

## Differentiation of monocytic U937 cells under static magnetic field exposure

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We present here a morphological, cytochemical and biochemical study of the macrophagic differentiation of human pro-monocytic U937 cells exposed to moderate intensity (6 mT) static magnetic fields (MF). It was found that the following substances induced differentiation in U937 cells to a progressively lower degree: 50 ng/mL 12-O-tetradecanoyl-13-phorbol acetate (TPA), low concentration of glutamine (0,05 mM/L), 10% dimethyl sulfoxide (DMSO) and 100 mM/L Zn<sup>2+</sup>. Differentiated U937 cells shift from a round shape to a macrophage-like morphology, from suspension to adhesion growth and acquire phagocytotic activity, the cytoskeleton adapting accordingly. Exposure to static MF at 6 mT of intensity decreases the degree of differentiation for all differentiating molecules with a consequent fall in cell adhesion and increased polarization of pseudopodia and cytoplasmic protrusions. Differentiation alone, or in combination with exposure to static MFs, affects the distribution and quantity of cell surface sugar residues, the surface expression of markers of macrophage differentiation, and phagocytotic capability. Our results indicate that moderate-intensity static MFs exert a considerable effect on the process of macrophage differentiation of pro-monocytic U937 cells and suggest the need for further studies to investigate the *in vivo* possible harmful consequences of this.

Key words: nitroblue tetrazolium, latex particles, cytoskeleton, surface molecules, phosphatidylserine

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The interest in the biological effects of non-ionizing electromagnetic fields (EMFs) on the whole organism, as well as on cellular systems, has considerably increased in recent years in consideration of their probable health risk for humans. Specifically, the influence of static magnetic fields (MFs) on biological systems has been a topic of considerable interest for many years (Rosen, 2003a; Tenforde, 1985). Induced electric currents are associated with these fields during activation and deactivation or with movement within the field (Rosen, 2003a; Tenforde, 1985).

Findings on the effects of exposure to electro and/or magnetic fields on humans are often contradictory and therefore difficult to interpret. Literature data concerning the biological effects of MFs and EMFs are also contradictory, mostly due to the multiplicity of experimental conditions (i.e., *in vitro* or *in vivo* models, intensity and type of field, oscillatory or static, time of exposure, metabolic state of the cells, etc). However, converging data indicate that the primary site of action of both MFs and/or EMFs is the plasma membrane (Santoro *et al.*, 1997; Rosen, 2003 a,b). The influence exerted by MFs (static or oscillating) on the plasma membrane has been described at different levels: on its surface (Paradisi, 1993), on the distribution of membrane proteins and membrane receptors (Bersani, 1997), on the cell-cell and cell-matrix junctions (Somosy, 1999), on cell membrane sugar residues (Bordiushkov *et al.*, 2000; Chionna *et al.*, 2003) and on trans-membrane fluxes of different ions, especially calcium (St Pierre and Dobson, 2000); in turn these perturbations influence the apoptotic rate, cellular shape and cytoskeleton (Chionna *et al.*, 2003; Fanelli *et al.*, 1999). Taking into account a link between childhood and worker

cancers and exposure to MFs (Comba *et al.*, 1995; Milham, 1996), and the fundamental role played by the physiological condition and/or the developmental history of the cells in the response to MFs exposure, a study into monocyte/macrophage differentiation, by using U937 cells continuously exposed to 6 mT static MF, could help in understanding this link. U937 cells, a histiocytic lymphoma cell line, preserve all the monoblastic characteristics of *in vivo* monocytes, including the ability to differentiate into mature macrophages under the effects of different inducers (Hosaya and Maranouchi, 1999). Differentiated U937 cells adhere to the culture plate and acquire phagocytotic capability. It is worth noting that many of the properties of mature macrophages are plasma membrane mediated, and, therefore, the influence of static MFs on the differentiation process of U937 (in particular for adhesion and phagocytosis) can be hypothesized. Unluckily, literature data regarding the effects of MFs on cell adhesion and phagocytosis are scarce. Inhibition of cellular adhesion for fibroblasts and human melanoma cells, have been reported in the studies by Blumenthal *et al.* (1997) and Short *et al.* (1992) using respectively EMFs or MFs exposure. Scarce are also the data on monocyte differentiation into macrophages by phorbol esters (Tao, 1999; Simko *et al.*, 2001) and those on phagocytosis (Simko *et al.*, 2001; Flipo *et al.*, 1998) under exposure to MFs. On the other hand, it is known that in the absence of MFs exposure, phorbol esters exert their biological effects by altering gene expression through the activation of protein kinase c (Nishizuka, 1984; Pedrinaci *et al.*, 1990), and by modulating the activity of transcriptional factors that bind cis elements, such as Nf-kB, AP-1, AP-2, AP-3 etc. (Garcia *et al.*, 1999). In this process, the expression of adhesion receptors notably changes (Garcia *et al.*, 1999).

The aim of the present work was to study the macrophagic differentiation of human pro-monocytic U937 cells under static MF exposure. The effect of 6 mT static MF on the macrophagic differentiation of U937 cells, with respect to the degree of differentiation, phagocytotic activity, cell surface markers, cell shape and F-actin integrity, is reported, giving evidence of the biological effects of static MF exposure. Differentiation was followed by biochemical, morphological and cytochemical observations and the analysis of the capacity to phagocytose latex particles.

## Materials and Methods

### Cells and cultures

U937 monoblastic cells were cultured in RPMI 1640 medium at 37°C supplemented with 10% inactivated fetal calf serum (FCS), 2 mM L-glutamine, 10.000 IU/mL nistatine, 100 IU/mL penicillin and streptomycin in a humidified atmosphere of 5% CO<sub>2</sub>; cells were used at a density of 1x10<sup>6</sup>cells/mL.

