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SHORT NOTE

Exposure to Low Frequency Pulsed Electromagnetic Fields Increases Interleukin-1 and Interleukin-6 Production by Human Peripheral Blood Mononuclear Cells

ANDREA COSSARIZZA,*,[1] STEFANO ANGIONI,† FELICE PETRAGLIA,† ANDREA R. GENAZZANI,† DANIELA MONTI,* MIRIAM CAPRI,* FERDINANDO BERSANI,‡ RUGGERO CADOSSI,§ AND CLAUDIO FRANCESCHI*

*Institute of General Pathology, University of Modena, via Campi 287, 41100 Modena, Italy; †Institute of Obstetrics and Gynecology, University of Modena, via del Pozzo 71, 41100 Modena, Italy; ‡Department of Physics, University of Bologna, via Irnerio 48, 40126 Bologna, Italy; and §Center for Experimental Hematology, University of Modena, via del Pozzo 71, 41100 Modena, Italy

The exposure of human peripheral blood mononuclear cells to extremely low frequency pulsed electromagnetic fields (PEMFs) increased both the spontaneous and the PHA- and TPA-induced production of interleukin-1 (IL-1) and IL-6. Our results suggest that cells of the monocytic lineage, which are good producers of both IL-1 and IL-6, can be important cellular targets for PEMFs. Taking into account that these cytokines are among the most pleiotropic ones, these data can help us understand the previous reported effects of PEMFs on the proliferation of human lymphocytes and the effects exerted by such fields on cartilage and bone cells, whose physiological activity is highly dependent on IL-1 and IL-6.

INTRODUCTION

The exposure of human beings to extremely low frequency (ELF) pulsed electromagnetic fields (PEMFs) can occur as a result of environmental (power lines and electrical apparatus), diagnostic (nuclear magnetic resonance), and therapeutic (bone fracture repair) influences [1–3]. A growing interest is thus present in studying the effects of such fields at both a cellular and molecular level [4–6]. Indeed, in recent years several groups have studied the effects of the exposure to magnetic fields on a variety of cellular functions and activities [7–10].

For many years, our group has studied the effects of ELF-PEMFs on immune cells [reviewed in 11]. In particular, we have reported that the exposure to such fields was able to increase mitogen-induced human lymphocyte proliferation, and that this effect was more evident when cells from aged donors or Down's syndrome patients were used [12, 13]. PEMFs also provoked an increased utilization of interleukin-2 (IL-2), a crucial growth factor for T lymphocytes [14].

We have studied the effects of PEMFs on the production by human peripheral blood mononuclear cells (PBMC) of two cytokines, i.e., IL-1β and IL-6, which exert several functions of primary importance for the activity of the immune cells and other cell types [15, 16].

The data presented in this paper suggest that the *in vitro* exposure to PEMFs increases the production of both cytokines.

MATERIALS AND METHODS

Subjects. A total of 16 healthy donors, students or university personnel, with ages between 21 and 35 years, were studied. All of them gave their informed consent.

Isolation of peripheral blood mononuclear cells. Immediately before the experiment, 20–30 ml of heparinized venous blood was obtained from young healthy donors and PBMC were separated by density gradient centrifugation, following standard methods.

Peripheral blood mononuclear cell cultures. Phytohemagglutinin (PHA-P, Difco Lab., U.S.A.)-stimulated cultures were performed as previously described [17]. Briefly, 0.1 ml of cell suspension containing 10^5 viable PBMC in complete medium (RPMI 1640, containing 2 m*M* glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum) was distributed in microplate wells and stimulated by addition of 0.1 ml of complete medium containing the mitogen. PHA-P, final concentration of 1 μl/ml, was used as mitogen for the evaluation of IL-6 production. The same dose of PHA-P plus 1 or 10 ng/ml 12-*O*-tetradecanoylphorbol-13-acetate (TPA, Sigma Chemical Co., U.S.A.) was used for the evaluation of IL-1β production.

PEMF-exposed or control cultures were incubated in a humidified atmosphere of 5% CO_2 and 95% air for 24 or 48 h, then supernatants were carefully removed, centrifuged to remove cells eventually present, collected, and frozen at −20°C until use.

Characteristics of PEMFs and exposure conditions. The exposure to pulsed electromagnetic fields was performed as previously described, using the same exposure system and electromagnetic signal [18]. Briefly, microtiter plates containing the cell cultures were placed between a pair of Helmoltz coils (maintained parallel to the plates)

[1] To whom reprint requests should be addressed.

