Cell shape and plasma membrane alterations after static magnetic fields exposure

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The biological effects of static magnetic fields (MFs) with intensity of 6 mT were investigated in lymphocytes and U937 cells in the presence or absence of apoptosis-inducing drugs by transmission (TEM) and scanning (SEM) electron microscopy. Lectin cytochemistry of ConA-FITC conjugates was used to analyze plasma membrane structural modifications. Static MFs modified cell shape, plasma membrane and increased the level of intracellular [Ca^{++}] which plays an antiapoptotic role in both cell types. Modifications induced by the exposure to static MFs were irrespective of the presence or absence of apoptotic drugs or the cell type. Abundant lamellar-shaped microvilli were observed upon 24 hrs of continuous exposure to static MFs in contrast to the normally rough surface of U937 cells having numerous short microvilli. Conversely, lymphocytes lost their round shape and became irregularly elongated; lamellar shaped microvilli were found when cells were simultaneosly exposed to static MFs and apoptosis-inducing drugs. In our experiments, static MFs reduced the smoothness of the cell surface and partially impeded changes in distribution of cell surface glycans, both features being typical of apoptotic cells. Cell shape and plasma membrane structure modifications upon static MFs exposure were time-dependent. Lamellar microvilli were clearly observed before the distortion of cell shape, which was found at long times of exposure. MFs exposure promoted the rearrangement of F-actin filaments which, in turn, could be responsible for the cell surface modifications. Here we report data that support biological effects of static MFs on U937 cells and human lymphocytes. However, the involvement of these modifications in the onset of diseases needs to be further elucidated.

Key words: Static MFs, apoptosis, lymphocytes, U937cells, cell surface, cytoskeleton

A growing interest in the influence of magnetic fields (MFs) on life processes is deriving from concerns about their possible harmful effects on human health. More and more evidence has been gathered during the last years about the hazardous consequences of the so-called *electromagnetic pollution*. Although the data reported in the literature are quite heterogeneous with regards to MF intensity (from 10^{-7} to 10T), type of fields (static or oscillatory), and subjects exposed to MFs (from *in vitro* cultured cells to humans), a link between MFs and tumor genesis has been suggested (McCann et al. 1993). However, a direct tumor genesis or mutagenesis has never been attributed to MFs (Morandi et al. 1996). Differences in exposure systems and conditions complicate the evaluation of studies indicating biological effects of MFs. MFs are claimed to stimulate multiple responses in the cells: i) interfere with the proliferation capability of mammalian cells *in vitro* (Ross 1990); ii) enhance the mutation rates of cells exposed to various mutagenic compounds (Nordenson et al. 1994; Miyakoshi et al. 1996); iii) increase the survival of tumor cells after cytocidal therapies (Liburdy et al. 19939); iv) increase the tumor rate in cancer-susceptible mouse strains (Morandi et al. 1996); v) influence neoplastic development through alterations in the expression of cancer related genes (Loberg et al. 2000). Other studies indicate effects of MFs on the different functions of subcellular organelles; MFs induce i) lipid peroxidation and generation of reactive oxygen species (Ishisaka et.al. 2000); ii) DNA damage (Fiorani et al. 1992); iii) intracellular calcium signaling (Lyle et al. 1997). A possible interference of static MFs with the apoptotic process has been reported as well, showing an increase of cell survival by both inhibiting apoptosis via modulation of capacitative Ca^{2+} influx (Fanelli et al. 1999), and altering the expression of apoptosis-related genes (bcl2, p53) (*manuscript in preparation*). Alterations in intracellular signaling, such as potentiation of the effect of phor-

bol-12-myristate 13-acetate (PMA) on O_2 generation and β-glucuronidase release in polymorphonuclear leukocytes, have also been described (Khadir et al. 1999).

Most of the theories addressing the mechanism of interaction between biological systems and MFs suggest that the primary site of interaction is the plasma membrane, whose structural and biophysical changes affect receptor binding or activation, and thereby affect cell function in general (Paradisi et al. 1993). The effects described for membrane responses to MFs are consistent with the demonstrated reorientation of diamagnetic molecular domains within the membrane (Rosen 1993).

A more precise knowledge of the plasma membrane and/or cell surface perturbation exerted by static MFs exposure could help to gain insight into the biological role of magnetic fields which, in turn, could have the same therapeutic advantages shown for electromagnetic fields in the healing of bone fractures or, in the opposite way, in metastatic cell detachment in cancer (Goodman et al. 1995; Blank 1995; Johnson et al. 2001). Moreover, it is general knowledge that morphological changes, mainly limited to the plasma membrane, are linked to functional alterations, i.e. phagocytosis. Therefore, in the present study, an investigation of the ultrastuctural changes (cell shape, plasma membrane and cytoskeletal changes) induced by the exposure to static MFs, in isolated peripheral lymphocytes and in the monocytic cell line U937, in the presence and absence of apoptotic-inducing drugs, was performed.

Materials and Methods

Cells and treatments
Cells and cultures
The pro monocytic U937 cells were cultured in RPMI 1640 medium supplemented with 10% inactivated fetal calf serum, 2 mM L-glutamine, 10,000 IU/mL nistatine, 100 IU/mL penicillin and streptomycin in a humidified atmosphere of 5% CO_2; cells were used at the density of 5×10^5.

Peripheral blood mononuclear cells were obtained after Ficoll gradient separation of buffy coats from blood donations of non-smoking healthy males, aged 25 to 45. Peripheral blood lymphocytes were separated from monocytes by double adherence to plastic; they were over 95% pure as evaluated by morphological criteria. During and after the treatment they were maintained at a cell density of 1×10^6

cells/mL, in complete culture medium at 37°C, supplemented with 10% inactivated fetal calf serum, 2 mM L-glutamine, 100 IU/mL penicillin and streptomycin in a humidified atmosphere of 5% CO_2; cells were used on the first day of explant.