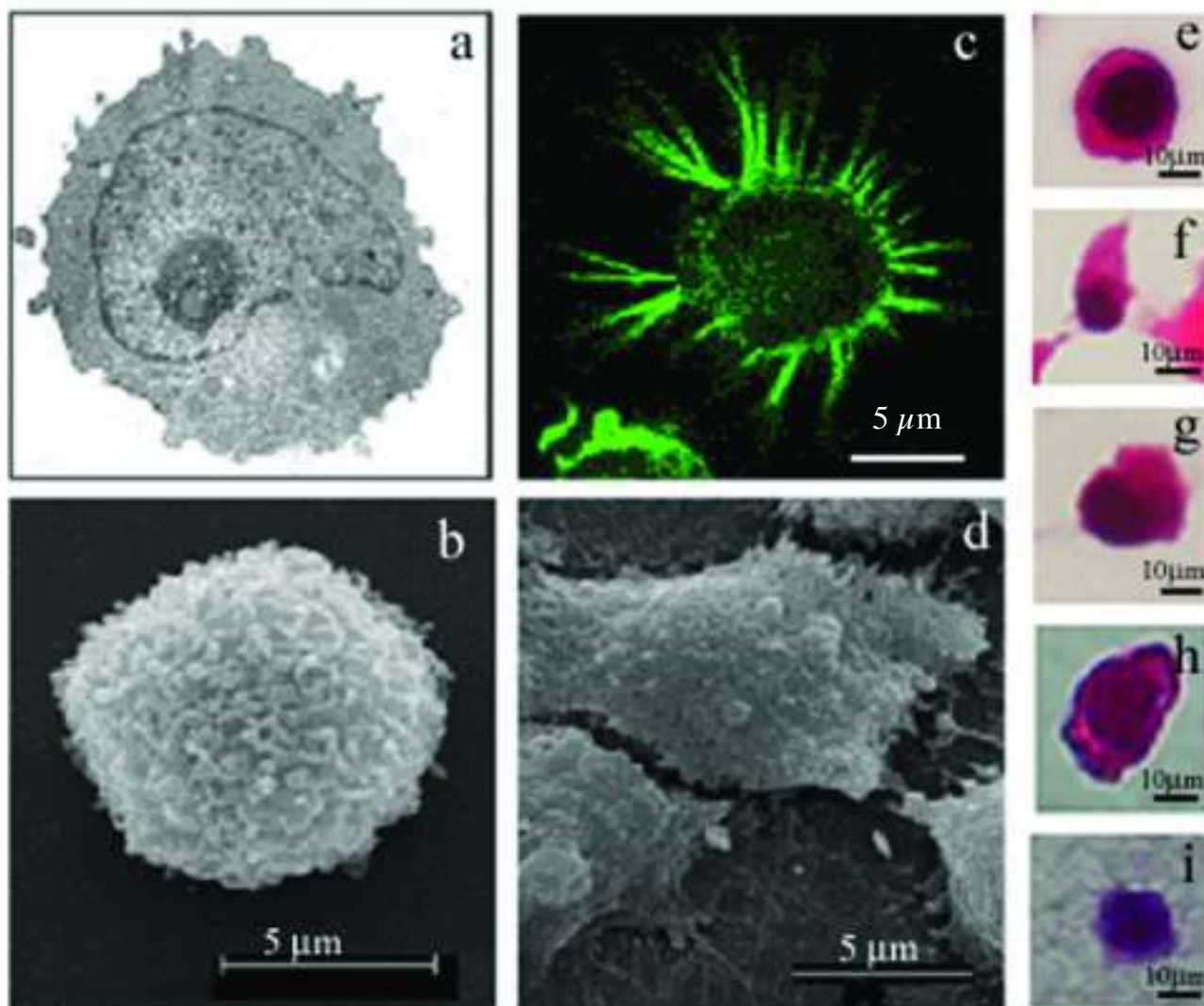
### Macrophagic differentiation

U937 cells were differentiated on a substrate of fibronectin 10 µg/mL left on the bottom of dishes for 18 hrs at 4°C, and then saturated with 1% bovine serum albumin- phosphate buffer pH 7.4 (BSA-PBS) for 90 min at 12°C. Macrophage differentiation was induced with: 50 ng/mL 12-O-tetradecanoil-13-phorbol acetate (TPA), 10% dimethyl sulfoxide (DMSO), 100 mM/L Zn<sup>++</sup> or with low concentration of glutamine 0,05 mM/L (using a culture medium not supplemented with L-glutamine).

Differentiation was monitored for 3 days following the addition of the inducers and its degree was evaluated by morphological criteria and Nitro blue tetrazolium testing (NBT) (Mendelsohn, *et al.* 1980). NBT can be used to determine U937 differentiation by both light microscopy observations of diformazan crystals and by spectrophotometric analysis. At fixed times, 100 µl of 10 mg/mL NBT in PBS were added to 3 mL cell medium; cells were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> for 30 min and for 2 hrs, before undergoing light microscopy and for spectrophotometric analysis respectively. Cells were washed three times with PBS pH 7.4, fixed with 4% formalin in the same buffer and then stained with May-Grunwald dye for light microscopy observation. Cells with diformazan crystals (differentiated cells) were counted. Spectrophotometric analysis (absorbance λ=560nm) was performed with cells washed three times with PBS pH 7.4 and in which diformazan crystals were dissolved in 2 mL of DMSO.

### Static magnetic field application

Static MF was produced by Neodymium magnetic disks (10 mm in diameter and 5mm in height) of known intensity supplied by Calamit Ltd (Milano, Italy) placed under the culture Petri dishes. The intensity of the field generated by the magnet was



**Figure 1. Morphology of undifferentiated (a, b) and differentiated (c, d) U937 cells. Undifferentiated cells have a round shape, short microvilli and bean-shaped nuclei (TEM micrograph, a; SEM micrograph, b). Differentiated U937 cells adhere to substrate, become flat with long pseudopodia (SEM micrograph, d; confocal micrograph, phalloidin-FITC labelled, c). Light micrographs of hematoxylin/eosin stained U937 cells, undifferentiated (e) or differentiated (see Materials and Methods section) with TPA (f), DMSO (g), Zn<sup>2+</sup> (h) or low concentrations of glutamine (i).**

checked by means of a gaussmeter (Hall-effect gaussmeter, GM04 Hirst Magnetic Instruments Ltd, UK). The field intensity of 6 mT is obtained on the bottom of the culture dish at 2.5 cm from the magnet. This distance was obtained by interposing between the magnetic disk and the Petri dish two disks of the same diameter as the culture dish, one metallic disk (in order to minimize the differences in the field intensity across the whole bottom of the dish) and one of inert material. The field intensities measured in three different zones of the dish bottom have been described in details in a previous work (Chionna *et al.*, 2004). During the entire peri-

od of experiment no increase in temperature was observed. Dishes of cells were always placed on the same two shelves in a tissue culture incubator where the ambient 50Hz magnetic field was 0.95/0.62 μT (heater on/off) and static magnetic flux density was 5.5 μT. In the laboratory areas where the cells were processed (between incubators and worktop) the field measured ranged between 0.08 and 0.14 μT (50 Hz) magnetic fields.

Static MFs were applied concomitantly with differentiation inducers. Exposures were carried out in a blind manner. Simultaneously experiments by omitting the magnets were performed as controls.

### Scanning electron microscopy

Morphological characteristics of differentiated and non-differentiated U937 cells were observed by scanning electron microscopy (SEM). 10<sup>6</sup> cells/mL were fixed with 2.5% glutaraldehyde in cacodilate buffer, pH 7.4, for 1 h at ice temperature, postfixed with 1% OsO<sub>4</sub> in the same buffer and dehydrated. SEM observations were performed on cells deposited on APES-treated (2% Acetone) coverslip slides for non-differentiated cells and on fibronectin 10 µg/mL coverslip slides for differentiated cells. Critical Point Dryer 020 Balzer and Sputter Coated 040 Balzer were used for the final preparation steps. Cells were examined under a Philips XL50 scanning microscope operating at 20 KV.

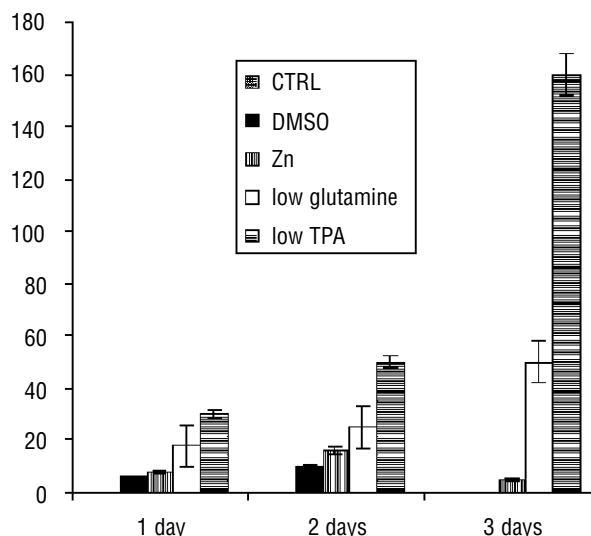
### Cytochemistry and immunofluorescence analysis