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TABLE 1

Production of IL-1β in PEMF-Exposed PBMC Cultures

Hours	Stimulus	Control	Exposed	*P*
24	None	11.3 ± 1.3	54.4 ± 13.3	<0.015
24	TPA 1 ng/ml + PHA 1 μl/ml	62.7 ± 26.4	119.1 ± 31.6	<0.009
24	TPA 10 ng/ml + PHA 1 μl/ml	159.0 ± 60.5	282.1 ± 63.9	<0.015

Note. The production of IL-1β in the supernatants of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) plus phytohemagglutinin (PHA)-stimulated PBMC cultures was measured by ELISA. Data are expressed in pg/ml (arithmetic mean ± SEM) and refer to 10 young healthy donors. Statistical analysis was performed by two-tail paired Student's *t* test.

powered by a pulse generator (Igea, Carpi, Italy). The pulse duration was about 2 ms and the repetition rate was 50 Hz, yielding a duty cycle of 1/10. The intensity of the magnetic field was 2.5 mT, the average time variation of the field being of the order of 1 T/s. The induced voltage was about 2 mV. The induced electric field inside each well was in the plane parallel to the microtiter plate surface and was estimated as 0.02 mV/cm [19]. The control cultures were maintained in the same incubator at a distance where no electromagnetic field was detectable, using a coil described elsewhere [20]. No thermic effect was produced by the exposure system used, as previously reported [12–14].

Cytokine measurements. The amount of IL-1β and IL-6 present in the supernatants was measured by the sandwich ELISA technique (Quantikine, British Bio-technology, Oxon, UK) following the manufacturer's instructions. Each point was run in triplicate.

Data calculation and statistical analysis. Statistical analysis was performed by two-tail paired Student's *t* test.

RESULTS

Table 1 shows the production of IL-1β in PEMF-exposed and control cultures of PBMC from 10 young healthy donors.

Table 2 shows the production of IL-6 in PEMF-exposed and control cultures of PBMC from 6 young healthy donors.

It is noteworthy that the exposure of human PBMC to PEMFs increases both the spontaneous and the PHA- and TPA-induced production of IL-1 and IL-6.

TABLE 2

Production of IL-6 in PEMF-Exposed PBMC Cultures

Hours	Stimulus	Control	Exposed	*P*
24	None	0.50 ± 0.22	0.58 ± 0.32	NS
24	PHA 1 μl/ml	3.24 ± 0.75	3.97 ± 0.68	<0.03
48	None	0.49 ± 0.25	0.94 ± 0.37	<0.04
48	PHA 1 μl/ml	3.88 ± 0.54	4.49 ± 0.52	<0.018

Note. The production of IL-6 in the supernatants of phytohemagglutinin (PHA)-stimulated PBMC cultures was measured by ELISA. Data are expressed in ng/ml (arithmetic mean ± SEM) and refer to six young healthy donors. Statistical analysis was performed by two-tail paired Student's *t* test.

DISCUSSION

Evidence from several laboratories, including ours, suggests that PEMFs have profound effects on immune cells [11, 21–23]. Immune responses are mediated by a variety of molecules collectively called cytokines. Besides immunocytes, these molecules have several targets outside the immune system, such as neuronal and endocrine cells, chondrocytes, fibroblasts, and bone cells, among others [15, 16]. In this paper, we demonstrate that the exposure to PEMFs was able to significantly increase the *in vitro* production of two important cytokines, such as IL-1β and IL-6.

IL-1 is mainly produced by activated monocytes, but also by endothelial cells, fibroblasts, and T and B lymphocytes, among others. Its main biological activities are the induction of fever, sleep, anorexia, bone resorption; at a cellular level, it is also able to induce the growth and differentiation of T and B cells, the growth of fibroblasts, and the synthesis of collagenase [reviewed in 24]. IL-6 is produced by macrophages, T and B lymphocytes, keratinocytes, endothelial cells, astrocytes, and bone marrow stroma cells. It is able to induce B cell differentiation, T cell activation and differentiation, neural cell differentiation, and bone resorption, among other functions [reviewed in 25].

Both spontaneous and mitogen-induced cytokine production were significantly affected by PEMFs, suggesting that this phenomenon might also occur under conditions similar to those present *in vivo*, where optimal concentrations of strong mitogens are unlikely to be present.

