ELF- magnetic fields and immune response modulation

Working Module Proposal

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COST "EMF-MED"

Proposed by

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1. Working Module Description

The effects of extremely low frequency magnetic fields (ELF MF) on biological systems have been studied for decades with focus on possible health risks. Many experimental studies reported various biological effects of exposure to ELF MF, however the biological relevance for these effects is unclear and a convincing evidence for mechanistic explanation is still missing. Nevertheless the modulation of oxidative response after ELF MF exposure has often been described and the release of reactive oxygen species (ROS) or other relevant effects were reported (Simkó, 2004, Simkó & Mattsson, 2004, Simkó, 2007, Mattsson & Simko, 2014). These effects are on a relatively low level (around 30-60 %). At low level "oxidative responses", ROS can act as second messengers and activate signaling cascades, which in turn can lead to physiological responses such as gene expression, metabolic changes, cell proliferation etc., which subsequently can lead to "cell activation". Immune-relevant cells use the reactive potential of ROS also to fulfill important physiological functions such as regulating the vascular tone and the cell functions controlled by oxygen concentration but also to activate the immune system functions.
The aim of this part of the Action is to focus on possible beneficial effects related to oxidative responses and modulation of immune system functions of ELF MF in order to 1) provide a better understanding of underlying physical and biological mode of action and 2) to contribute to the development of innovative EMF-based medical treatment.

Cellular stress can be driven by intrinsic or extrinsic factors causing different downstream processes leading to cellular effects, which in turn can be damaging or cause induction of signal transduction. One of the main research activities proposed here therefore refers to the regulation of redox homeostasis during and after the activation of oxidative processes and the associated cell signalling pathways. The main questions are:

- Is it possible to use weak stress conditions, like moderate increase of oxidative processes initiated by ELF MF, as a positive cell activator e.g. in immune cells?
- Is there a cell type dependency of such activation, mediated e.g. through surface-receptor configurations?
- When is a cellular oxidative process strong enough to cause activation?

We suggest to study ELF-MF affected regulatory mechanisms on the molecular level also in comparison with other stressors. Previous investigations show the cell activating capacity of 1 mT MF. We think, that downstream processes are understood, however it is unknown which cell component is the first target interacting with MF. Is it a receptor on the outside of the cell membrane, or is it inside, as a component of a receptor or of the protein cascade? (Fig. 1).

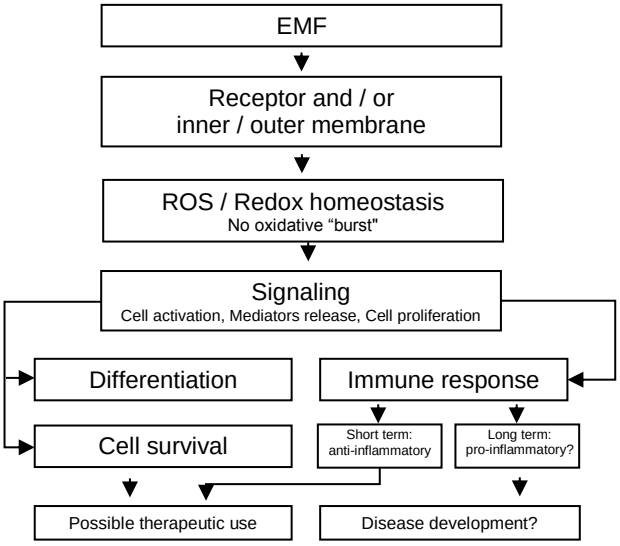

Figure 1

Hypothetical interactions between ELF-MF and living systems
ELF-MF interacts with cellular receptors and/or the inner or outer site of the membrane and activates specific molecular pathways leading to a change of the redox homeostatic capacity of the cell. This can be due to the release of free radicals triggering cell signalling that influences cell activation, release of intermediates, and/or the activation of cell proliferation. Cell signalling can also lead to the activation of differentiation and cell survival, being a helpful effect in therapeutical use. On the other hand, signalling can also trigger the immune response. In a short term perspective, it seems that anti-inflammatory responses are activated, which could be a useful tool for treatment of inflammation. Long term effects however, could trigger pro-inflammatory pathways causing the amplification of or the development of diseases (Mattsson & Simko, 2012, Mattsson & Simkó, 2014)

Specific goals are to elucidate the efficacy and the primary mechanisms of EMF-induced effects alone, and/or in combination with other agents such as nanoparticles. In particular, the induced modulation of the cell type-dependent redox homeostasis by EMF should be investigated on the molecular level. Certain cell membrane receptors, G-proteins and kinases, and intracellular antioxidants are in focus to detect the regulatory mechanisms. The goal is to discover target molecules and/or functional units which are affected by EMF and/ or chemical factors in immune relevant cells.

