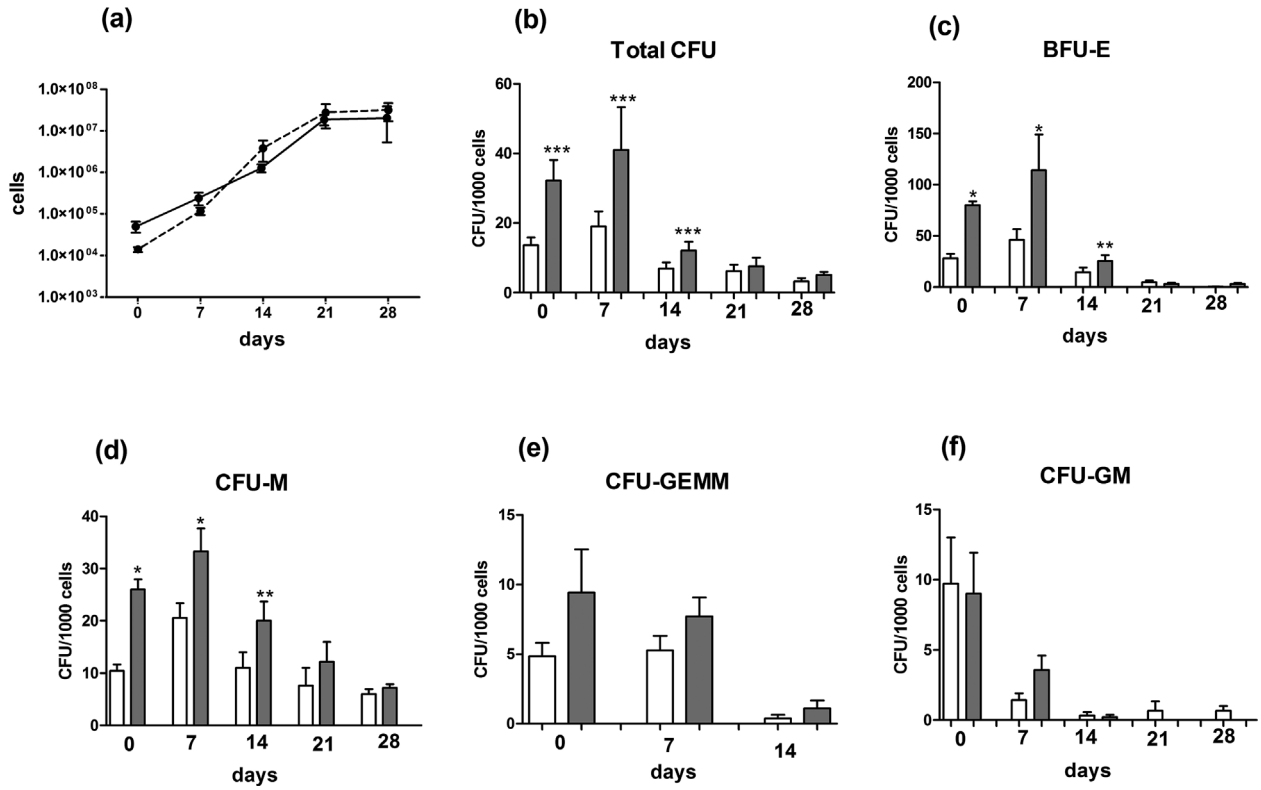

Fig. 3. Effects of GMF exposure according to 3 T-protocol on proliferation and clonogenic potential of human CD34+ cells. Data were obtained at baseline (day 0) and at days 7, 14, 21, and 28 after GMF exposure. Results represent mean ± SEM values of three different experiments performed in duplicate ($n = 2$ samples) or in triplicate ($n = 1$ sample). $^{*}P < 0.05$ and $^{**}P < 0.01$ Student's *t*-test between GMF exposed (dotted line and grey bars) and sham controls (continuous line and white bars).

by immuno-magnetic beads after exposure. The clonogenic potential of CD34+ cells selected from GMF-exposed mononuclear cells or from sham-exposed controls were then evaluated. In this case, we observed a slight increase of CFU-GEMM, while more committed progenitors, including CFU-M and BFU-E, were produced at similar level.