The results presented here, and those previously reported on the increased utilization of IL-2 by lymphocytes exposed to the same PEMFs [14], suggest that cytokine production may be one of the most important functions affected by these nonionizing radiations. At the cellular level, monocytes are probably important targets for PEMF action, as they are present in the peripheral blood cell population we have used in our studies and are considered among the most important producers of IL-1β and IL-6 [16, 24, 25]. The phenomenon described here can have far reaching consequences, tak-

ing into account that a variety of phagocytic cells spread out all over the organism, such as astrocytes, Kupffer cells, and Langerhans cells, among others, derive from circulating monocytes. Our data might also help in explaining other biological effects exerted by PEMFs and, in particular, those concerning bone repair, a phenomenon in which IL-1 and IL-6 play a crucial role.

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REFERENCES

1. Carstensen, E. L. (1987) *in* Biological Effects of Transmission Line Fields, Elsevier, New York.
2. Budinger, T. F. (1979) *IEEE Trans. Nucl. Sci.* **26,** 2821–2825.
3. Bassett, C. A. L., Pawluk, R. J., and Pilla, A. A. (1977) *Science* **184,** 575–577.
4. Jauchem, J. R. (1990) *Lancet* **336,** 884.
5. Pool, R. (1990) *Science* **249,** 1378.
6. Pool, R. (1991) *Nature* **349,** 554.
7. Cadossi, R., Hentz, V. R., Kipp, J., Eiverson, R., Ceccherelli, G., Zucchini, P., Torelli, G., Franceschi, C., and Cossarizza, A. (1989) *Exp. Hematol.* **17,** 88–95.
8. Goodman, R., and Henderson, A. S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3928–3932.
9. Reiter, R. J., and Richardson, B. A. (1992) *FASEB J.* **6,** 2283–2287.
10. Rodemann, H. P., Bayreuther, K., and Pfleiderer, G. (1989) *Exp. Cell Res.* **182,** 610–621.
11. Cadossi, R., Bersani, F., Cossarizza, A., Zucchini, P., Emilia, G., Torelli, G., and Franceschi, C. (1992) *FASEB J.* **6,** 2667–2674.
12. Cossarizza, A., Monti, D., Bersani, F., Cantini, M., Cadossi, R., Sacchi, A., and Franceschi, C. (1989) *Biochem. Biophys. Res. Commun.* **160,** 692–699.
13. Cossarizza, A., Monti, D., Bersani, F., Scarfi, M. R., Zannotti, M., Cadossi, R., and Franceschi, C. (1991) *Aging* **3,** 241–246.
14. Cossarizza, A., Monti, D., Cantini, M., Paganelli, R., Montagnani, G., Cadossi, R., Bersani, F., and Franceschi, C. (1989) *FEBS Lett.* **248,** 141–144.
15. Mizel, S. B. (1989) *FASEB J.* **3,** 2379–2388.
16. Akira, S., Hirano, T., Taga, T., and Kishimoto, T. (1990) *FASEB J.* **4,** 2860–2867.
17. Franceschi, C., Licastro, F., Chiricolo, M., Bonetti, F., Zannotti, M., Fabris, N., Mocchegiani, E., Fantini, M. P., Paolucci, P., and Masi, M. (1981) *J. Immunol.* **126,** 2161–2164.
18. Cantini, M., Cossarizza, A., Bersani, F., Cadossi, R., Ceccherelli, G., Tenconi, R., Gatti, C., and Franceschi, C. (1986) *J. Bioelect.* **5,** 91–104.
19. Mc Leod, B. R., Pilla, A. A., and Sampsel, M. W. (1983) *Bioelectromagnetics* **4,** 357–370.
20. Cossarizza, A., Monti, D., Moschini, G., Cadossi, R., Bersani, F., and Franceschi, C. (1989) *Radiat. Res.* **118,** 161–168.
21. Cossarizza, A., Borghi, V., Bersani, F., Cantini, M., De Rienzo, B., Zucchini, P., Montagnani, G., Mussini, D., Troiano, L., Grassilli, E., Tropea, F., Monti-Biasi, D., and Franceschi, C. (1989) *J. Bioelectr.* **8,** 227–237.
22. Cadossi, R., Ceccherelli, G. B., Emilia, G., Torelli, G., Ruggieri, M. P., Bersani, F., Cossarizza, A., and Franceschi, C. (1988) *Bioelectrochem. Bioenerg.* **19,** 315–322.
23. Cadossi, R., Zucchini, P., Emilia, G., Franceschi, C., Cossarizza, A., Santantonio, M., Mandolini, G., and Torelli, G. (1991) *Exp. Hematol.* **19,** 196–201.
24. Dinarello, C. A. (1991) *Blood* **78,** 1627–1652.
25. Kishimoto, T. (1989) *Blood* **74,** 1–10.

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