

Exposure of rats to extremely low-frequency electromagnetic fields (ELF-EMF) alters cytokines production

Iraj Salehi¹, Karim Ghazikhanlou Sani² & Alireza Zamani^{3,4}

¹Department of Physiology, School of Paramedical Sciences, Hamadan University of Medical Sciences, Hamadan, Iran, ²Department of Radiology, School of Paramedical Sciences, Hamadan University of Medical Sciences, Hamadan, Iran, ³Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran, and ⁴Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Investigations indicate a potential link between exposure to extremely low-frequency electromagnetic field (ELF-EMF) and some cancers. Carcinogenesis of ELF-EMF may be mediated by effect on the immune system. During an immune response, naïve T cells differentiate to effector type 1 helper T cells (T_H1), T_H2, or T_H17 subsets according to existence of different cytokines and T_H1 is important in defense against tumors. Therefore, it will be reasonable to test whether ELF-EMF can change cytokines like interferon gamma (IFN- γ), interleukin-4 (IL-4), IL-6, and IL-12 that regulate T_H1/T_H2/T_H17 balance. Forty adult male rats were randomly separated into ELF-EMF-exposed and sham-exposed control groups. The ELF-EMF group was exposed to a flux density of 100 μ T, frequency 50 Hz, 2 h/day for 3 months. The controls were placed in identical chamber without ELF-EMF. The results showed there were no significant differences between the mean mass of rats, thymuses, and spleens in ELF-EMF exposed group compared with controls. Serum IL-12 level was decreased from 418 \pm 47 pg/ml in controls to 300 \pm 23 pg/ml ($p < 0.05$) in ELF-EMF-exposed group. Phytohemagglutinin activated *in vitro* production of IL-6 by the whole spleen culture (1356 \pm 92 pg/ml) and total blood culture (418 \pm 40 pg/ml) of ELF-EMF-exposed rats were higher ($p < 0.001$) comparing with controls (905 \pm 74 pg/ml), (182 \pm 26 pg/ml), respectively. However, the levels of IFN- γ , IL-4, and IL-6 of serum and IFN- γ , IL-4, and IL-12 in spleen culture and total blood culture of two groups were not significantly different. It seems that ELF-EMF may change T_H1/T_H2/T_H17 balance toward down regulation of T_H1 and upregulation T_H17 type responses.

Keywords Cytokine, T-lymphocytes, Electromagnetic fields, Interleukin, Interferon

INTRODUCTION

Exposure to extremely low-frequency electromagnetic field (ELF-EMF) is increasing with wide demand and our daily use of ordinary electrical appliances and communication devices. Whether ELF-EMF induces health hazards has been

Address correspondence to Alireza Zamani, Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran. E-mail: a_zamani@umsha.ac.ir

disputed since the issue first became prominent (Marino, 1978) and the results in this respect are equivocal. Therefore, more experimental works relevant to the identification of possible mechanisms are needed (Roosli et al., 2010; Hardell and Sage, 2008). According to the some investigations, a potential link between exposure to ELF-EMF and cancer (Hardell and Sage, 2008) can be due to the effect of ELF-EMF on the immune system which is critical for protecting an organism against development and growth of tumors (Mevissen et al., 1998).

In an immune system response, naïve CD4⁺T cells which mature in the thymus have a major role. During immune response, antigens are presented by antigen presenting cells (APCs) to these cells and they become activated and differentiate into effector type 1 helper T cell (T_H1), type 2 helper T cell (T_H2), or type 17 helper T cell (T_H17) subsets according to different cytokines milieu (Harrington et al., 2005; Mosmann et al., 1986; Mosmann and Coffman, 1989; Park et al., 2005). T_H1 cells are essential for clearing tumors, intracellular bacteria, and viruses. T_H1 differentiation is induced by interleukin12 (IL-12) and interferon gamma (IFN- γ) which are secreted, respectively, by APCs and already differentiated T_H1 and natural killer cells. T_H2 cells are essential in eliminating cellular pathogens and their differentiation depend on interleukin4 (IL-4). The T_H17 subset has strong proinflammatory effect and interleukin6 (IL-6) can induce naïve CD4⁺T cells differentiate to T_H17 subset (Xu et al., 2010; Saito et al., 2010). It can be concluded that cytokines are important modulators of immune functions and especially detection of IFN- γ , IL-4, IL-6, IL-12 can be a sensitive indicator for perturbation of the immunoregulator network by external or internal factors (Aldinucci et al., 2003; Jonai et al., 1996).