U937 cells, fixed with 4% formalin in phosphate buffer pH 7.4 for 10 min. and deposited on gelatinated microscopy slides (for non adhering cells) were analyzed for surface localization of sugars by using 40 µg/mL Concanavalin-A (Con-A) (mannose) and 2 µg/mL fluorescein isothiocyanate (FITC)-conjugated *Ricinus communis* (D-galactose) for 30 min in the dark. FITC-conjugated Phalloidin was used at a concentration of 10 µM for 20 min for actin detection. FITC-conjugated AnnexinV was used to localize phosphatidylinserine (PS) on the cell surface; it was used at a concentration of 20 µM for 20 min at room temperature in the dark. After induction with TPA for 3 days in the absence or presence of static MFs, cells were washed twice with ice-cold PBS and incubated at 4°C with the following FITC-conjugated antibodies: anti-CD11b, anti-CD14, anti-CD 33, anti-CD15. As a negative control, we used an irrelevant FITC-conjugated monoclonal antibody. Samples were observed with a Nikon PCM 2000 microscope (Nikon, Japan) with Plan Fluor objectives (Nikon,

**Table 1. Evaluation of differentiation of U937 cells by light microscopy analysis of cells containing diformazan crystals.**

	Nitro Blue Tetrazolium Test					
	1 d		2 d		3 d	
	- M.F	+ M.F.	- M.F.	+ M.F.	- M.F.	+ M.F.
TPA	++	++	+++	°+++	++++	++++
Zn <sup>++</sup>	+	+	++	++	++	°+
DMSO	+		+	+	--	--
LCG*	+	+	++	++	++++	+++

\*LCG= Low Concentration of Glutamine; +++++ = very high differentiation; ++++ = high differentiation; +++ = moderate differentiation; +/++ = low/ scarce differentiation



**Figure 2. Number of substrate-adhering cells after 1, 2 and 3 days of treatment with different inducers of macrophagic differentiation for light microscopy field 20x. The data are the average of 50 fields examined.**

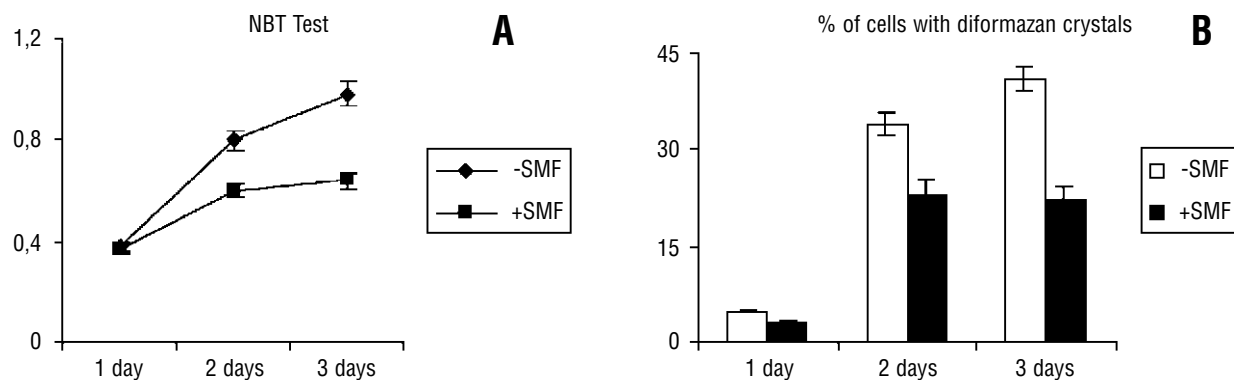
Japan). Confocal microscopy was performed utilizing a confocal Nikon PCM 2000 laser scanning head based on a Nikon Eclipse 600 microscope, equipped with an Argon Laser ED HeNe 488 nm 543 nm source. Acquisition and visualization were completely computer controlled with the EZ 2000 software (Coord-Nikon, The Netherlands).

### Phagocytosis of latex beads

Fluorescent latex beads, 2 µm in diameter, were added, in a ratio of 10 beads for 1 cell, to the culture medium of U937 cells, differentiated in the presence and absence of static MFs. After 15 min., 30 min., 1, 2, and 3 h, cells were washed at least 10 times in PBS and then fixed with 4% formalin. U937 cells were further stained with FITC-conjugated phalloidin. Samples were observed with conventional and confocal fluorescent microscopy, and cells that had adhered or phagocytosed latex beads were counted.

### Results

U937 cells are round shaped cells with short microvilli, scarce cytoplasm and a large bean-shaped nucleus. U937 cells can be induced to differentiation by a large number of substances (TPA, DMSO, Zn<sup>++</sup> and low concentration of glutamine). In their differentiated stage these cells assume a typical macrophagic aspect, adhere to substrate, and change morphology: they assume a more irregular flat shape



**Figure 3. Evaluation of differentiation of U937 cells by NBT test. A) spectrophotometric analysis of the insoluble, blue coloured compound (diformazan) formed by the reducing substances (i.e. esterase acid) synthesized during differentiation induced by TPA; B) percentage of cells with diformazan crystals in the cytoplasm evaluated by morphological analysis at light level.**

with long pseudopodia and cytoplasmic protrusions, and acquire phagocytotic capability and a positive reaction to NBT (Figures 1, 2 and Table 1).

### ***TPA is the most effective macrophage-inducer of U937 cells***

In our experiments, the most efficient molecule able to induce macrophage differentiation of U937 cells (as revealed by the large number of substrate-adhering cells, NBT, the typical morphology of macrophages and phagocytotic capability) was TPA, followed in decreasing order by low glutamine concentration, Zn<sup>2+</sup> and DMSO (Figure 2 and Table 1). Differentiation of U937 cells induced by TPA started soon after the first day of incubation and increased progressively up to the third day (Figure 3). U937 cells were not induced to differentiate when cultured in the presence of fibronectin substrate; indeed, these cells maintained their round shape and were used as controls in all experiments. The degree of U937 cells differentiation was time-dependent; it increased during the second and the third day of treatment. The number of adhering cells was, in turn, a function of the degree of differentiation (Figure 2): very low after two days of culture, dropping almost to zero at the third day for control cells and for Zn<sup>2+</sup> and DMSO treated cells; conversely, for low glutamine concentration and, particularly, for TPA-treated cells, the number of substrate-attached cells increased after 3 days, thus indicating their ability to induce macrophage differentiation. TPA-differentiated U937 cells were used in all the experiments described below unless specified.