2. State-of-the-Art

The exposure to environmental extremely low frequency (ELF) electromagnetic fields (EMF) emitted from different sources like power lines, has a connection to cancer, especially childhood leukaemia. The International Agency for Research on Cancer (IARC) and the World Health organisation (WHO) have agreed that EMFs are a possible carcinogen to humans, class 2B (IARC, 2002). However, there is no supporting evidence for this classification from in vivo or in vitro studies. Furthermore, there are no data on mode of action(s) that can provide an explanation for any effect on biological structures at these flux density levels (daily averages exceeding 0.3–0.4 μT). Based on our own results and on the data from the literature, an interaction between EMF and cellular systems is plausible. In our studies we have found that ELF-MF induces ROS production in different immune relevant cell types (Simkó, 2004, Simkó & Mattsson, 2004, Simkó, 2007, Mattsson & Simko, 2014). Further, we could show that MF-exposure induce a slight (moderate) oxidative process. It seems that the induced cell reactions are dependent on the cell type. After stimulation by MF, specific cell reactions are induced and passed on to intracellular signal transduction pathways. The question is which mechanisms are induced, and to what degree these are cell type specific. Our results indicate the activation of cell specific responses and not a specific cell response. It is noteworthy to say that by knowing the mode of

action between cells and MF, the targeted and positive use of MF is more plausible than an application “into the blind”.

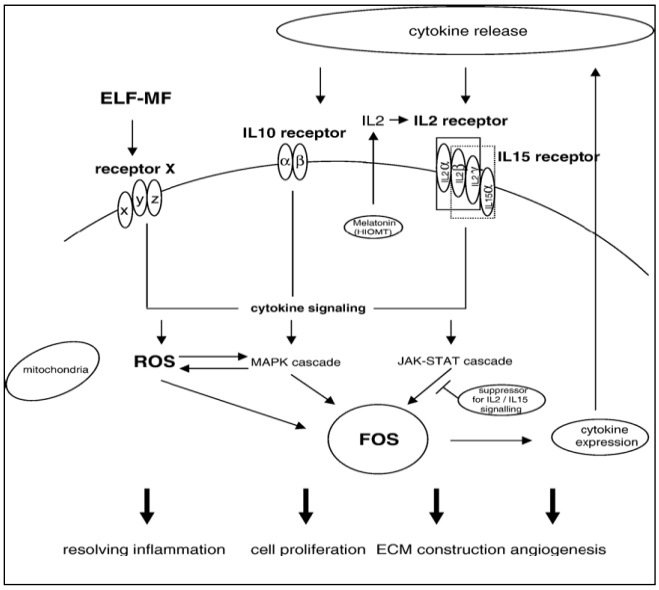

Figure 2 Induction of the alternative activation pathway in human monocytes during 1.0 mT ELF-MF exposure. Cell activating capacity was immediately detected as an increased ROS production after ELF-MF exposure, where we assume that the cell activation is mediated via a non-identified receptor-dependent pathway. Subsequently, ROS initiates the expression of cytokine signalling by an increased expression of the transcription factor FOS, resulting in an increased cytokine release causing the expression of the cytokine receptor IL15RA and IL2RA – two pro-inflammatory cytokine receptors and IL10RA, indicating an anti-inflammatory response. Thus, cytokine signalling maintains ROS release and FOS expression via the APK cascade, while JAK-STAT cascade via IL2 and IL15 receptor might be suppressed by the decreased melatonin production, indicated by a decreased expression of the HIOMT gene, and by the expression of genes encoding proteins for inhibiting IL-2 production (e.g., TCF8) and JAK-STAT signalling (SOCS4). Therefore, we assume that no inflammation is induced by ELF-MF. Furthermore, gene expression profiling and gene kinetic analysis confirm that inflammation is resolved and cell proliferation, ECM construction, and angiogenesis are promoted, indicating the involvement of the alternative pathway (Lupke *et al.* 2006).