These days, most of the world's population is chronically exposed to electromagnetic fields of less than 0.1 microtesla and, according to the International Commission on Non-Ionizing Radiation Protection (ICNIRP), the maximum limit of 100 μ T for 50 Hz exposure is recommended (Bioinitiative Report, 2007). Therefore, the present study was conducted to evaluate the effect of 100 μ T of ELF-EMF on serum levels of IFN- γ , IL-4, IL-6, and IL-12 and their *in vitro* production when activated with Phytohemagglutinin (PHA) in whole spleen culture supernatant and total blood culture supernatant in rats.

MATERIALS AND METHODS

Animals

Forty albino Wistar three-month-old-male rats were obtained from the animal facilities of Razi Institute, Karaj, Iran. The rats were housed in plastic cages (5 in each) under 21–22°C conditions, humidity 55–65%, and light (6 am–6 pm) and dark (6 pm–6 am) cycles. After one week of adaptation, the animals were randomly separated into ELF-EMF-exposed and sham-exposed control groups. Food and water were available *ad libitum*.

We used the one-exposure system. First of all, the ELF-EMF-exposed group were exposed to a flux density of 100 μ T (i.e., 1 Gauss), frequency 50 Hz, 2h/day for 3 months from 10–12 am, and then all the sham-exposed controls were placed in identical chamber with no electromagnetic field from 12–14.

All animals were weighed and sacrificed under Thiopentone (50 mg/kg) anesthesia from 12–2 pm. Then, the total blood of the animals was drained from vena cava vein and the blood were collected in two bottles, one containing ethylenediamine tetraacetic acid (EDTA), as anticoagulant, and the other without anticoagulant. At the end, spleens and thymuses were aseptically removed and weighed (Zamani et al., 2009).

Electromagnetic Exposure System

The exposure unit was comprised of a solenoid coil with length of two m and radius of 20 centimeters that the turns were 1,000 per meter (Fig. 1). The diameter of the copper wire was two millimeters. A 220 V and 50 Hz sinusoidal power frequency current was fed through the solenoid in the exposure system. An adjustable resistance circuit was connected to the power source to set out the electric field and consequently the magnetic field. The prepared circuit was able to generate an effective magnetic field in the range of 0–2000 μT , with sinusoidal wave of frequency of 50 Hz. The magnetic flux density was measured with a teslameter (HI-3604,

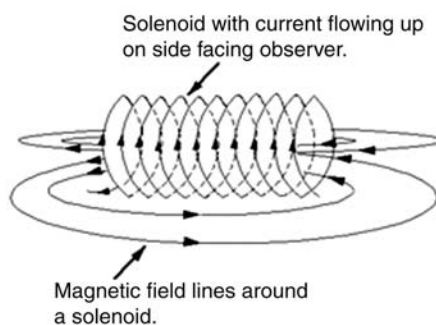


FIGURE 1 The electromagnetic exposure system. The ELF-EMF-exposed group were exposed to ELF-EMF in their home cages at the middle of the exposure system and the sham-exposed controls were placed in the system with no electromagnetic field. The temporal homogeneity is defined by measuring the magnetic field strength at different times. The calculated magnetic field strength was $99 \pm 2 \mu\text{T}$. Due to the excessive length of the solenoid (2 m), the spatial homogeneity was not assessable. But according to physical rules, the magnetic field lines are parallel to each other and the homogeneity must be in an acceptable range. The background magnetic strength in rats kept in place was about $0.07 \pm 0.03 \mu\text{T}$.

Holaday, USA) and the density was adjusted by varying the coil current using external resistance circuit (Mevissen et al., 1998).