### ***Static magnetic fields inhibit TPA-induced differentiation of U937 cells***

When U937 cell differentiation took place under

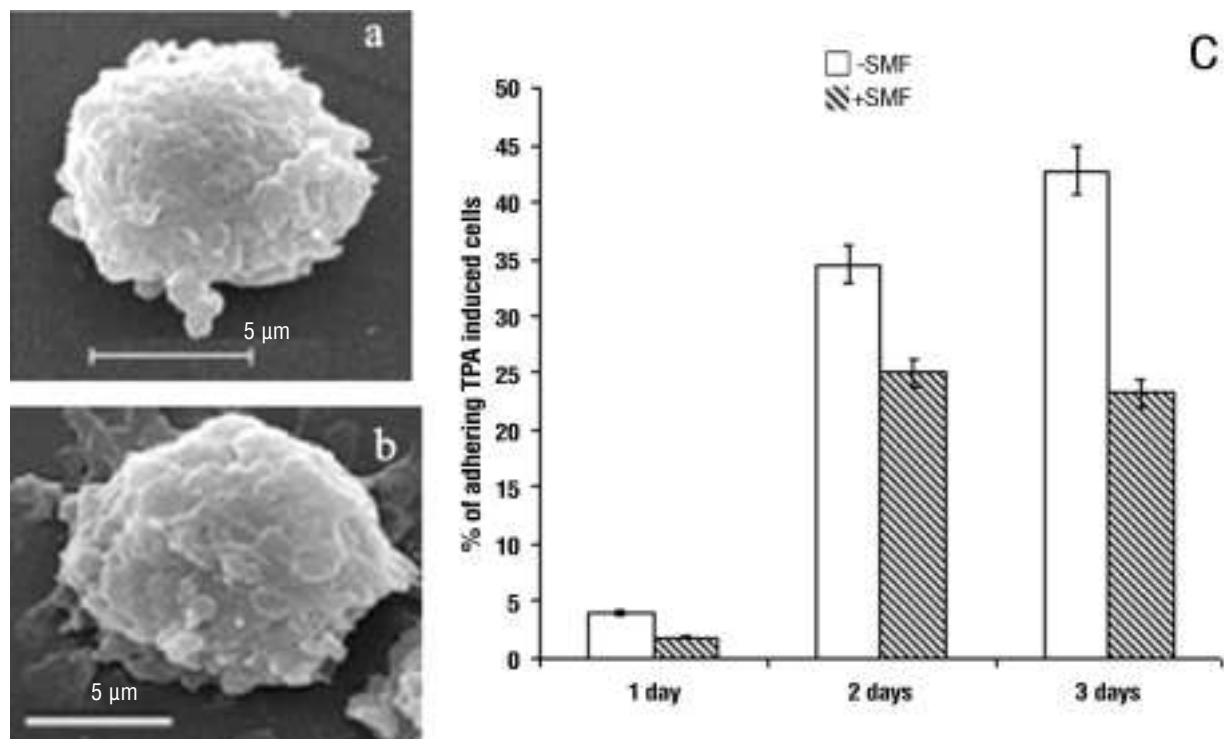
static MF exposure, a decreased degree of differentiation was found at all times and for all parameters measured (morphology, NBT, phagocytosis), thus showing the inhibitory effect of SMFs during macrophage differentiation of U937 cells induced by TPA (Figure 3).

### ***Static MFs affect cellular shape and substrate adhesion in differentiated U937 cells***

Differences regarding cellular shape were also analysed in macrophage differentiated cells in the presence of static MFs; the microvilli of static MF-exposed undifferentiated cells became lamellar or bubble like (compare Figure 1 b and Figure 4 a). U937 cells differentiated under MFs were characterized by an only slightly flattened shape (Figure 4 b) and, often at one pole of the cell, by protrusions and pseudopodia that conferred an elongated shape and foamy appearance on the cells. In general, the adhesion rate of exposed cells was lower than that of non-exposed cells, at all measuring times. When cells were treated with TPA in the presence of static MFs, the number of substrate-attached cells fell (Figure 4 c); this is in agreement with the lower degree of differentiation of exposed cells compared to those maintained in cultures in the absence of static MFs.

### ***Static magnetic fields interfere with cell surface molecules expression***

Mature macrophages are characterized by the expression of a specific set of molecules on their cell surface (i.e., integrins, adhesion molecules, phosphatidyl serine, etc.). By immuno and cytochemical fluorescence investigations the expression of PS, CD11, CD14, CD15, CD33 and sugar residues were studied.



**Figure 4.** Influence of static MF 6 mT exposure on the morphology of undifferentiated (a) and TPA-differentiated (b) U937 cells. Differentiation under exposure to 6mT static MF significantly affected the number of adhering cells (c).

PS is constitutively expressed on the surface of mature macrophage (Callahan *et al.*, 2000), and U937 cells induced with phorbol 12-myristate 13-acetate (PMA) to differentiate into mature macrophages develop PS on their surface (Williamson and Schlegel, 2002). In our study, which used TPA, PS was also expressed during differentiation on the developing adhesion uropodes (Figure 4 a). The exposure to static MF of undifferentiated U937 cells led to heavy labelling of the surface with AnnexinV-FITC conjugates. A similar increase in PS expression on the cell surface was found in cells differentiated in the presence of static MF when compared with those differentiated in the absence of static MF (Figures 5 b, c). TPA induced rapid adhesion to the plastic surface, accompanied by a significant increase in cell surface expression of CD11 molecules and the myelomonocytic antigen CD14 (Figure 6). In contrast, CD33 did not change and CD15 was down-modulated (data not shown). Exposure to static MF leads to a further increment of surface expression of CD11 and CD14 during the macrophage differentiation as well as in undifferentiated cells (Figure 6). Non significant changes of CD33 and CD15 surface expression were observed for both

undifferentiated and differentiated cells exposed to static MF.

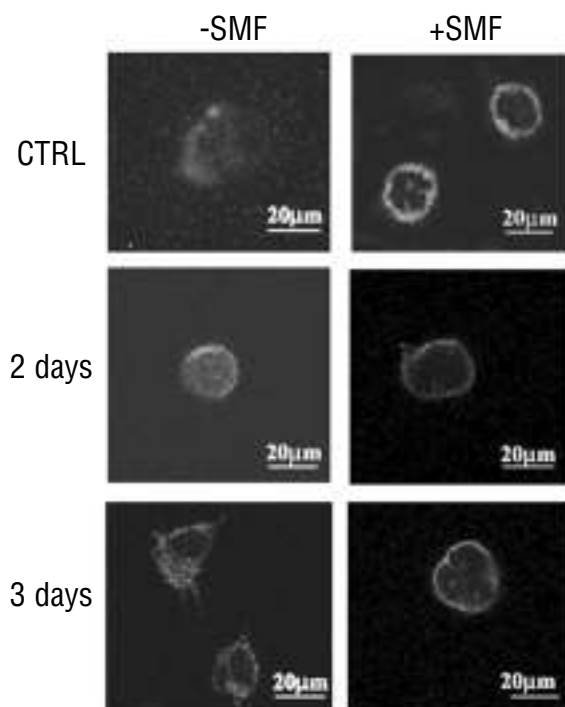
By using FITC-conjugated lectins (Con-A for mannose residues and *Ricinus communis* for galactose residues), the expression of mannose and galactose moieties were localized on differentiated U937 cells in the presence and absence of static MFs. In a previous study, we showed that undifferentiated U937 cells, in the absence of static MFs, express abundant residues of both mannose and galactose on their surfaces (Chionna *et al.*, 2003). When cells are cultured for 24 h in the presence of static MFs, the intensity of the lectin-FITC fluorescence decreased, indicating a decrement of mannose and galactose moieties on the cell surface (Chionna *et al.*, 2003).

In differentiated U937 cells, the mannose residues disappeared from the surface; however, Con-A binding sites were detected only after Triton pre-incubation of cells, thus indicating preferential cytoplasmic localization of mannose residues (the highest intensity was shown after three days of incubation with TPA) in the mature macrophages (Figure 7). Con-A-FITC labelling inside the cytoplasm was further enhanced with the presence of static MFs during TPA induction. Conversely, galac-

tose residues, whose surface expression was higher in differentiated cells with respect to non-differentiated ones, were more abundantly expressed on the surface of cells differentiated in the absence of static MFs than on the surface of cells differentiated in the presence of static MFs. With or without exposure to static MFs, galactose residues inside the cells were very scarce (data not shown).