Induction of apoptosis
Apoptosis was induced with 10^{-2} M cycloheximide (CHX) for up to 18hrs or 24hrs, followed by a 1hr recovery period in fresh medium; 1 mM H_2O_2 for 1 hr, followed by a 1hr recovery period; 1 μg/mL puromycin (PMC) for up to 6 hours.

Magnetic field application
Static MFs were applied as reported by Fanelli et al. (1999). Briefly, static MFs were produced by metal magnetic disks of known intensities; magnets produce static without producing alternating MFs. Unlike electric field-driven, solenoid-generated MFs, static MFs do not induce any temperature increase. Static MFs intensity is given in millitesla (1 $T=10^4 G$). The intensity (6 mT) chosen according to Fanelli et al. (1999), corresponds to the antiapoptotic plateau.

Magnets were placed under the plastic culture Petri dishes. Since static MFs intensity decreases according to the square of the distance, the actual field intensity on the cells was calculated considering the thickness of the bottom of the Petri dish (1.2 mm). Static MFs were applied concomitantly with the apoptogenic treatments, unless otherwise specified.

Recovery
After apoptogenic treatment in the presence or absence of 6 mT static MFs, drugs were washed out and lymphocytes and U937 cells were seeded in quadruplicate at the same density (1×106 cells/mL). For each treatment, two Petri dishes were placed outside, and two inside, the 6mT static MFs. At the times indicated, apoptosis was quantitated and the number of viable cells was estimated in a hemocytomer.

Analysis of apoptosis
Cell viability was assessed by membrane impermeability to trypan blue and propidium iodide, positive staining with vital dyes, normal nuclear shape and texture revealed upon vital staining with Hoechst 33342, hematoxylin and eosin staining (Bonanno et al. 2002). Apoptosis was detected by light

microscopy and by cytofluorimetry. Nuclear fragmentation was detected after staining with Hoechst 33342 (1 μg/mL) according to nuclear morphological features (Bonanno et al. 2002).

Quantitation of apoptosis

The percentage of apoptotic and viable cell fractions was performed by cytometry with an EPICS XL cytofluorimeter (Coulter Electronic Inc., Hialeah, FL, USA) equipped with a 5-W argon laser having a 488-nm excitation wavelength. The fixed cells were stained with propidium iodide (10 μg/mL) in phosphate buffered saline containing 40 units/mL RNase and 0.5% Tween 20. The 635-nm emission wavelength was monitored for propidium iodide emission. The apoptotic fraction was calculated by evaluation of the subG1 DNA. For each flow cytometric analysis, at least 10,000 events were calculated.

Trasmission and scanning electron microscopy

Ultrastructure of lymphocytes and U937 cells was obtained by trasmission (TEM) and scanning (SEM) electron microscopy. A quantity of 10^6 cells/mL were fixed with 2.5% glutaraldehyde in cacodylate buffer, pH7.4, for 1h at ice temperature and postfixed with 1% OsO_4 in the same buffer; afterwards, samples were dehydrated, embedded in Spurr resin and examined under a Philips CM12 TEM. SEM observations were performed on cells deposited on APES (2% in acetone)-treated coverslip slides. A Critical Point Dryer 020 Balzer and a Sputter Coated 040 Balzer were used for the final preparation steps. Cells were examined under a Philips XL50 scanning microscope.

Lectins and phalloidin cytochemistry

U937 cells, fixed with 4% paraformaldehyde in phosphate buffer, pH 7.4, for 10 min and deposited on gelatin-treated microscopy slides, were analyzed for surface localization of sugars by using *Concanavalin-A* (Con-A) (40 μg/mL mannose) and *Ricinus communnis* (2 μg/mL D-galactose) FITC conjugates for 30 min in the dark. Phalloidin-FITC conjugate was used at a concentration of 20 μM for 20 min. Samples were observed by using a Nikon PCM 2000 microscope (Nikon, Japan) with Plan Fluor objectives (Nikon). Confocal microscopy was performed using a confocal laser scanning head Nikon PCM 2000 based on a Nikon Eclipse 600 microscope. Acquisition and visualization are completely computer-controlled through EZ 2000 software (Coord-

Nikon, The Netherlands) (Diaspro, 2002).

Samples labelled with FITC-conjugate lectins were excited with an Argon Laser ED HeNe 488 nm, equipped with 543 nm emission source.

Measurements of Ca^{2+} levels

Cells (5×10^7 cells at a concentration of 1×10^6/mL) were washed twice with loading buffer (120 mM NaCl, 5.4 mM KCl, 4.2 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.3 mM $MgSO_4$, 20 mM Hepes, 15 mM glucose, 2% BSA equilibrated with CO_2), re-suspended at a final concentration of 2×10^7 cells/mL and then loaded with 4 μM fura-2 acetoxymethylesther (AM) for 30 min at room temperature. After dye loading procedure, cells were washed twice with the same loading buffer and then re-suspended in fresh loading buffer at the final concentration of 3×10^6 cells/mL. Cells were stored at room temperature until use and pre-warmed at 37°C for 2 min before measurements. The fluorescence of fura-2 was measured using a Jasco FP-750 spectrofluorometer, equipped with an electronic stirring system and a thermostabilized (37°C) cuvette holder and controlled by a personal computer running the Jasco Spectra Manager software for Windows 95 (Jasco Europe s.r.l., Lecco, Italy). Excitation wavelengths were 340 nm and 380 nm and emission wavelength 510 nm, respectively and slit widths were set to 10 nm. In a glass cuvette, 2 mL of cell suspension, at the final concentration of 3×10^6 cells/mL, were added. Fluorescence values were converted to [Ca^{2+}] values according to Grynkiewicz et al. (1995).