***In Vitro* Production of IFN- γ , IL-4, IL-6, and IL-12 by the Whole Spleens**

To determine *in vitro* production of IFN- γ , IL-4, IL-6, and IL-12, each spleen was washed three times with Hanks' solution and completely suspended in 2 ml RPMI1640 medium (Gibco-BRL, Australia) containing 100 U/ml penicillin G (Hayan, Iran), 10% FCS (Gibco-BRL, Australia), 100 μ g/ml streptomycin (Hayan, Iran), and 1 μ g/ml PHA (Sigma, Germany), and incubated for 24 h in CO₂ incubator at 37°C. Thereafter, the supernatants were collected and frozen until cytokines measurement (Taniguchi et al., 1994).

***In Vitro* Production of IFN- γ , IL-4, IL-6, and IL-12 by the Total Blood Cells**

1 ml of fresh blood containing anticoagulant was suspended in 1 ml complete RPMI₁₆₄₀ medium (like above), placed in 12-well plates and incubated for 24 h in CO₂ incubator at 37°C. After incubation, the supernatants were collected and kept frozen until cytokines assay (Taniguchi et al., 1994).

Cytokine Measurement by ELISA

Serum levels of IFN- γ , IL-4, IL-6, and IL-12, as well as supernatant levels of these cytokines, were determined by sandwich enzyme-linked immunosorbent assays according to the manufacturer's instructions (Bender Medsystem, Vienna, Austria for IFN- γ , IL-4, IL-6; Invitrogen, California, USA for IL-12). All assays were carried out in duplicate.

Statistical Analysis

Results were expressed as mean \pm standard error. Unpaired Student's *t*-test was used to compare the mean of the examined groups. All comparisons were two-sided with *P* values that are noted for each group to indicate statistical significance and *P* < 0.05 was considered as the minimum level of significance. The statistical software used for this analysis was SPSS version 16.

RESULTS

The data in Table 1 show that three months of treatment with a flux density of 100 μ T, frequency 50 Hz, 2 h/day, and 7 days a week was not able to increase or decrease the mass of the rats, spleens, and thymuses. Therefore, in respect to spleens and thymuses, ELF-EMF exposure possibly has no effect on the amount of cells in these two important organs of the immune system in the rats.

Although the levels of IFN- γ , IL-4, and IL-6 in the serum of the rats were not significantly altered in both groups (Figs. 2A-C), the level of serum IL-12 was

TABLE 1 Effect of ELF-EMF on the mass of the rats, spleens, and thymuses.

Mass	Sham-exposed control (grams)	No.	ELF-EMF exposed	No.	<i>P</i> Value
Rats (pre-exposed)	201 \pm 12	20	196 \pm 16	20	0.662
Rats (post-exposed)	315 \pm 15	20	302 \pm 16	20	0.569
Spleen	1.466 \pm 0.14	20	1.468 \pm 0.23	20	0.994
Thymus	0.296 \pm 0.02	20	0.259 \pm 0.03	20	0.302

*Results are presented as mean \pm SEM

Unpaired Student's *t*-test was used to compare the mean of two groups. All comparisons were two-sided with *P* values that are noted for each group to indicate statistical significance.

decreased to 300 ± 23 pg/ml in ELF-EMF-treated rats from 418 ± 47 pg/ml in sham-exposed control ($p < 0.05$) (Fig. 2D).

The levels of IFN- γ , IL-4, and IL-12 were not changed in the supernatant of the whole spleens cultures and the total blood culture of two groups when activated with PHA for 24 h (Figs. 2A, B, and D) but production of IL-6 by the whole spleens cultures (1356 ± 92 pg/ml) and the total blood culture (418 ± 40 pg/ml) of ELF-EMF-exposed rats were higher than production of IL-6 by the whole spleens cultures (905 ± 74 pg/ml) and the total blood culture (182 ± 26 pg/ml) ($p < 0.001$) of sham-exposed controls, respectively (Fig. 2C).