### Static MFs affect F-actin distribution

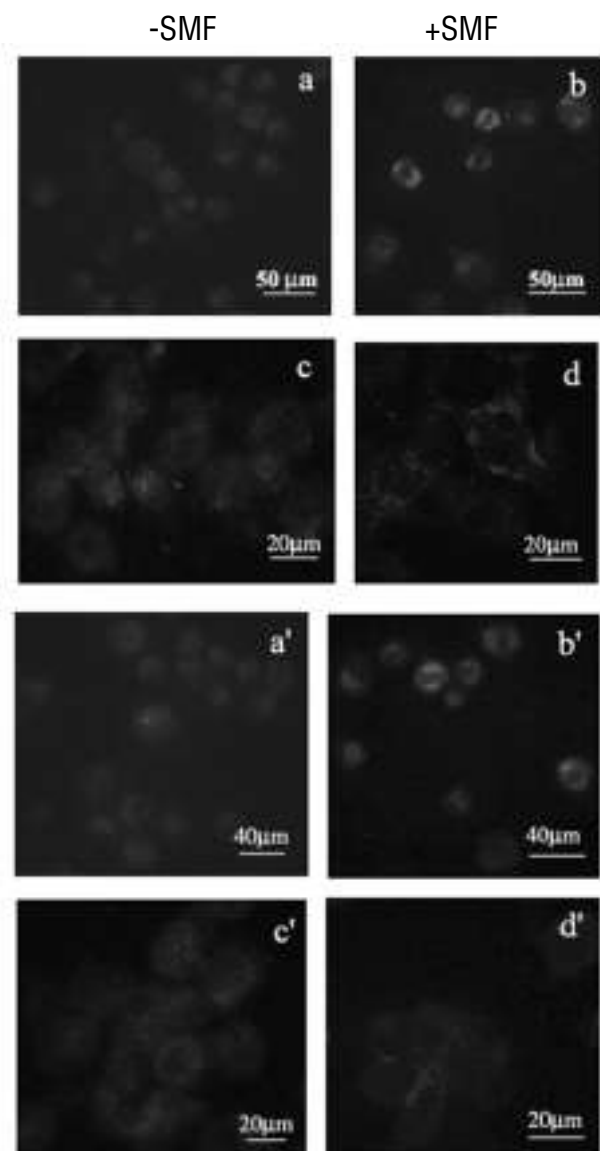
In addition to their roles in cell adhesion, integrins regulate cytoskeletal organization and mediate transmembrane signal transduction (Yamada and Miyamoto, 1995). Cytoskeleton modification affects cell shape; this is supported by the observation that F-actin in the differentiated cells, as detected by FITC-conjugated phalloidin, is highly expressed in the cytoplasmic protrusions. F-actin in non-differentiated U937 cells is de-arranged following exposure to static MFs (Chionna *et al.*, 2003), while in the static MF-exposed differentiated cells the fluorescence is polarized in the cytoplasmic protrusions and pseudopodia (Figure 7).



**Figure 5.** AnnexinV-FITC binding to TPA-differentiated U937 cells at the second and third day of treatment) and to undifferentiated cells exposed 24 h to 6mT static MF.

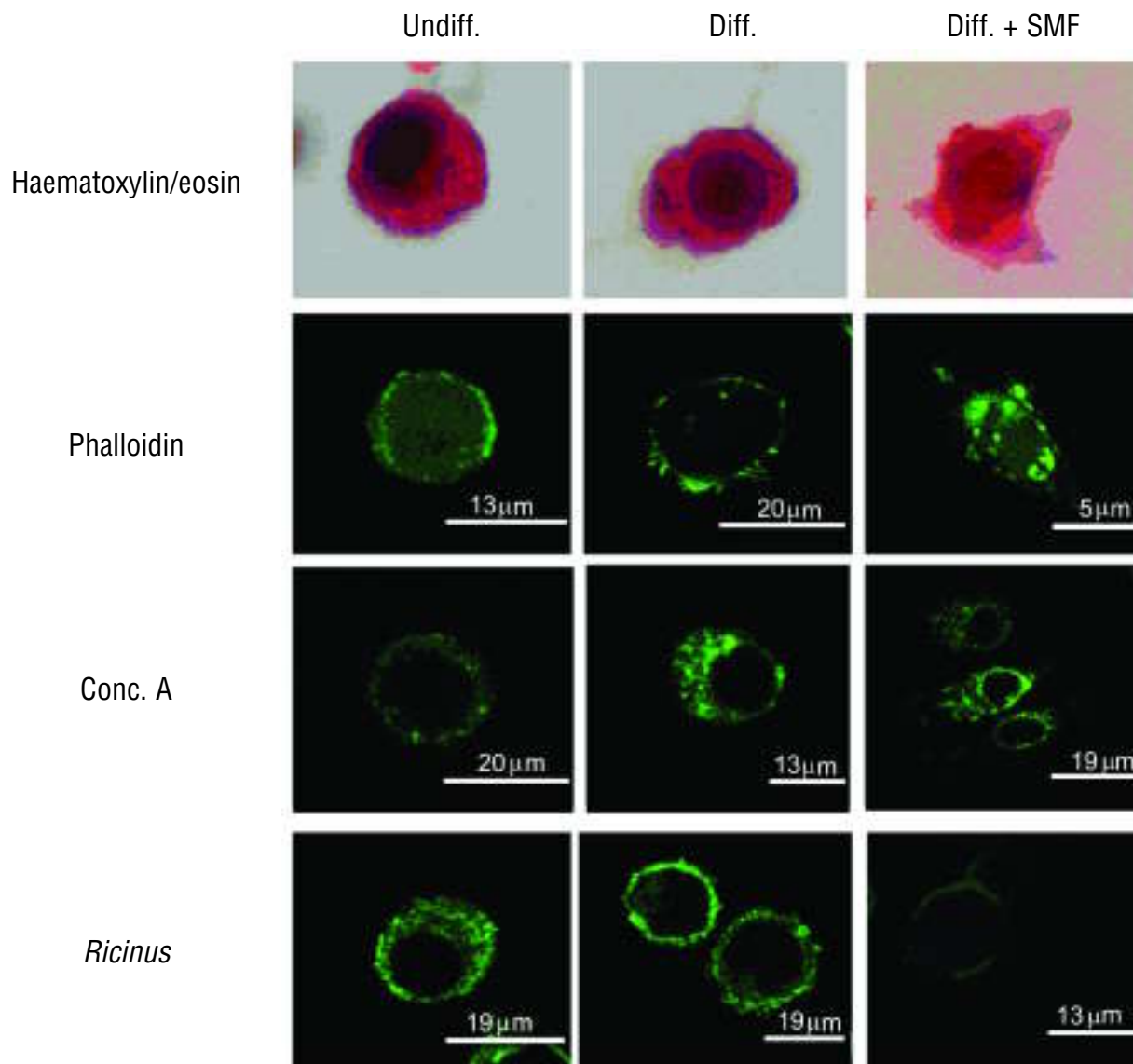
### The influence of static MFs on phagocytosis

One of the typical properties of mature macrophages is their ability to phagocytose. The ability of TPA-differentiated U937 cells to phagocytose latex particles was tested in the presence or absence of static MFs for 3 days. U937 cells acquire the ability to phagocytose only after macrophagic differentiation. Differentiated U937 cells actively phagocytosed latex particles, but exposure to static MFs for up to 2 h reduced the number of cells internalizing them. However, the phagocytotic indices of both exposed and non-exposed cells were almost comparable after 3 h of



**Figure 6.** Fluorescence light microscopy of anti CD11 and CD14 FITC-labelled antibodies to undifferentiated (a, b, a', b') or TPA differentiated (c, d, c', d') U937 cells in the absence (a, c, a', c') or presence (b, d, b', d') of 6mT static MF.