The interaction of ELF-MF with cells has been suggested to induce alterations in cell physiological processes such as differentiation, proliferation, and signal transduction. There is evidence that exposure of e.g. mouse macrophages to MF (50 Hz, 1 mT) result in modifications of physiological and biochemical processes leading to immune cell activation (Rollwitz *et al.*, 2004, Frahm *et al.*, 2006). Other functional studies showed the activating capacity of MF in macrophages and monocytes, leading to free radical (ROS) and cytokine (IL-1ß) formation as well as increased phagocytic activity (Simkó *et al.*, 2001, Lupke *et al.*, 2004, Rollwitz *et al.*, 2004, Frahm *et al.*, 2006). Gene expression profiling indicates the alteration of several genes involved in metabolism, cellular physiological processes, signal transduction and immune response in human umbilical cord monocytes (e.g. (Lupke *et al.*, 2006). Lupke et al. (2006) showed, using real-time RT-PCR analysis, that the kinetics of the expression of IL15RA, and IL10RA after EMF exposure suggest the regulation of cell activation via the *alternative pathway* of macrophages, whereas the delayed gene expression of FOS, IL2RA and the melatonin synthesizing enzyme HIOMT suggests the suppression of inflammatory processes (Lupke *et al.*,

2006), Fig 2 and Fig. 3). The alternative pathway of macrophages leads to resolving inflammation, and to the activation of healing, in contrast to the classical pathway (see Fig. 3). An extensive protein screening approach demonstrated a statistically significant modification in the expression level of 124 out of ca. 900 investigated proteins after exposure to 1 mT MF for 45 min. The kinetics of certain proteins involved in important cellular signal transduction pathways, such as redox homeostasis, endocytic processes, and cellular stress were analysed. Using flow cytometry, microscopy and immunochemistry, alterations in the expression of PI3-kinase, PKB, PP2A, gp91phox, clathrin, Hsp70 and Hsp110 were detected (Frahm *et al.*, 2010, Mannerling *et al.*, 2010). These data give further evidence for the modulation of redox regulatory processes in response to MF and for the activation of the alternative pathway of macrophages. Furthermore, we documented that MF induces molecular changes of important proteins which act in intracellular vesicle transport, and PI3-kinase/PKB mediated regulatory processes (Frahm *et al.*, 2010).

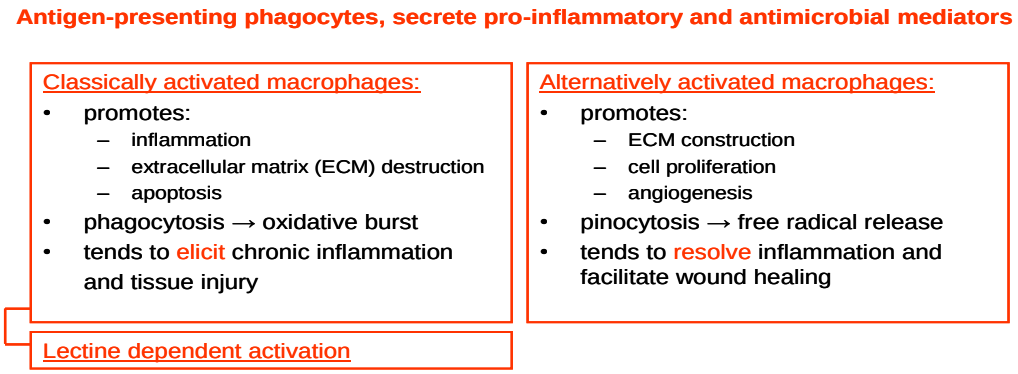

Figure 3 Monocyte/macrophage activation pathways. The left side shows the classical pathway leading to inflammation, apoptosis etc., whereas the alternative pathway (right side) shows the activation of cell proliferation, angiogenesis and resolving inflammation (e.g. activation of healing)

3. Gaps and Challenges

Numerous laboratory studies have examined ELF-EMF effects on cellular processes. Effects are seen from the functional level, like cell proliferation to the molecular level, including modulation of signal transduction pathways and also DNA-damage. Several investigators studied immunological processes after or during EMF-exposure, suggesting an anti-tumoricidal effect of EMF. Other studies found increased cytokine and free radical secretion by immune cells after EMF exposure. Therefore, and due to the findings of the epidemiologic studies, one potential target for ELF-EMF seems to be the immune system.

However, there are many gaps in the knowledge base:

- Interaction mechanisms
- Exposure requirements
- How and if cellular effects will influence tissues and ultimately the organisms
- How reproducible are noted effects

4. Objectives to be achieved

The following motivational points can be identified as trigger for this Working Module:

- Which is the primary interaction site between applied ELF MF and the animal cell?
- Which are the exposure characteristics that allow such an interaction?
- Can exposures affect expression of cell characteristics and activities that promote tissue healing and regeneration?
- Are cellular effects of such magnitude and character that they promote healing or improvement of disease conditions?