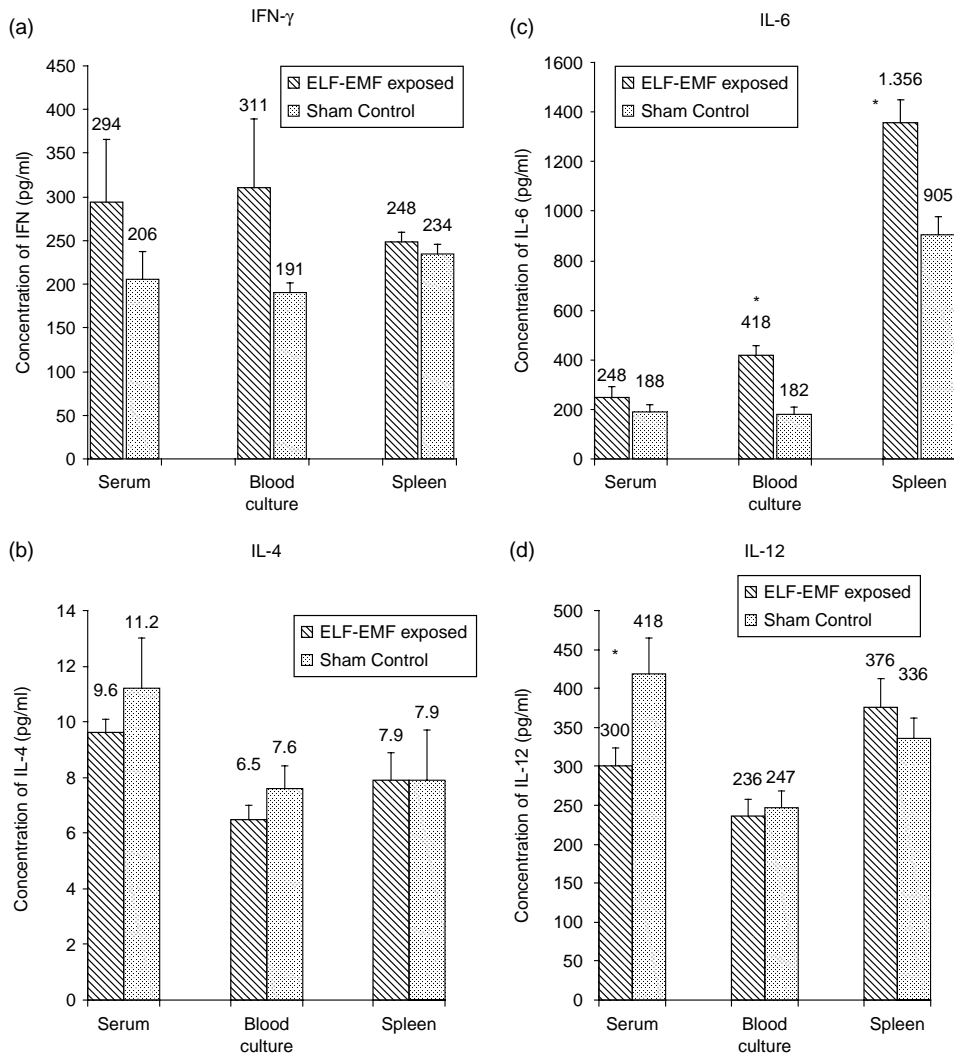


FIGURE 2 Production of IFN- γ , IL-4, IL-6, and IL-12 in serum, supernatant of spleen, and total blood cultures of ELF-EMF and sham-exposed control groups. We could not observe the statistically significant differences between ELF-EMF-exposed and sham-exposed animals in the production of IFN- γ and IL-4 (A, B), whereas whole spleens and total blood cultures activated by PHA ($1 \mu\text{g/ml}$) for 24 h were able to increase *in vitro* production of IL-6 levels in the supernatant of spleens and blood culture cells of ELF-EMF-exposed compared to the sham-exposed controls (C). Serum IL-12 showed a significantly lower level in the ELF-EMF-exposed rats compared to serum IL-12 levels in the sham-exposed rats (D). Data are presented as mean concentration \pm SEM.

DISCUSSION

There is one clear characteristic in our study that first ELF-EMF exposure was carried out on rats for three months and then the cells were challenged with PHA. To our knowledge, the previous studies all analyzed only *in vivo* or *in vitro* production of cytokines, except Boscolo et al. (2001). Boscolo et al. (2001) measured *in vitro* production of IFN- γ and IL-4 by peripheral blood mononuclear cells (PBMCs) activated with PHA and followed detection of them in the serum of EMF-exposed people.