**Figure 7.** Conventional (first line) light micrographs of U937 cells, undifferentiated and differentiated in the presence or in absence of 6mT static MF, stained with haematoxylin/eosin. Confocal micrographs (from second to fourth line) of U937 cells, undifferentiated and differentiated in the presence or in absence of 6mT static MF, labelled with phalloidin-FITC, ConA-FITC and *Ricinus Communis*-FITC conjugates. Confocal images were obtained as described in Materials and Methods section).

phagocytosis (Figure 8), thus suggesting that exposure to static MFs merely delays the process of phagocytosis. Conversely, a comparison of the percentage of cells phagocytosing latex particles with the percentage of cells with latex particles adhering to the cell surfaces, shows that adhesion is favoured under exposure to static MFs, in particular at the longer time examined. Therefore, there is an increase in the percentage of cells that bind but do not ingest latex particles. It appears that exposure to static MFs favours adhesion but slows down ingestion (Figure 8).

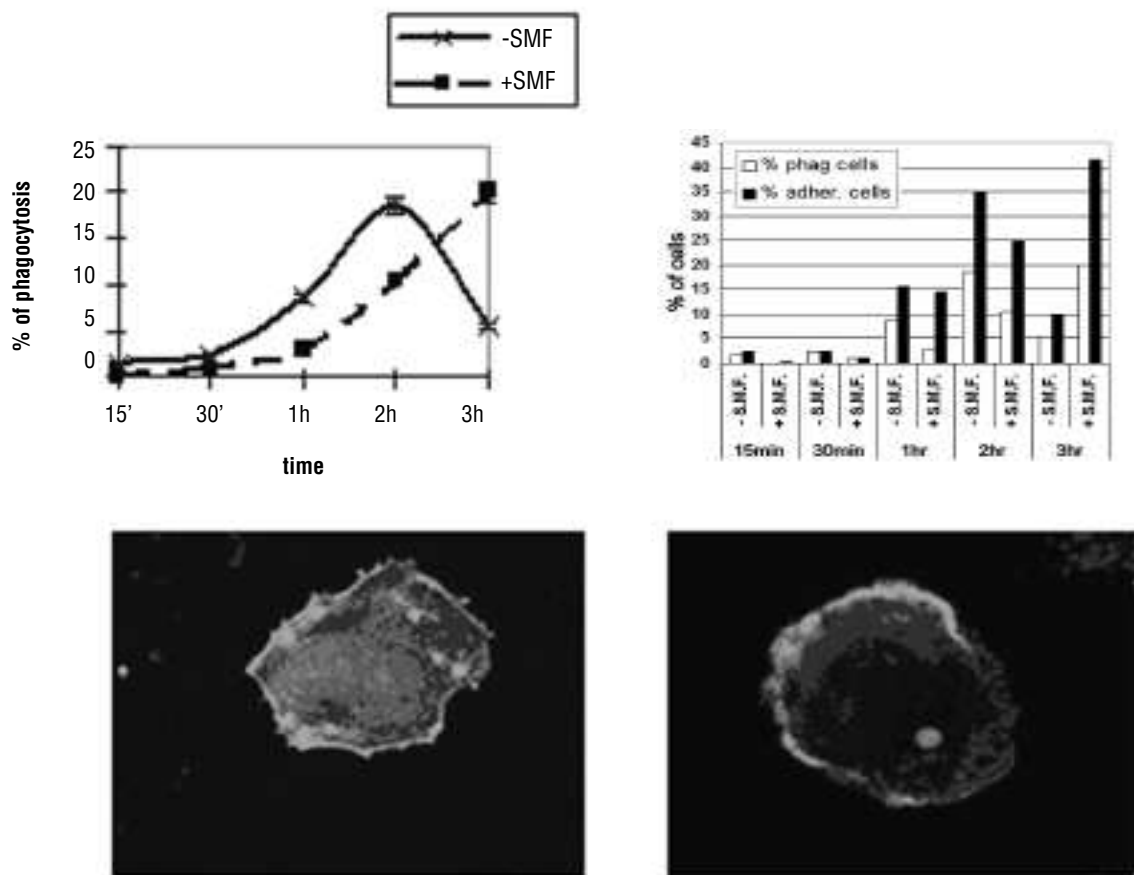
## Discussion

The influence of static MFs on biological systems has drawn the interest of many researchers (Rosen, 2003a; Tenforde, 1985), aimed to explain the biological effects of these fields. Static MFs have been classified into weak (< 1 mT), moderate (1 mT to 1 T), strong (1 T to 5 T), and ultra-strong (>5 T). A number of organisms are using weak geomagnetic fields for spatial orientation and navigation; indeed, biogenic magnetite is used as a field sensor. The ability of strong and ultra-strong fields to alter



the orientation of a variety of diamagnetic anisotropic organic molecules, has been considered the cause of many of their biological effects. Conversely, the effects in several biosystems of moderate-intensity static MFs (used in the present work) are still controversial. The phenomenological descriptions of the initial reports, have been followed by studies aimed to define their mechanism of action. A virtual explanation to all the bio-effects attributed to moderate static MFs could be due to the effects of these fields on the molecular structure of excitable membranes. As a consequence, the function of embedded ion-specific channels is modified. Our data support the widely-held opinion that the plasma membrane is the primary site of action of static MFs (Rosen, 2003b; Tenforde, 1985; Santoro *et al.*, 1997; Panagopoulos *et al.*, 2000;

Cho *et al.*, 1999). Exposure to static MFs gave rise to a series of modifications irrespective of the differentiated or undifferentiated state of U937 cells; cell surface and shape and F-actin distribution were both affected, as was the expression of integrins, surface antigens and sugar residues (Chionna *et al.*, 2003). The effect of 6 mT static MFs on the macrophage differentiation of U937 cells, measured by monitoring the production of reducing substances, by morphological and cytochemical observations and by evaluation of the ability to phagocytose latex particles, is reported here, providing evidence of the biological effects of moderate static MFs. In our study, the molecule which induced macrophage differentiation of U937 cells most efficiently was TPA. TPA-differentiated cells (in particular those treated for three days) acquired typi-



**Figure 8.** Phagocytosis indices of differentiated U937 cells allowed to internalise latex particles of 2  $\mu$ 82m diameter in the absence and presence of 6mT static MF. a) time course of latex particles phagocytosed by exposed or non-exposed TPA-differentiated U937 cells; b) time course of the percentage of cells internalising latex particles or just binding them to their surface in the absence or presence of 6mT static MFs. The values are related to one representative experiment out of four performed; c, d) fluorescent micrographs of TPA-differentiated U937 cells showing fluorescent internalised latex particles labelled with phalloid-FITC. The difference in the internalisation capability in the absence (c) or presence (d) of 6mT static MFs is clearly indicated by the different number of particles inside the cells.

cal macrophage morphology, expression of specific surface antigen and integrins and phagocytotic capability. Indeed, phorbol esters are known to induce, with high efficiency, differentiation of monocytic cells, most likely through interaction with PKC (Hosaya and Maranouchi, 1999; Takada *et al.*, 1999; Hass *et al.*, 1989; Hass *et al.*, 1989; Ferriera *et al.*, 1991; Liu and Wu, 1992; Roth and Polin, 1992; Jarvinen *et al.*, 1993). Conversely, our data are not in agreement with those of other authors (Jarvinen *et al.*, 1993; Tobias *et al.*, 1987; Prieto *et al.*, 1994; Katoh *et al.*, 1998; Koyama *et al.*, 2000), which show that fibronectin substrate saturated with BSA alone is able to induce U937 cells to differentiate; they are in agreement however with data showing that Zn<sup>++</sup>, DMSO and low concentrations of glutamine are poor inducers of macrophagic differentiation (Splitter *et al.*, 1997).