5. Proposed Research Activities

The following main research areas could be performed:

I: Identification of the interface between cells and ELF MF

- Comparative analysis of cell activation markers and or products such as the release of various reactive intermediates, antioxidant enzymes etc. in e.g. human immune relevant cells in co-exposure to other stressors.
- Proteomic and genomic analysis of potential protein and gene candidates to detect target molecules such as receptors etc. for interactions
- Specific studies of signal transduction pathways that are influenced by redox changes
- Investigation of cell activation to understand the "best timing" of exposure

II: Evaluation of the relevance of the influenced physiological effects

- Investigations to the beneficial effects of EMFs ("mild stress")
- Establishment of co-cultures e.g. free radical releasing and epithelial cells to investigate whether activated cells contribute to cell communication in non-immune cells to allow e.g. the healing process.
- Identification and evaluation of target tissue(s) for EMF exposure

III: Identification of exposure conditions for cell activity modulation

- Identification of the need of specific exposure parameters such as frequency, frequency modulation, field strength, exposure duration and constancy, cell condition, etc.

IV: Development of a targeted exposure setup for the activation of immune system for medical application

- Establishment of algorithms for targeted EMF application
- Development of personal EMF application by considering and even using already existing metal implants
- Designed medical application for different purposes

6. References

Frahm J, Mattsson MO & Simkó M (2010) Exposure to ELF magnetic fields modulate redox related protein expression in mouse macrophages. *Toxicol Lett* **192**: 330-336.

Frahm J, Lantow M, Lupke M, Weiss DG & Simkó M (2006) Alteration in cellular functions in mouse macrophages after exposure to 50 Hz magnetic fields. *J Cell Biochem* **99**: 168-177.

IARC (2002) Non-ionizing radiation, Part 1: static and extremely low-frequency (ELF) electric and magnetic fields. Vol. 80 p.^pp. 1-395.

Lupke M, Rollwitz J & Simkó M (2004) Cell activating capacity of 50 Hz magnetic fields to release reactive oxygen intermediates in human umbilical cord blood-derived monocytes and in Mono Mac 6 cells. *Free Radic Res* **38**: 985-993.

Lupke M, Frahm J, Lantow M, Maercker C, Remondini D, Bersani F & Simkó M (2006) Gene expression analysis of ELF-MF exposed human monocytes indicating the involvement of the alternative activation pathway. *Biochim Biophys Acta* **1763**: 402-412.

Mannerling AC, Simkó M, Mild KH & Mattsson MO (2010) Effects of 50-Hz magnetic field exposure on superoxide radical anion formation and HSP70 induction in human K562 cells. *Radiat Environ Biophys* **49**: 731-741.

Mattsson M-O & Simkó M (2014) Grouping of experimental conditions as an approach to evaluate effects of extremely low frequency magnetic fields on oxidative response in in vitro studies. *Frontiers in Public Health* **2**.

Mattsson MO & Simko M (2012) Is there a relation between extremely low frequency magnetic field exposure, inflammation and neurodegenerative diseases? A review of in vivo and in vitro experimental evidence. *Toxicology* **301**: 1-12.

Mattsson MO & Simko M (2014) Grouping of Experimental Conditions as an Approach to Evaluate Effects of Extremely Low-Frequency Magnetic Fields on Oxidative Response in in vitro Studies. *Front Public Health* **2**: 132.

Rollwitz J, Lupke M & Simkó M (2004) Fifty-hertz magnetic fields induce free radical formation in mouse bone marrow-derived promonocytes and macrophages. *Biochim Biophys Acta* **1674**: 231-238.

Simkó M (2004) Induction of cell activation processes by low frequency electromagnetic fields. *ScientificWorldJournal* **4 Suppl 2**: 4-22.

Simkó M (2007) Cell type specific redox status is responsible for diverse electromagnetic field effects. *Curr Med Chem* **14**: 1141-1152.

Simkó M & Mattsson MO (2004) Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: possible immune cell activation. *J Cell Biochem* **93**: 83-92.

Simkó M, Droste S, Kriehuber R & Weiss DG (2001) Stimulation of phagocytosis and free radical production in murine macrophages by 50 Hz electromagnetic fields. *Eur J Cell Biol* **80**: 562-566.