The results of the present study showed that long-term exposure of rats to ELF-EMF could not change the mass of rats, spleens, and thymuses compared to the sham-exposed control. These results are in agreement with the several other studies and can prove that there is no significant change in the amount of cells in these two lymphoid organs during ELF-EMF exposure (Al-Akhras, 2008; House et al., 1996; Marino et al., 2000; Sommer and Lerchl, 2006).

This study demonstrated that the serum levels of IFN- γ , IL-4, and IL-6 were not different in EL-EMF-exposed and sham-exposed control rats. This is in favor of Lin et al. (2009) and Boscolo et al. (2001), who showed no significant changes in inflammatory cytokines, including IL-6 in plasma of mice after static magnetic field (250,000 μ T) treatment for 2 h and IL-4 in serum of 7 men and 8 women employed in a museum when were exposed in a room to an electromagnetic field (range 0.2–3.6 μ T) for 2 years (Lin et al., 2009; Boscolo et al., 2001). In contrast, the serum level of IFN- γ in the study by Boscolo et al. (2001) and IL-6 level in two other studies that investigated the effects of extremely low-magnetic field on acute and chronic arthritis of rats and Pulsed electromagnetic fields (PEMF) effect on rats bone fractures were decreased (Bao et al., 2006; Boscolo et al., 2001; Shen and Zhao, 2010).

Among all cytokines measured in the serum, IL-12 was decreased in the serum of the ELF-EMF-exposed rats compared to sham-exposed group. As we have searched the literature, this is the first study on measurement of serum IL-12 level in ELF-EMF-exposed-animals. To discuss this reduction of IL-12, it seems that melatonin plays a critical role. Melatonin increases the production of cytokines, such as IL-12, and alters the balance of T cells mainly towards T_H1 responses (Srinivasan, 2008a, b; Majewska, 2007). There is increasing evidence from laboratory studies that exposure to ELF-EMF can suppress pineal function and reduce circulating concentration of melatonin in the body (Mevissen et al., 1998; Simko, 2004). Therefore, it can be concluded that the effect of ELF-EMF in reduction of IL-12 may be, in part, mediated by reduction of melatonin and down regulation of IL-12 and T_H1 cells during ELF-EMF exposure may result in the failure of immune response system against cancers.

In vitro production of IFN- γ , IL-4, and IL-12 from PHA-induced whole spleen and total blood cultures of ELF-EMF-exposed and sham-exposed control rats were not statistically significant in our study. These results are in consistent with the results of Aldinucci et al. (2003) and Ikeda et al. (2003) studies that *in vitro* production of IFN- γ in the presence of 475,000 μ T and 100–500 μ T, respectively, and Boscolo et al. (2001) work in the presence of 0.2–3.6 μ T IL-4 production from human PBMC (Aldinucci et al., 2003; Boscolo et al., 2001; Ikeda et al., 2003). In contrast, other works show the difference in the *in vitro* production of IFN- γ and IL-4 in electromagnetic field-exposed cases (Boscolo et al., 2001; Jonai et al., 1996; Kaszuba-Zwoinska et al., 2008; Salerno et al., 1999). It must be taken into account that the reasons underlying the discordance in some results of electromagnetic studies may be due to difference in the density of electromagnetic field.

In vitro production of IL-6 was increased in the supernatant of whole spleens and total blood cultures of the ELF-EMF-exposed rats. This is in accordance with the results of some studies including: static magnetic field exposure (400,000 μ T) for 12 h that increased the level of LPS-induced IL-6 (Lin et al., 2008), extremely low-frequency (ELF) sinusoidal magnetic field (MF) exposure induced the expression of IL-6 receptor in HL60 (a human promyelocytic leukemia cell line) culture with exposure in density of 100–800 μ T for 12 h (Zhou et al., 2002) and exposure to low-frequency pulsed electromagnetic fields (2500 μ T) increases IL-6 production by human PMBCs when activated with PHA (Cossarizza et al., 1993). In addition, ELF-EMF exposure of rats and *in vitro* production of IL-6 failed to change in some investigations (Aldinucci et al., 2003; Salerno et al., 1999; Jonai et al., 1996; Morandi et al., 1994). Moreover, a systematic review by Heikkila et al. (2008) that showed IL-6 concentration in serum of cancer patients was higher than controls in most studies and IL-6 may have a role in cancer. To realize how IL-6 can increase cancer frequency it can be concluded that IL-6 regulates T_H17 differentiation and chronic inflammation which create cellular microenvironment beneficial to cancer growth. IL-6 is also involved in the control of cell proliferation and apoptosis (Heikkila et al., 2008).