Knowledge of the effects of static MFs on U937 cell differentiation is scarce and controversial. The data here reported indicate that exposure to static MFs interferes with U937 cell differentiation. Indeed, exposure to static MFs diminished the number of TPA-differentiated cells. Differences with respect to our results, likely due to different experimental conditions i.e. the type of field (static as opposed to oscillating) and its intensity, have been reported by Tao (1999), who showed that exposure of HL-60 cells to 60-Hz EMF at 1 G is approximately equivalent (in terms of differentiation) to treatment of the cells with 250-500 pg/mL TPA. Furthermore, both EMF and TPA treatment have an enhancing effect on differentiation at low TPA concentrations (Tao, 1999).

Cell adhesion is a fundamental step in many physiological processes such as development, cell-cell contact, differentiation etc. It is not surprising that any alteration of the surface molecules affects adhesion. The external surface of differentiated and undifferentiated U937 cells exposed to static MFs for at least 24 hrs is dramatically different from that of non-exposed cells (Chionna *et al.*, 2003). However, here we report that not all cell surface molecules are modified in the same way, or to the same extent (quantity and distribution). The reason could be due to the different diamagnetic properties of the individual molecules (Rosen, 1992; 2003a,b). In particular, integrin expression is enhanced, depressed or unaffected at the same time for the different molecules. This could help to explain why substrate adhesion is influenced by

static MFs. Once again, the use of different fields can give different results, as for example with the inhibition of fibroblast adhesion in the presence of 60 and 1000 Hz EMFs, and melanoma cell adhesion in the presence of a static MF using a 4.7-Tesla superconducting magnetic resonance imaging (MRI) magnet (Blumenthal *et al.*, 1997; Short *et al.*, 1992). Mature macrophages are the professional phagocytes of the organism. The ability to bind to and ingest particles is a feature of the macrophagic differentiation of monocytes, which conversely are unable to internalize any kind of particle. Phagocytosis is a very complex phenomenon that requires molecules for the recognition of foreign particles, followed by the rearrangement of the phagocyte shape leading to the engulfment of the particles to be ingested (Rabinovitch, 1995; Aderem and Underhill, 1999; Chimini and Chavier, 2000). Differentiated U937 cells are able to ingest latex particles at a very efficient rate, which however is strongly reduced during the first two hours of incubation with latex particles under static MFs. However, the phagocytotic index reached the values as the control after three hours of incubation with the particles. Surprisingly, the ability to bind to but not to ingest latex particles was always higher under exposure to static MFs, thus confirming the fundamental role of the modifications to cell surface molecules achieved after exposure to static MFs. The reason for such a modified phagocytotic index is not clear. However, it could be hypothesized that many consecutive causes converge, such as variation of Ca<sup>++</sup> concentration, which modulates the cytoskeleton, in turn affecting the ability to engulf; modifications to the cell surface can then affect binding to latex particles. However, data from the literature regarding phagocytosis under static MFs or 50 Hz EMFs, are also controversial; it has been reported to increase as well as decrease for lymphocytes and macrophages (Simko *et al.*, 2001; Flipo *et al.*, 1998).

From what has been reported above, there is substantial evidence to indicate that moderate-intensity static MFs are capable of influencing differentiation of monocytic cells in mature macrophages. Specifically, all the altered values here reported are closely linked to the plasma membrane. Most of the reported effects of moderate static MFs may be explained on the basis of alteration of membrane calcium ion flux (Rosen, 1996). Indeed, Ca<sup>++</sup> has been demonstrated to be affected by static MFs

(Fanelli *et al.*, 1999). In a previous paper, we showed that in lymphocytes and U937 cells, normal or triggered to apoptosis, exposure to static MF of 6 mT caused the concentration of Ca<sup>++</sup> to increase (Chionna *et al.*, 2003). Probably Ca<sup>++</sup> also has a key role in the reduction and/or delay here reported of differentiation and phagocytosis under exposure to static MFs. Ca<sup>++</sup> is very important for PKC induction by TPA and for cytoskeleton structure, whose perturbations influence phagocytosis and the expression and distribution of surface molecules (Chimini and Chavier, 2000; Carraway and Carraway, 1989). Specifically, the exposure of PS on the external surface of undifferentiated U937 cells can be due to modifications in Ca<sup>++</sup> concentration. PS is largely confined, in normal cells, to the inner leaflet of the plasma membrane. In apoptotic cells, PS becomes exposed at the outer leaflet of the membrane. This results from transmembrane lipid scrambling that is not counteracted by the flippases that maintain lipid asymmetry in normal cells (Greenberg and Grinstein, 2002). The kinetics and specificity of the lipid movements induced in these apoptotic cells resemble those seen after activation of the scramblases by elevating cytosolic Ca<sup>++</sup> in normal lymphocytes (Williamson *et al.*, 2001).

In conclusion, our data seem to show a considerable effect of moderate intensity static MFs on the process of macrophage differentiation of monocytic U937 cells induced by TPA. Further studies of the biological effects of static MFs (weak, moderate and low intensity) could help determine the degree to which exposure to static MFs increases the incidence of cancer as indicated by epidemiological studies. These studies might also help to find new strategies for protecting against exposure to MFs as well as to EMFs, which are found everywhere in the environment since they are generated by natural and artificial sources, from the generation and transmission of electricity, to domestic appliances and industrial equipment, telecommunications and broadcasting.