Results

Viability, apoptotic rate and Ca^{2+} concentration

In the present work, static MFs at an intensity of 6mT were used and applied for up to five days to freshly isolated human lymphocytes and to U937 cells. This usage did not exert toxic or apoptotic effects and only slightly interfered with the normal rate of cell growth (plus 10% of mitosis in U937 cells). However, static MFs exposure of cells resulted in an altered apoptotic rate when the cells were incubated with apoptotic drugs during the exposure. In particular, in our experimental model, an inhibitory effect on apoptosis by static MFs was measured in both lymphocytes and U937 cells. The apoptotic rate of U937 cells was reduced by 20-30% when incubated with PMC, CHX or H_2O_2 in the presence
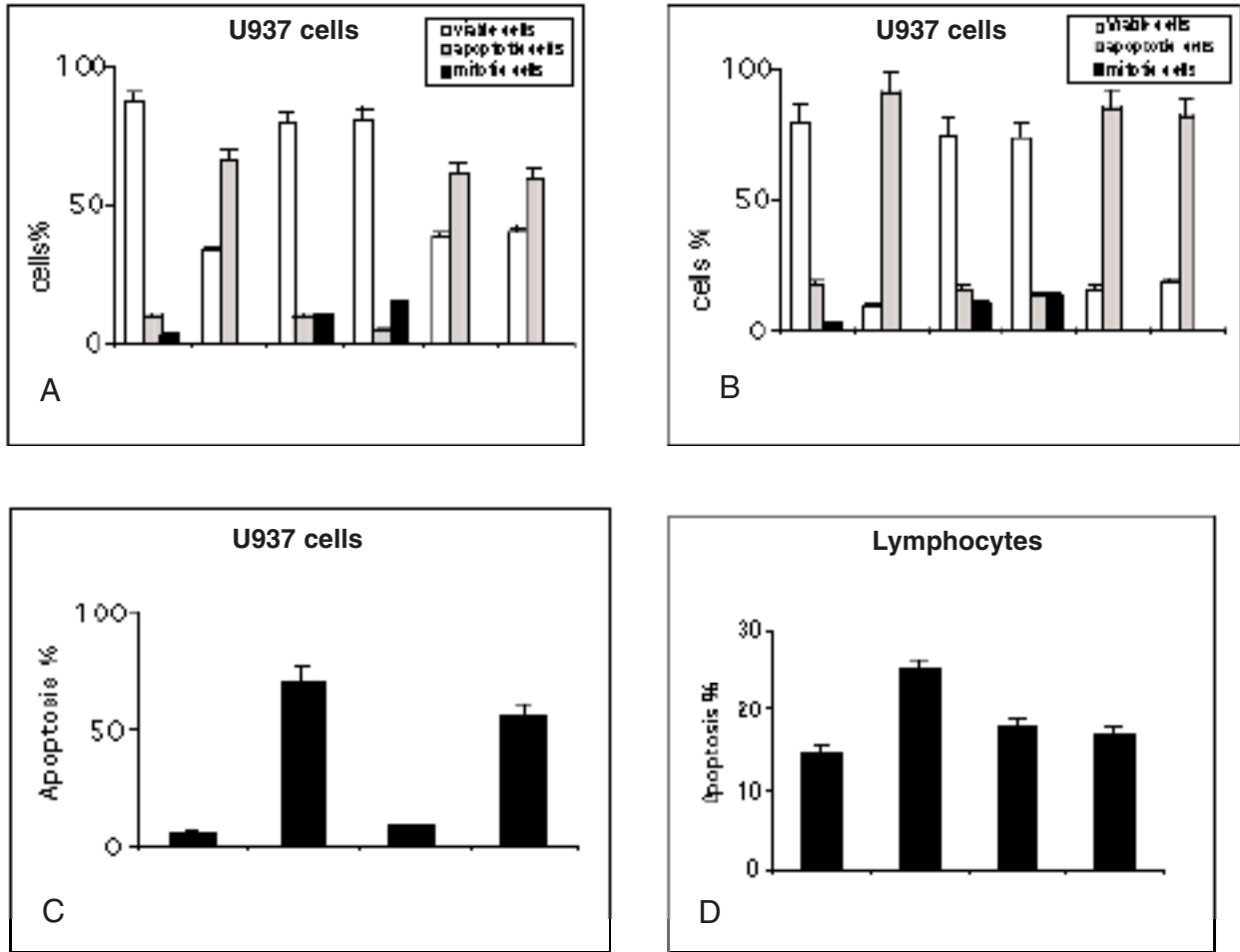
Figure 1. Cytofluorimetric evaluation of the viability, as well as the apoptotic and mitotic rates of U937 cells (a-c) and lymphocytes (d) in the presence and absence of apoptosis inducing drugs and static MFs of 6 mT intensity. 6 mT magnetic fields reduce H2O2, CHX or PMC–induced apoptosis. Values are average values ±SD of five (U937 cells) or three (lymphocytes) independently performed experiments, each in duplicate. PMC = puromycin (1 µg/mL); CHX = cycloheximide (10^{-2}M); H_2O_2 = hydrogen peroxide (1 µM).

of static MFs (Figure 1 a, b, c) and by 10-15% in lymphocytes treated with CHX or H_2O_2. The rate of apoptosis was affected by static MFs independently of the inducers used, thus showing that MFs reduced apoptosis by interfering with the apoptotic process and not with the inducers.

Since a reduction in the apoptotic rate has already been reported upon static MFs exposure of U937 cells and lymphocytes, due to modulation of Ca^{2+} influx (Fanelli et al.1999), the static MFs effects on intracellular levels of Ca^{2+} in control and apoptosing U937 cells and lymphocytes was monitored in parallel. Data reported in Figure 2 show the intracellular increase of Ca^{2+} measured upon static MFs exposure. The exposure to static MFs significantly modified the Ca^{2+} levels in both cell types.