In conclusion, it can be concluded that ELF-EMF may change the T_H1/T_H2/T_H17 balances toward down regulation of T_H1-type response and upregulation in T_H17-type response of the immune system.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the staff of the Research Center for Molecular Medicine (Hamadan Iran) for helping them carry out this project, especially Mr. Fazli and Mrs. Ghiasvand. This project was funded by the research deputy of Hamadan University of Medical Sciences (Hamadan Iran).

Declaration of interest

The authors have no conflict of interest.

REFERENCES

- Al-Akhras, M. A. (2008). Influence of 50 Hz magnetic field on sex hormones and body, uterine, and ovarian weights of adult female rats. *Electromagn. Biol. Med.* 27:55–163.
- Aldinucci, C., Garcia, J. B., Palmi, M., et al. (2003). The effect of strong static magnetic field on lymphocytes. *Bioelectromagnetics* 24:109–117.
- Bao, X. Q., Shi, Y. J., Zhang, Y. X. (2006). [Effects of extremely low magnetic field on acute and chronic arthritis of rats]. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 22:505–508 (English Abstract).
- Blackman, C., Blank, M., Kundi, M., et al. (2007). A Rationale for a Biologically-based Public Exposure Standard for Electromagnetic Fields (ELF and RF). *BioInitiative Report*, 1–610
- Boscolo, P., Bergamaschi, A., Di Sciascio, M. B., et al. (2001). Effects of low frequency electromagnetic fields on expression of lymphocyte subsets and production of cytokines of men and women employed in a museum. *Sci. Total Environ.* 270:13–20.
- Cossarizza, A., Angioni, S., Petraglia, F., et al. (1993). Exposure to low frequency pulsed electromagnetic fields increases interleukin-1 and interleukin-6 production by human peripheral blood mononuclear cells. *Exp. Cell Res.* 204:385–387.
- Hardell, L., Sage, C. (2008). Biological effects from electromagnetic field exposure and public exposure standards. *Biomed. Pharmacother.* 62:104–109.
- Harrington, L. E., Hatton, R. D., Mangan, P. R., et al. (2005). Interleukin 17-producing CD4⁺ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* 6:1123–1132.
- Heikkila, K., Ebrahim, S., Lawlor, D. A. (2008). Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. *Eur. J. Cancer* 44:937–945.
- House, R. V., Ratajczak, H. V., Gauger, J. R., et al. (1996). Immune function and host defense in rodents exposed to 60-Hz magnetic fields. *Fund. Appl. Toxicol.* 34:228–239.