DISCUSSION AND CONCLUSIONS

The biological effects of exposure to MFs such as those from MRI scanners have recently been investigated with special emphasis on possible beneficial as well as potential adverse effects [Santini et al., 2005; Desai et al., 2009; Leone et al., 2014; Belyaev et al., 2015].

In this context, an important issue deals with "actual" exposure level of workers during MRI examination [McRobbie, 2012; Andreuccetti et al., 2013; Yamaguchi-Sekino et al., 2014]. Although a classification for MRI workers has been recently proposed by Hansson Mild et al. [2013] based on different exposure sources (exposure to static field only; exposure to static plus switched gradient fields; exposure to static plus switched gradient plus radio-frequency fields), the picture emerging from studies performed so far is that exposure levels can vary significantly depending not only on specific work duties and medical protocols but also on the distribution of stray fields around scanners [Yamaguchi-Sekino et al., 2014]. With reference to GMF exposure, it is worth noting that this may occur only when presence of medical (in particular anesthesiologist) or paramedic staff is required in the magnet room during specific diagnostic examinations.

Establishing a link between MF exposure and effects on human health is further complicated by limited accessibility/availability of samples from exposed subjects to be investigated at cellular and molecular levels.

Within this scenario, here we realized an exposure system very reliably reproducing (cross

correlation index higher than 0.9) GMFs generated by real sources. Indeed, the customized exposure system can generate two actual EPI signals previously acquired near 1.5 T and 3 T scanners at Bambino Gesù Children's Hospital (Rome, Italy): these signals can be associated only to analyzed scanners and a generalization is not possible. The maximum value of reproduced gradient EPI signals is about 100 μT: this value corresponds to maximum B-field amplitude measured in positions very close to the bores of both scanners (1.5 T and 3 T) and is comparable to values reported in Andreuccetti et al. [2013] and measured near other scanners and employing other types of sequences. In these positions, the corresponding maximum computed occupational WPIs for both EPI signals were <1, still compliant with international safety guidelines. Thus, B-field exposures levels employed for in vitro experiments, presented in this paper, represent actual exposure of the operators working near analyzed scanners.

We used this homemade device to expose in vitro human hematopoietic stem cells (CD34+ isolated from peripheral blood samples of adult healthy donors) and evaluate effects of GMFs on proliferation and clonogenic potential. These biological observable properties were also investigated in a pilot study on hematopoietic stem cells isolated from whole blood units donated by three healthcare workers at the same MRI scanners from which EPI signals were acquired.

Normal hematopoietic stem cells are functionally defined by their ability to give rise to long term hematopoiesis in recipients subjected to bone marrow radiation; these cells are CD34+ at immunophenotype, and include a hierarchy of more mature progenitor cells [Eaves, 2015]. CD34+ cells were chosen as biological sample to evaluate effects of GMFs because their peculiar biological properties (i.e., self-renewal and multipotency) would be expected to make them more susceptible to MFs than differentiated cells, as previously demonstrated by studies on low-frequency electromagnetic fields and radiation [Reipert et al., 1997; Wright, 1998; Sarvestani et al., 2010].

Collectively, our results show that GMF exposure did not negatively affect hematopoiesis and it promoted commitment of CD34+ cells towards erythroid and monocyte lineages. Indeed, we found that 72 h in vitro exposure of CD34+ cells to 1.5 T or 3 T-protocols resulted in increase of BFU-E and CFU-M. This effect was not accompanied by alteration in other type of colonies, suggesting that GMF exposure probably favors regulatory mechanisms underlying the generation of these progenitors from undifferentiated CD34+ cells. In line with this hypothesis, commitment of CD34+ cells towards erythroid and monocytes lineages appeared to persist over time only when cells were exposed to GMF 3 T-protocol. Different effects of 1.5 T and 3 T-protocols could be possibly related to characteristics of gradient sequence waveforms (with different frequency content) used for diagnostic procedures.