Lymphocytes

Normal lymphocytes are characterized by a regular round cellular shape with microvilli randomly distributed all over the cell surface (Figure 3a). When lymphocytes were treated with 1 mM H_2O_2 for 1 hr or 10^{-2}M CHX for 18hrs, the rate of apoptosis in the culture increased up to about 22% and 28%, respectively (Figure 1d). SEM investigation of apoptotic cells showed rounded lymphocytes with smooth surfaces due to the loss of microvilli (Figure 3c). When lymphocytes, incubated with apoptogenic drugs, were concomitantly exposed to static MFs a marked alteration of their cellular shape was observed. In particular, application of static MFs to lymphocytes treated with CHX (10^{-2}M for 18hrs) caused structural modifications of membrane
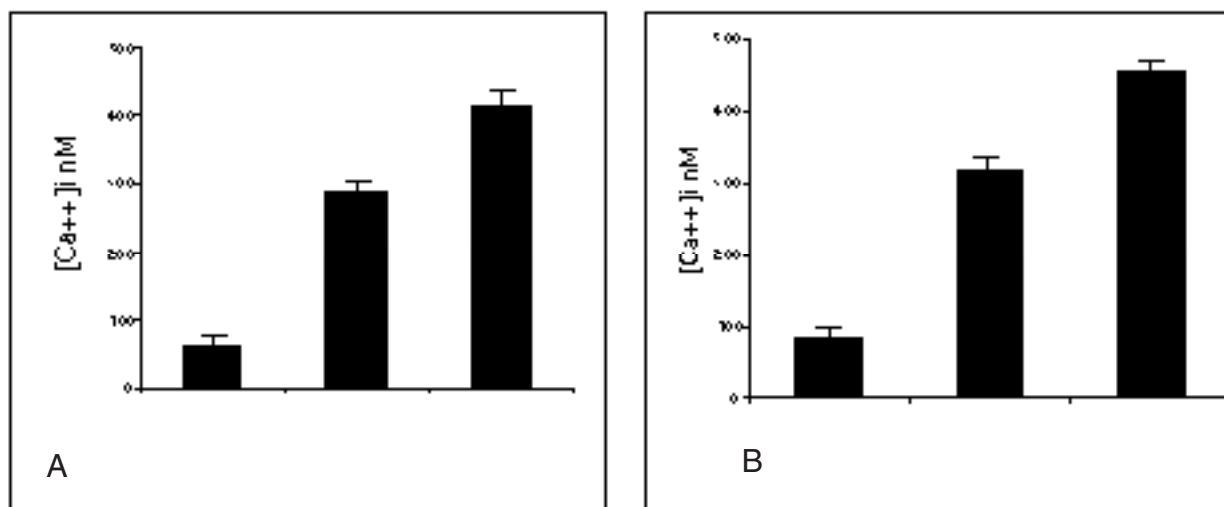
Figure 2. Evaluation of concentration of calcium ions by fura-2 (see *Materials and Methods*) in U937 cells (a) and lymphocytes (b) treated with PMC (1 µg/mL) or CHX (10⁻²M), respectively, in the presence of static MFs of 6 mT of intensity. Magnetic fields increase the concentration of calcium ions of three and two folds, respectively, both in apoptotic U937 cells and lymphocytes.

microvilli, which became lamellar (Figure 3d). In addition, the lymphocytes lost they round shape and became irregularly elongated (Figure 3b). When cells were exposed to static MFs only, a slight reshaping of the cell surface was monitored. In fact, the modifications were less pronounced than in apoptotic drug-treated cells (Figure 3d). The possible effects of prolonged exposure to static MFs on the cellular shape of lymphocytes has not yet been investigated.

Cell surface modifications accompanied both apoptosis and static MFs exposure (Figure 3e-h). In fact, Con-A-FITC binding was altered by the apoptotic treatment (Figure 3f) as well as by the static MFs exposure (Figure 3g-h). During apoptosis, the exposure of sugar residues increased and their distribution was changed. Following exposure to static MFs, in control human lymphocytes the Con-A-FITC binding pattern was modified, and fluorescence concentrated on the cell periphery with a crescent shape. The modifications exerted by the apoptotic treatment were prevented by static MFs exposure: the labeling was similar to those observed for untreated lymphocytes.

U937 cells

Static MFs applied on U937 cells mostly led to cell surface modifications. In fact, abundant lamellar-shaped microvilli were observed upon 24 hrs of continuous exposure to static MFs (Figure 4 b, b'),

in contrast to the normal rough surface of the cells with many, short microvilli (Figure 4 a, a'). The modifications in cell shape and microvilli to static MFs exposure were time-dependent. In fact, lamellar microvilli were observed well before the distortion of the cell shape, which was found at longer time periods of exposure (Figure 5 b', c'). Possible modifications of organelles were investigated by TEM. However, even at the longer time periods of exposure to static MFs, the morphology of cytoplasmatic organelles and nucleus was unmodified; in particular, beam shaped nuclei with evident nucleolus and zones of condensed and non-condensed chromatin were detected.

After incubation with CHX for 24 hrs or with PMC for 4 hrs, apoptotic morphologies were detected. Chromatin condensed along the envelope and nucleus fragmented by budding (Figure 4 c, d) or by cleavage (Figure 5 a). The surface of apoptotic U937 cells become smooth (Figures 4 d, d'; 5 a, b). When U937 cells were simultaneously incubated with pro-apoptotic molecules and static MFs for 24 hrs (Figures 4 e, e'; 5 b,c), the smoothness of the cell surface, typical of apoptotic cells, was reduced. In fact, attempts at microvilli reformation with a faint lamellar shape were observed.