## References

- Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. *Annu Rev Immunol* 1999; 17:593-623.
- Bersani F, Marinelli F, Ognibene A, Matteucci A, Checchi S, Santi S, et al. Intramembrane protein distribution in cell cultures is affected by 50 Hz pulsed magnetic fields. *Bioelectromagnetics* 1997; 18:463-9.
- Blumenthal NC, Ricci J, Breger L, Zychlinsky A, Solomon H, Chen GG, et al. Effects of low-intensity AC and/or DC electromagnetic fields on cell attachment and induction of apoptosis. *Bioelectromagnetics* 1997; 18:264-72.
- Bordiushkov IN, Goroshinskaia IA, Frantsiants EM, Tkacheva GN, Gorlo EI, Neskubina IV. Structural-functional changes in lymphocyte and erythrocyte membranes after exposure to alternating magnetic field. *Vopr Med Khim* 2000; 46:72-80.
- Callahan MK, Williamson P, Schlegel RA. Surface expression of phosphatidylserine on macrophages is required for phagocytosis of apoptotic thymocytes. *Cell Death Differ* 2000; 7:645-53.
- Carraway KL, Carraway CAC. Membrane-cytoskeleton interactions in animal cells. *Biochim Biophys Acta* 1989; 988:147-71.
- Chimini G, Chavier P. Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. *Nat Cell Biol* 2000; 2: E191-96.
- Chionna A, Dwikat M, Panzarini E, Carlà EC, Verri T, Pagliara P, et al. Cell shape and plasma membrane alterations after static magnetic fields exposure. *Eur J Histochem* 2003; 47: 299-308.
- Chionna A, Tenuzzo B, Panzarini E, Dwikat M, Abbro L, Dini L. Time-dependent modifications of Hep G2 cells during exposure to Static Magnetic Fields. *Bioelectromagnetics* 2004 in press.
- Cho MR, Thatte HS, Silvia MT, Golan DE. Transmembrane calcium influx induced by ac electric field. *Faseb J* 1999; 13:677-83.
- Comba P, Grandolfo M, Lagorio S, Polichetti A, Vecchia P. Rischio cancerogeno associato a campi magnetici a 50/60 Hz. *Rapporti ISTI-SAN* 1995; 29.
- Fanelli C, Coppola S, Barone R, Colussi C, Gualandi G, Volpe P, Ghibelli L. Magnetic fields increase cell survival by inhibiting apoptosis via modulation of Ca<sup>++</sup> influx. *Faseb J* 1999; 13:95-100.
- Ferreira OC Jr, Valansky JE, Sheridan K, Wayner EA, Bianco C, Garcia-Pardo A. Phorbol ester-induced differentiation of U937 cells enhances attachment to fibronectin and distinctly modulates the alpha5 beta1 and alpha 4 beta1 fibronectin receptors. *Exp Cell Res* 1991; 193:20-6.
- Flipo D, Fournier M, Benquet C, Roux P, Le Boulaire C, Pinsky C, et al. Increased apoptosis, changes in intracellular Ca<sup>2+</sup>, and functional alteration in lymphocytes and macrophages after in vitro exposure to static magnetic field. *J Toxicol Environ Health A* 1998; 54:63-76.
- Garcia A, Serrano A, Abril E, Jimenez P, Real LM, Canton J, et al. Differential effect on U937 cell differentiation by targeting transcriptional factors implicated in tissue- or stage-specific induced integrin expression. *Exp Hematol* 1999; 27:353-64.
- Greenberg S, Grinstein S. Phagocytosis and innate immunity. *Curr Opin Immunol* 2002; 14:136-45.
- Hass R, Bartels H, Topley N, Hadam M, Koheler L, Goppel-Strude M, et al. TPA-induced differentiation and adhesion of U937 cells: changes in ultrastructure, cytoskeletal organization and expression on cell substrate antigens. *Eur J Cell Biol* 1989; 48:282-93.
- Hosaya H, Maranouchi T. Differentiation and dedifferentiation of the human monocyte leukemia cell line, U937. *Cell Struct Funct* 1999; 17:263-9.
- Jarvinen M, Ylanne J, Virtanen I. The effect of differentiation on the integrin expression K562 erythroleukemia cells. *Cell Biol Int* 1993; 17:399-407.
- Katoh K, Mohri H, Ogawa K, Okubo T. Human plasma fibronectin mediates adhesion of U937 cells by RGD and CS1. *J Thromb Thrombolysis* 1998; 5:125-34.
- Koyama Y, Norose-Toyoda K, Hirano S, Kobayashi M, Ebihara T, Someki L, et al. Type I collagen is a non-adhesive extracellular matrix for macrophages. *Arch Histol Cytol* 2000; 63:71-9.
- Liu MY, Wu MC. Induction of human monocyte cell line U937 differentiation and CSF-1 production by phorbol ester. *Exp Hematol* 1992; 20:974-9.
- Medelsohn N, Gilbert H, Christman J, Acs G. Effects of maturation on the response of human promyelocytic leukaemia cells (HL-60) to the tumor promoter 12-O-tetradecanoyl phorbol-13-acetate. *Cancer Res* 1980; 40:1469-74.
- Milham S. Increased incidence of cancer in a cohort of office workers exposed to strong magnetic fields. *Am J Ind Med* 1996; 30: 702-4.
- Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature* 1984; 308: 693-8.
- Panagopoulos DJ, Messini N, Karabarbounis A, Philippetis, AL, Margaritis LH. A mechanism for action of oscillating electric fields on cells. *Biochem Biophys Res Commun* 2000; 272:634-40.
- Paradisi S, Donelli G, Santini MT, Straface E, Malorni W. A 50-Hz magnetic field induces structural and biophysical changes in mem-

- branes. *Bioelectromagnetics* 1993; 14:247-55.
- Pedrinaci S, Ruiz-Cabello F, Gomez O, Collado A, Garrido F. Protein kinase C-mediated regulation of the expression of CD14 and CD11/CD18 in U937 cells. *Int J Cancer* 1990; 45:294-8.
- Prieto J, Eklund A, Patarroyo M. Regulated expression of integrins and other adhesion molecules during differentiation of monocytes into macrophages. *Cell Immunol* 1994; 156:191-211.
- Rabinovitch M. Professional and non professional phagocytes: an introduction. *Trends Cell Biol* 1995; 5:85-7.
- Rosen AD. Membrane response to static magnetic fields: Effect of exposure duration. *Biochim Biophys Acta* 1992; 1148:317-20.
- Rosen AD. Inhibition of calcium channel activation in GH3 cells by static magnetic fields. *Biochim Biophys Acta* 1996; 1282:149-55.
- Rosen AD. Mechanism of action of moderate-intensity static magnetic fields on biological systems. *Cell Biochem Biophys* 2003a; 39:163-73.
- Rosen AD. Effect of a 125 mT static magnetic field on the kinetics of voltage activated Na<sup>+</sup> channels in GH3 cells. *Bioelectromagnetics* 2003b; 24:517-23.
- Roth P, Polin RA. Induction of monocytic cell adherence to matrix-bound fibronectin by phorbol ester. *J Clin Lab Immunol* 1992; 37:51-63.
- Santoro N, Lisi A, Pozzi D, Pasquali E, Serafino A, Grimaldi S. Effect of extremely low frequency (ELF) magnetic field exposure on morphological and biophysical properties of human lymphoid cell line. *Biochem Biophys Acta* 1997; 1357: 281-90.
- Short WO, Goodwill L, Taylor CW, Job C, Arthur ME, Cress AE. Alteration of human tumor cell adhesion by high-strength static magnetic fields. *Invest Radiol* 1992; 27:836-40.
- Simko M, Droste S, Kriehuber R, Weiss DG. Stimulation of phagocytosis and free radical production in murine macrophages by 50 Hz electromagnetic fields. *Eur J Cell Biol* 2001; 80:562-6.
- Somosi Z. Radiation response of cell organelles. *Review Micron* 1999; 31:165-81.
- Spittler A, Oehler R, Goetzinger P, Holzer S, Reissener CM, Leutmezer F, et al. Low glutamine concentrations induce phenotypical and functional differentiation of U937 myelomonocytic cells. *J Nutr* 1997; 127:2151-7.
- St Pierre TG, Dobson J. Theoretical evaluation of cell membrane ion channel activation by applied magnetic fields. *Eur Biophys J* 2000; 29:455-6.
- Takada Y, Hachiya M, Osawa Y, Hasegawa Y, Ando K, Kobayashi Y, et al. 12-O-tetradecanoylphorbol-13-acetate induced apoptosis is mediated by tumor necrosis factor alpha in human monocyte U937 cells. *J Biol Chem* 1999; 274:28286-92.
- Tao Q, Henderson A. EMF induces differentiation in HL-60 cells. *J Cell Biochem* 1999; 73:212-7.
- Tenforde TS. Mechanisms for the biological effects of magnetic fields. In Grandolfo M, Michaelson SM, Rindi AV, eds. *Biological effects and dosimetry of static magnetic fields and ELF electromagnetic fields*. Plenum Press, New York, 1985, pp. 71-92.
- Tobias JW, Bern MM, Netland PA, Zetter BR. Monocyte adhesion to subendothelial components. *Blood* 1987; 69:1265-8.
- Triantafillos C, Andreas EM, Klaus TP, Sandip MK. Molecular mechanism of sinc-dependent leukocyte adhesion involving urokinase receptor and beta2-integrins. *Blood* 1999; 93:2976-83.
- Williamson P, Christie A, Kohlin RA, Schlegel P, Comfurius P, Harmsma M, et al. Phospholipid scramblase activation pathways in lymphocytes. *Biochemistry* 2001; 40:8065-72.
- Williamson P, Schlegel RA. Transbilayer phospholipid movement and the clearance of apoptotic cells. *Biochim Biophys Acta* 2002; 1585: 53-63.
- Yamada KM, Miyamoyo S. Integrin transmembrane signalling and cytoskeletal control. *Curr Opin Cell Biol* 1995; 7:681-89.