GMF effect on clonogenic potential of CD34+ was not associated with changes in proliferative activity and did not cause premature exhaustion of clonogenic potential of CD34+ cells, as shown by the fact that exposed CD34+ cells, but not control cells, were able to generate BFU-E also after 4 weeks of culture. Effects of 1.5 T and 3 T-exposure protocols were consistently and reproducibly seen in all independent experiments performed (three samples for each GMF protocol used with experiments performed at least in duplicate). Interestingly, a previous study has shown that proliferation and differentiation of human CD34+ from human placental and umbilical cord blood were both affected by exposure to 10 T static magnetic field [Monzen et al., 2009]. In particular, authors showed that after 16 h exposure there was significant increase of BFU-E and this effect was accompanied by a significant up-regulation of cell cycle-related genes and genes involved in early-hematopoiesis such as *c-KIT*, *GATA2*, and *RUNX1*. Although we have not investigated molecular mechanisms underlying effects of GMF on CD34+ cell differentiation towards the erythroid and monocytes lineages, it is plausible to hypothesize that regulation of gene expression by GMF has also occurred in our experimental conditions. Of note, recent evidence pointed to chromatin remodeling as a critical determinant in mediating effects of electromagnetic field on neural stem cell proliferation and differentiation [Leone et al., 2014, 2015].

Our pilot experiments suggest that in vivo and in vitro GMF exposure exerts different influences on the hematopoietic system. In fact, experiments performed on CD34+ cells isolated from two radiologists and one anesthesiologist working at selected hospital MRI facilities (i.e., exposed in vivo to GMFs) behave like CD34+ cells never exposed to GMFs.

These findings, that admittedly need to be confirmed in future studies on a larger number of subjects working at the selected MRI scanners, are not surprising, considering that hematopoietic stem cells are normally embedded within bone marrow specialized niches, which selectively promote their resting or proliferation and differentiation [Shen and Nilsson, 2012; Scadden, 2014]. Niches are composed by several kinds of cells, including osteoblasts, mesenchymal, and endothelial cells and their influence on

CD34+ cells subjected to in vitro GMF exposure has not been explored in our experimental conditions. Similarly, we cannot rule out that CD34 negative hematopoietic cells themselves could influence (either through cell-to-cell contact or through production of soluble molecules) the effect of GMF on the CD34+ cell population. Accordingly, when we exposed umbilical cord blood CD34+ cells to GMFs together with the whole mononuclear cell fraction, the unilineage erythroid and monocyte progenitors were not affected. The expansion of multipotent progenitors (i.e., CFU-GEMM) we observed in umbilical cord CD34+ cells may be due to the fact that these cells, compared to those from other sources, are enriched with rare subsets of highly immature progenitors, with extensive proliferation ability [Traycoff et al., 1994; Hao et al., 1995].

Nonetheless, our observations on effects of GMF on hematopoietic cells deserve to be further investigated, in particular, considering our previous data on neural stem cells [Leone et al., 2014]. Future studies focusing on possible involvement of epigenetic mechanisms, which are important regulators in stem cell physiology and pathology [Beerman and Rossi, 2015], are advisable. Notably, evidence of a direct link between GMF exposure and differentiation potential of CD34+ cells might have a relevant clinical impact, with possible implication also on engraftment of GMF exposed cells.

Collectively, our results showed that in vitro exposure to GMF generated by 1.5 T and 3 T MRI scanners did not negatively affect hematopoiesis and it promoted the commitment of CD34+ cells towards erythroid and monocyte lineages.

Although data obtained from in vivo exposure of workers need to be validated in a larger population, our results do not seem to raise significant concerns about adverse effects on hematopoiesis by occupational exposure levels we referred to in our study. On the other hand, GMF effects we observed in vitro encourage further investigations to assess whether MRI occupational exposure causes changes at molecular and epigenetic levels, that would provide evidence of long-term biological effects of GMFs on exposed workers.

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