Modifications of cell surface expression of glycans, as detected by ConA and *Ricinus communis*-FITC conjugates, were studied by confocal microscopy on control and apoptosis induced U937 cells. ConA and

- SMFs 6mT

+ SMFs 6mT
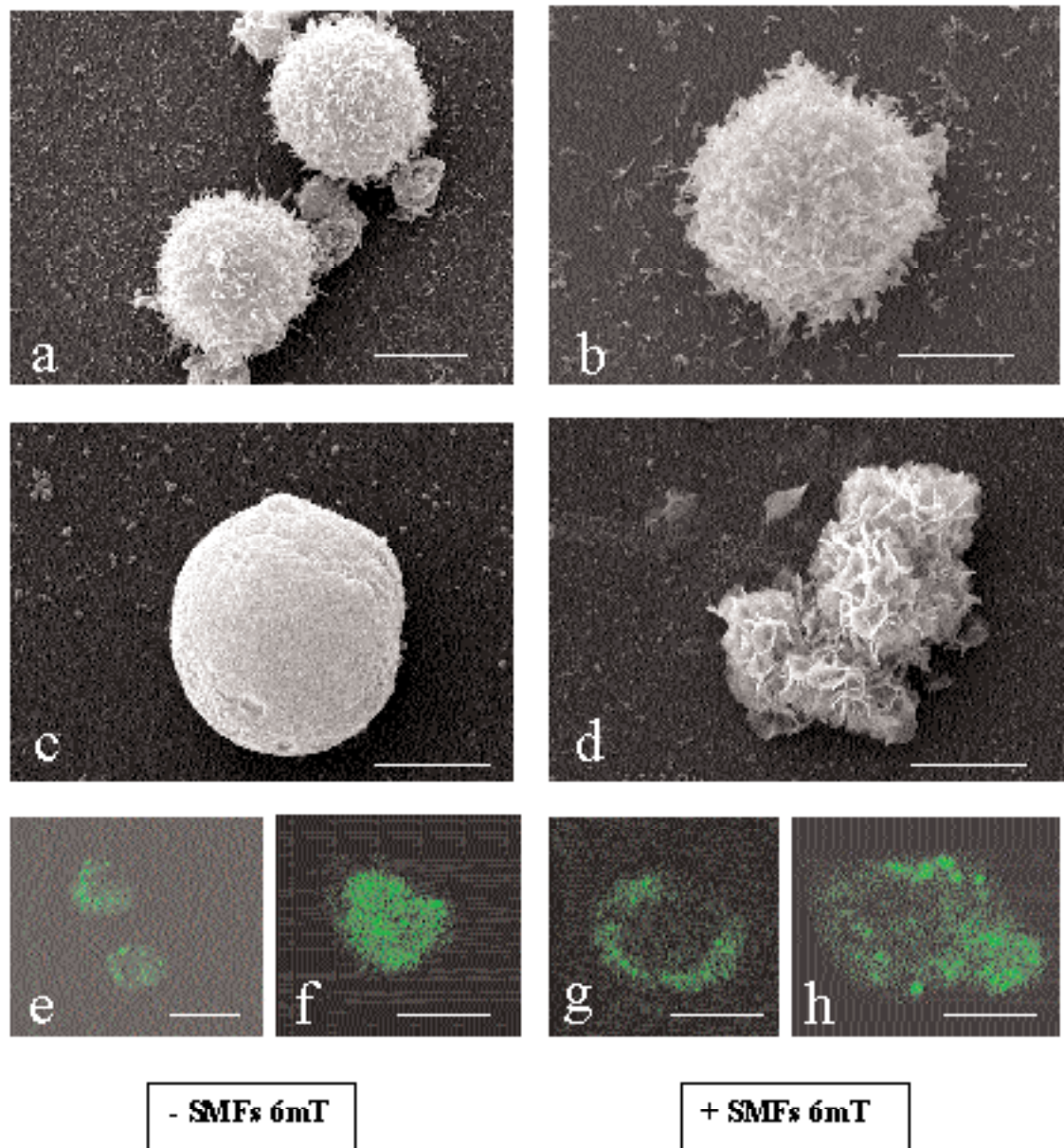
Figure 3. SEM micrographs (a-d) showing cell shape modifications, and confocal fluorescent micrographs (e-h) showing ConA-FITC labeling to human lymphocytes following the apoptotic treatment in the presence or absence of 6mT StaticMFs. a) Controls of human lymphocytes are characterized by a round shape with microvilli randomly distributed all over the cell surface. A slight remodeling of the microvilli shape is observed (b) after 18 hrs of exposure to static MFs. c) Round smooth apoptotic lymphocytes without microvilli after treatment with H_2O_2 100 mM for 1hr. The presence of static MFs during the apoptotgenic treatment dramatically modifies cell shape, which becomes elongated, and microvilli, which appear lamellar (d). Bar = 5 μm. e) Controls of human lymphocytes. Patches of fluorescence on the entire cell surfaces f). CHX treated human lymphocytes for 24 hrs. Increased binding of ConA-FITC conjugates randomly distributed all over the cell surface. G) 24 hrs exposure to static MFs of controls of human lymphocytes gives crescent shape distribution of ConA-FITC binding sites, which is lost (h) when cells are contemporaneously exposed to PMC and staticMFs. Bar = 5 μm.