- Ikeda, K., Shinmura, Y., Mizoe, H., et al. (2003). No effects of extremely low frequency magnetic fields found on cytotoxic activities and cytokine production of human peripheral blood mononuclear cells in vitro. *Bioelectromagnetics* 24:21–31.
- Jonai, H., Villanueva, M. B., Yasuda, A. (1996). Cytokine profile of human peripheral blood mononuclear cells exposed to 50 Hz EMF. *Indiv. Health* 34:359–368.
- Kaszuba-Zwoinska, J., Cieciko-Michalska, I., Madroszkiewicz, D., et al. (2008). Magnetic field anti-inflammatory effects in Crohn's disease depends upon viability and cytokine profile of the immune competent cells. *J. Physiol. Pharmacol.* 59:177–187.
- Lin, C. T., Lee, S. Y., Chen, C. Y., et al. (2008). Long-term continuous exposure to static magnetic field reduces polysaccharide-induced cytotoxicity of fibroblasts. *Int. J. Radiat. Biol.* 84:219–226.
- Lin, S. L., Chang, W. J., Lin, Y. S., et al. (2009). Static magnetic field attenuates mortality rate of mice by increasing the production of IL-1 receptor antagonist. *Int. J. Radiat. Biol.* 85:633–640.
- Majewska, M., Zajac, K., Zemelka, M., et al. (2007). Influence of melatonin and its precursor L-tryptophan on Th1 dependent contact hypersensitivity. *J. Physiol. Pharmacol.* 58(Suppl 6):125–132.
- Marino, A. A., Becker, R. O. (1978). High-voltage lines: hazard at a distance. *Environment* 20:6–15.
- Marino, A. A., Wolcott, R. M., Chervenak, R., et al. (2000). Nonlinear response of the immune system to power-frequency magnetic fields. *Amer. J. Physiol. Regul. Integr. Comp. Physiol.* 279:R761–R768.
- Mevisen, M., Haussler, M., Szamel, M., et al. (1998). Complex effects of long-term 50 Hz magnetic field exposure in vivo on immune functions in female Sprague-Dawley rats depend on duration of exposure. *Bioelectromagnetics* 19:259–270.
- Morandi, M. A., Del Rio, J. A., Caren, R. P., et al. (1994). Effects of short term exposure to 60 Hz electromagnetic fields on interleukin 1 and interleukin 6 production by peritoneal exudate cells. *Life Sci.* 54:731–738.
- Mosmann, T. R., Cherwinski, H., Bond, M. W., et al. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136:2348–2357.
- Mosmann, T. R., Coffman, R. L. (1989). TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immunol.* 7:145–173.
- Park, H., Li, Z., Yang, X. O., et al. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* 6:1133–1141.
- Roosli, M., Frei, P., Mohler, E., et al. (2010). Systematic review on the health effects of exposure to radiofrequency electromagnetic fields from mobile phone base stations. *Bull. World Health Organ* 88:887–896F.
- Saito, S., Nakashima, A., Shima, T., et al. (2010). Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *Amer. J. Reprod. Immunol.* 63:601–610.
- Salerno, S., Lo Casto, A., Caccamo, N., et al. (1999). Static magnetic fields generated by a 0.5 T MRI unit affects in vitro expression of activation markers and interleukin release in human peripheral blood mononuclear cells (PBMC). *Int. J. Radiat. Biol.* 75:457–463.
- Shen, W. W., Zhao, J. H. (2010). Pulsed electromagnetic fields stimulation affects BMD and local factor production of rats with disuse osteoporosis. *Bioelectromagnetics* 31:113–119.
- Simko, M., Mattsson, M. O. (2004). Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: possible immune cell activation. *J. Cell. Biochem.* 93:83–92.
- Sommer, A. M., Lerchl, A. (2006). 50 Hz magnetic fields of 1 mT do not promote lymphoma development in AKR/J mice. *Radiat. Res.* 165:343–349.
- Srinivasan, V., Spence, D. W., Pandi-Perumal, S. R., et al. (2008a). Therapeutic actions of melatonin in cancer: possible mechanisms. *Integr. Cancer Ther.* 7:189–203.
- Srinivasan, V., Spence, D. W., Trakht, I., et al. (2008b). Immunomodulation by melatonin: its significance for seasonally occurring diseases. *Neuroimmunomodulation* 15:93–101.
- Taniguchi, A., Kohsaka, H., Carson, D. A. (1994). Competitive RT-PCR ELISA: a rapid, sensitive and non-radioactive method to quantitate cytokine mRNA. *J. Immunol. Meth.* 169:101–109.
- Xu, M., Mizoguchi, I., Morishima, N. (2010). Regulation of antitumor immune responses by the IL-12 family cytokines, IL-12, IL-23, and IL-27. *Clin. Dev. Immunol.* 2010:1–9.
- Zamani, A., Vahidinia, A., Ghannad, M. S. (2009). The effect of garlic consumption on Th1/Th2 cytokines in phytohemagglutinin (PHA) activated rat spleen lymphocytes. *Phytother. Res.* 23:579–581.
- Zhou, J., Li, C., Yao, G., et al. (2002). Gene expression of cytokine receptors in HL60 cells exposed to a 50 Hz magnetic field. *Bioelectromagnetics* 23:339–346.