Ricinus communis binding sites, whose amount is not negligible, are randomly distributed on the surfaces of U937 control cells (Figure 6 a, A). Conversely, the apoptotic treatment decreased cell surface mannose and galactose residues of PMC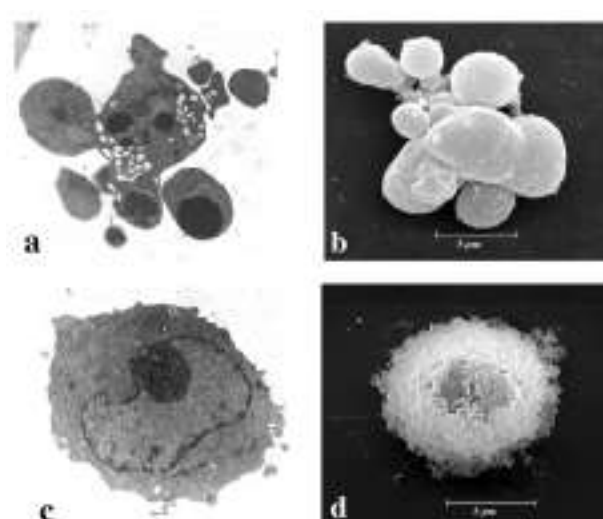-treated U937 cells (Figure 6 c, C). Fluorescence (Con-A-FITC) concentrated to one pole of the cell was found. After exposure to static MFs the normal fluorescence distribution observed in control cells was not altered (Figure 6 b, B). However, the exposure to static MFs of PMC-treated U937 cells partially impeded the modifications in the distribution
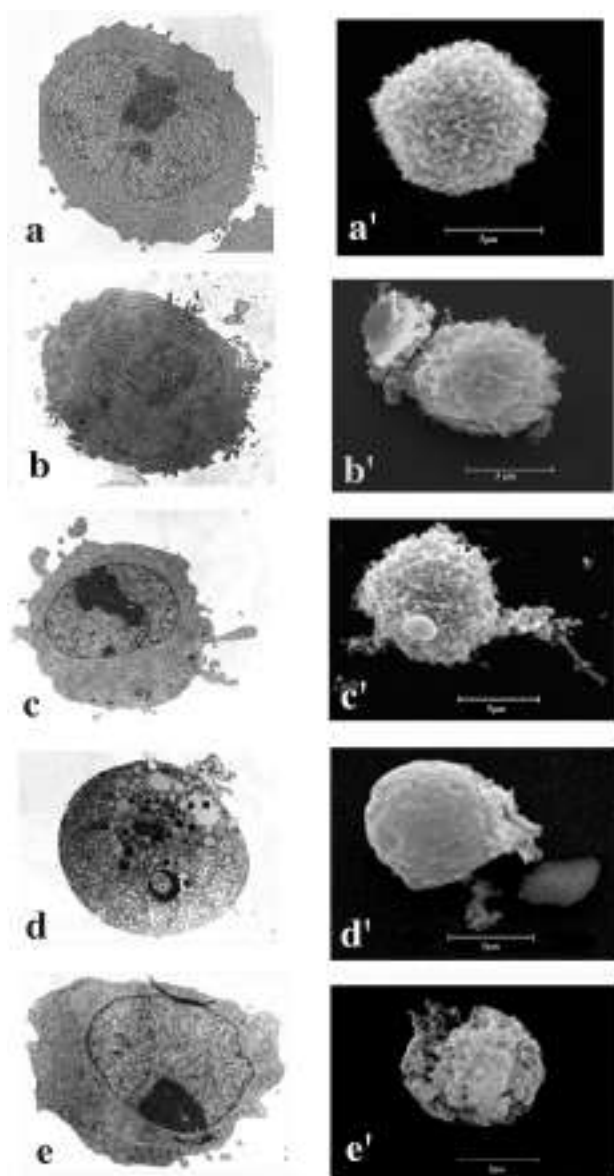
Figure 4. TEM (a,b,c,d,e) and SEM (a',b',c',d',e') micrographs of U937 cells showing cell shape modification following the apoptotic treatment (CHX 10^{-2}M for 24 hrs) in the presence of 6mT staticMFs. The 24 hrs continuous exposition to static MFs leads to cell shape distortion and/or lamellar microvilli presence (b,b',c,c') when compared to controls of U937 cells (a,a'). 10^{-2}M CHX for 24 hrs induces apoptosis in U937 cells, showing the characteristic roundness and smoothness of the cell shape (d,d'). Exposure for 24 hrs to static MFs reduces the apoptotic rate and the smoothness of cell surfaces (e,e'). Bar= 5 µm.

Figure 5. TEM and SEM micrographs of U937 cells showing cell shape modification following PMC treatment (1 µg/mL for 4 hrs.) in the presence of 6mT staticMFs. One µg/mL PMC for 4 hrs induces massive apoptosis and cellular fragmentation (a, b). When 6mT static MFs and PMC (1 µg/mL for 4hrs.) are applied simultaneously to U937 cells, a significant reduction of apoptosis and of nuclear and cellular fragmentation together with the onset of lamellar microvilli is observed (c,d). Bar = 5 µm.

of cell surface glycans exerted by the apoptogenic inducer (Figure 6 d).

One possible cause of the above described modifications could be cytoskeletal alterations, besides the reorientation of molecular domains within the membrane (Rosen 1993). Figure 7 shows the modifications in the binding of phalloidin-FITC to U937 cells after exposure to static MFs only and/or after the contemporary treatment of static MFs and PMC. The pro-apoptotic drug induced a remodeling of F-actin distribution (Figure 7b arrows), further remodeled by the contemporary application of static MFs (Figure 7d).

Discussion

In this study, we showed that static MFs exert a strong and reproducible effect on the cell shape and on the plasma membrane, as well as on apoptosis in the different cell types; this latter effect is mediated by the MFs ability to increase Ca^{2+} influx (Fanelli et al. 1999). Other possible effects of static MFs, such as an alteration of the pattern of gene expression, cannot be excluded (*manuscript in preparation*).

These findings are in agreement with other literature data which indicate that the plasma membrane is a very sensitive target for the action of MFs. MFs of very low intensity can generate cell modifications as was shown by the described experiments with the use of 6mT magnets. Therefore, in spite of the fact that this is not a high value of magnetic fields, it is nevertheless able to produce changes in the cell structure and function.

ConA-FITC

Ricinus communis FITC

-SMFs 6mT
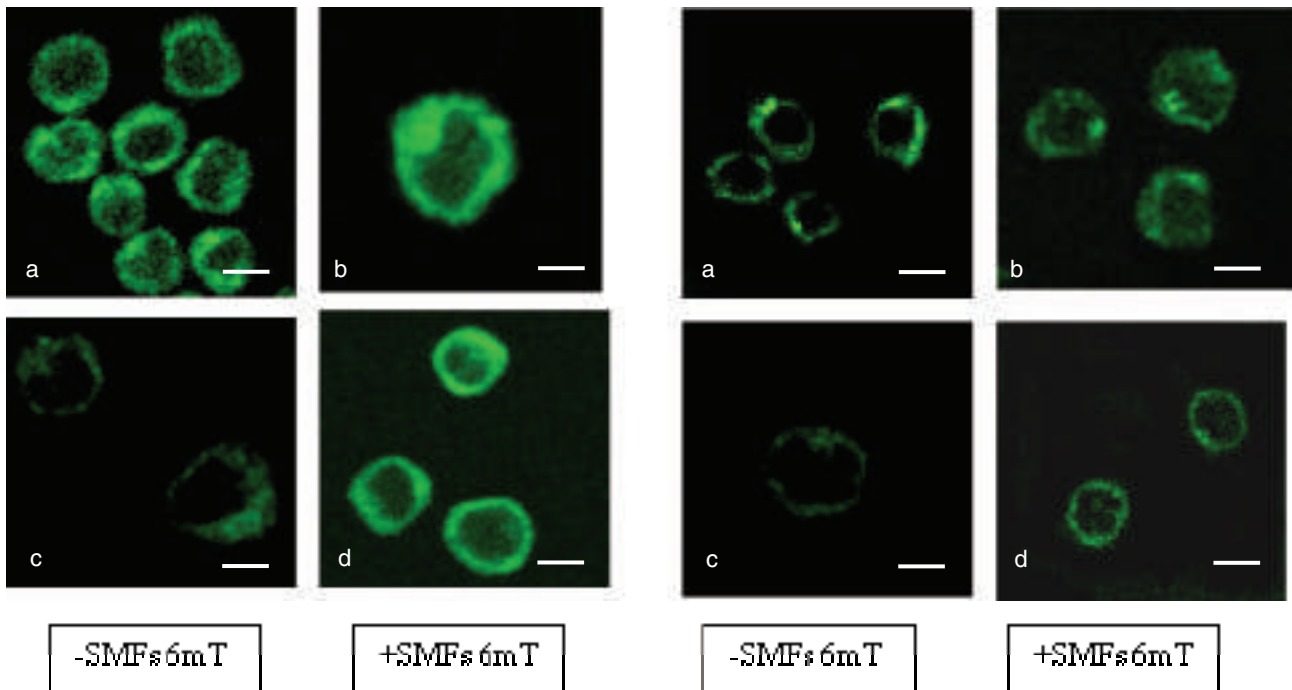
+SMFs 6mT

-SMFs 6mT

+SMFs 6mT

Figure 6. Confocal micrographs of ConA (a-d) and *Ricinus communis* (A-D) binding sites on U937 cells. Lectin FITC conjugates were used. (a, A') Control U937 cells. Surface random distribution of fluorescence. (c, C) Puromycin treated U937 cells for 4 hrs. Loss of ConA and *Ricinus communis* binding sites parallels the onset of apoptosis. ConA-FITC accumulates preferentially at one pole of the cell. (b, B) 4 hrs of exposure of untreated cells to 6mT static MFs does not alter the fluorescence distribution or intensity observed for control cells. (d, D) The loss of ConA and Ricinus communis binding sites is impeded when cells are simultaneously exposed to static MFs and PMC. Bar= 5 µm.
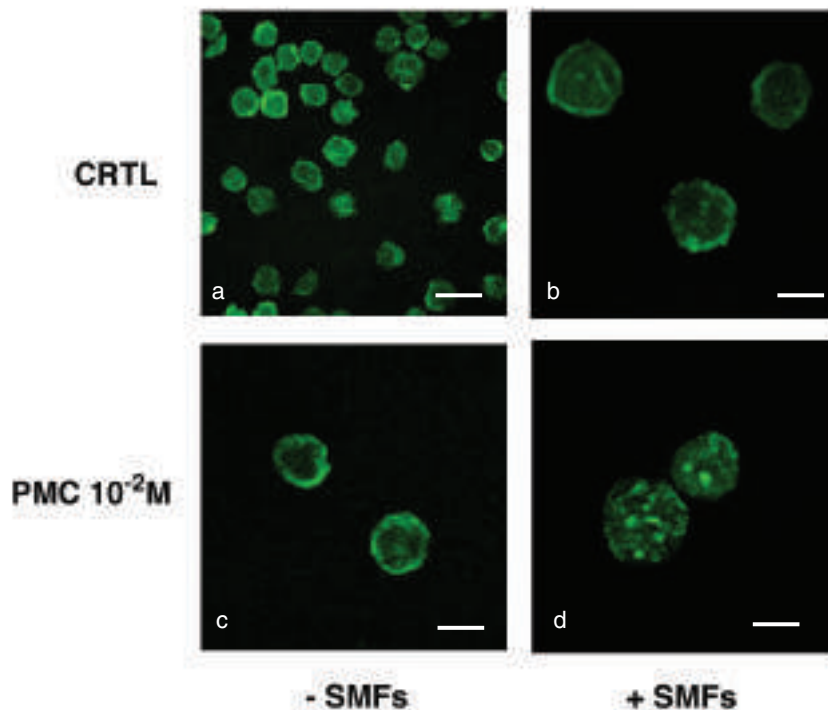

CRTL

PMC 10^{-2}M

- SMFs

+ SMFs

Figure 7. Confocal micrographs of phalloidin-FITC labeling to U937 cells. a) Random distribution of fluorescence, showing a thick fluorescent ring under the plasma membrane corresponding to the terminal web. b) deaggregation of the thick ring is observed after 4 hrs of exposure of control cells to static MFs (arrow). c) In PMC treated U937 cells, phalloidin-FITC is visible as a thick ring of fluorescence. d) simultaneous exposure to static MFs and PMC destroyed the ring, showing small patches of fluorescence. Bar= 5 µm.

The intensity-induced field effects have been investigated elsewhere (Fanelli et al. 1999), while in this paper the time-dependent effects of field exposure have only partially been investigated: i.e. in U937 cells. Our results, in agreement with the data reported by Rosen (1993), in which the duration of the exposure (within certain limits) is important in determining the extent of the plasma membrane as a response to static MFs, show strict differences in the morphology of U937 cells after a short or a long-term exposure to 6mT static MFs. Moreover, the effects measured are a function of the duration of static MFs exposure up to a *limit*, above which, longer durations are not associated with further plasma membrane modification. The same results are confirmed when apoptotosis-inducing drugs are given concomitantly with static MFs. Further time course studies are in progress.

When control cells are submitted to static MFs, electron microscopy observations showed alterations in the cell shape and the lamellar microvilli, but without other morphological changes. In fact, the plasma membrane, by virtue of its bioelectrical properties, is the site where EMFs influences are most likely to be exerted (Kell 1986). Uniform field exposure influences the formation of cell protrusions (Patel and Poo, 1982; Onuma and Hui, 1988; Ross et al. 1988). A current of electrons or ions can cause a movement of proteins in the membrane and determine a re-arrangement of their position on the membrane surface (Chiabrera et al. 1984). This might be one possible explanation for lectin binding site rearrangements. It could not be excluded that variations in the lipid dynamics described within the 48 hrs from cell seeding in Friends erythroleukemia cell membrane (Volpe et al. 1998), could also favor the movement of ConA and Ricinus communis binding sites.

The changes of cell shape and cell surface micro-morphology are closely related to the reorganization of the cytoskeleton elements within the exposed cells (Popov et al. 1991; Santoro et al. 1997). Cytoskeleton reorganization is not due to disruption of its elements, but promotion of their assembly and modulation of their orientation by high magnetic fields in vitro (Bras et al. 1998) might occur. Our preliminary data are more in favor of cytoskeleton re-organization than of cytoskeleton disruption.

One important event during the MFs exposure is the modification in Ca^{2+} influx. In our experimental model, a two-fold increase in calcium ions has been measured for both cell types. This seems to be a cru-cial event in the cell, since Ca^{2+} can contribute to many cell functions. For example, some of the available studies suggest that the mechanism of reorganization and breakdown of different cytoskeleton elements is related to modified Ca^{2+} homeostasis or altered phosphorylation/dephosphorylation state of proteins in exposed cells (Santoro et al. 1997); decreased phagocytic uptake of latex microspheres accompanied by an increased intracellular Ca^{2+} level in macrophages (Flipo et al. 1998); reduced apoptotic rate (Fanelli et al. 1999).

The decreased rate of apoptosis measured for U937 and lymphocytes is in agreement with other studies which demonstrated that static MFs decrease the rate of apoptosis mainly by interfering with Ca^{2+} influxes (Fanelli et al. 1999). In fact, variation in the Ca^{2+} concentration is responsible for entering apoptosis in certain cell types, while others undergo apoptosis by pathways that are independent of the Ca^{2+} influxes. Static MFs exposure was found to be protective for both lymphocytes and U937 from entering apoptosis. In fact, to confirm this result, for both cell types used, high concentrations of Ca^{2+} were measured upon concomitant exposure to static MFs and apoptogenic drugs, thus giving further insight into the phenomenon.

It could be hypothesized that some sublethal damage persists in the cells, even when the cell morphology still seems to be normal; in fact, it is known that MFs interfere with DNA repair processes (Chow and Tung, 2000; Robinson et al. 2002) or with altered cell metabolism, thus explaining why MFs act as co-tumorigenic or co-carcinogenic factors without by themselves inducing these processes (Looscher and Liburdy, 1998). Alternatively, marked fields alterations of cellular shape and binding sites distribution, may be, in a cascade, responsible for many other perturbations of cell functions such as: decreased phagocytic uptake (Flipo et al. 1998); alteration of receptors (Blumental et al. 1997); drastic alteration of cell morphology leading to the detachment from the culture dish as shown for rat tendon fibroblasts (Blumental et al. 1997); developmental anomalies in chick embryos after exposure to 50Hz EMF, due to modifications of cell-cell or cell-substratum interactions (Lahijani and Ghafoori, 2000); cell-mediated immune response alteration in T-cell responses to mitogens (Lahijani and Ghafoori, 2000; Thun-Battersby et al. 1999).

To sum up, the data described in the present paper are in favor for a biological effect of static MFs;.

even if the perturbations on either U937 cells or lymphocytes by static MFs of 6mT intensity are sub-lethal. These perturbations can, especially at longer times of exposure, evolve into progressive accumulation of modifications, i.e. gene expression (personal communication), which, in turn, can act as co-tumorigenic or co-carcinogenic factors. Therefore, the importance of understanding deeply the true mode of action of the MFs, as well as that of elec-tromagnetic fields, on living organisms arises from the necessity of protecting human health in the future.

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