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Extremely low frequency pulsed electromagnetic fields increase interleukin-2 (IL-2) utilization and IL-2 receptor expression in mitogen-stimulated human lymphocytes from old subjects

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The effects of the exposure of mitogen-stimulated human lymphocytes from aged subjects to low-frequency pulsed electromagnetic fields (PEMFs) were studied by measuring the production of interleukin-2 (IL-2) and the expression of IL-2 receptor. PEMF-exposed cultures that presented increased [^{3}H]thymidine incorporation showed lower amounts of IL-2 in their supernatants, but higher percentages of IL-2 receptor-positive cells and of T-activated lymphocytes. Taken together, these data suggest that PEMFs were able to modulate mitogen-induced lymphocyte proliferation by provoking an increase in utilization of IL-2, most likely acting on the expression of its receptor on the plasma membrane.

Pulsed electromagnetic field; Interleukin-2; Interleukin-2 receptor; (Human lymphocyte)

1. INTRODUCTION

Numerous sources of electrophoretic fields exist in nature and in occupational and residential environments. In nearly all instances, these fields pose no obvious threat to human health or safety and are generally considered as an inevitable by-product of modern technology. However, public awareness of the ubiquitous nature of these fields and growing controversy over their potential effects on living systems are currently stimulating the scientific community to investigate accurately their biological effects. In fact, there is evidence that such fields, which do not produce macroscopic thermal effects, affect the growth and DNA synthesis of many cell types, including lymphocytes [1-7].

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In previous studies, we showed that PEMFs, although devoid of any effect on DNA repair synthesis [8], were able to increase phytohaemagglutinin (PHA)-induced lymphocyte proliferation, and that such an effect was particularly evident when cells from old donors were studied [9,10]. Here, we report that PEMFs could favour lymphocyte proliferation by increasing utilization of the growth factor interleukin-2 (IL-2) and expression of the IL-2 receptor on the plasma membrane.

2. MATERIALS AND METHODS

2.1. *Subjects*

Lymphocytes from aged subjects (86-90 years old, mean age 88.2 ± 0.8 years) were used to assess production and utilization of interleukin-2 (IL-2). Expression of the IL-2 receptor (IL-2R) on cell membranes was evaluated in 10 other aged subjects (84-96 years old, mean age 87.4 ± 1.2 years); in six of them (85-96 years old, mean age 88.0 ± 1.6 years) the percentage of activated T-lymphocytes was also determined. All aged subjects were in good condition, as assessed by accurate clinical evalua-

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tion; in addition, more than 70 haematological and biochemical parameters were evaluated for each subject in order to confirm the clinical status.

2.2. *Isolation of peripheral blood lymphocytes and mitogen-stimulated cultures*

Immediately before experiments, 30–40 ml heparinized venous blood was obtained and peripheral lymphocytes were separated by density gradient centrifugation, according to Böyum [11]. PHA-stimulated cultures were performed as described [12]. Briefly, 0.1 ml cell suspension containing 10^5 viable lymphocytes in complete medium (RPMI 1640, containing 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% inactivated human AB serum from a pool of 10 donors) was distributed in microplate wells (Falcon Plastic, Los Angeles, CA) and stimulated by adding 0.1 ml complete medium containing different doses of mitogen (PHA-P, Difco, Detroit, MI); final concentrations 1 and 10 μl/ml). Cultures were incubated in a humidified atmosphere of 5% CO_2 and 95% air for different periods of time, i.e. 18, 24, 48 or 72 h. [^{3}H]Thymidine ([^{3}H]TdR) was added for the final 6 h of culture, and microplates were treated as described in [12].

2.3. *In vitro production and measurement of interleukin-2*

(Peripheral blood lymphocytes (PBLs) from aged subjects were cultured for 24 or 48 h at 10^6 cells/ml under the conditions described above, either without or with PHA at 1 or 10 μl/ml, in quadruplicate, and were exposed to PEMFs or left unexposed. At the end of the culture periods cell supernatants were collected after centrifugation, and stored at −20°C until assay. Separate cultures under the same experimental conditions were kept for 72 h and pulsed with [^{3}H]TdR to correlate the effect of PEMF exposure on IL-2 production to that on DNA synthesis. The quantitative microassay described by Gillis et al. [13] was used for measurement of in vitro produced IL-2 activity. Briefly, 5000 CTLL-A cells (a cytolytic murine T cell line) were seeded in duplicates in each well of a 96-well culture microplate, with serial dilutions of supernatants to be assayed and known standards of recombinant IL-2 (Biogen SA, Basel, Switzerland), or immunochemically purified IL-2 (gift from Professor S. Venuta, Naples, Italy) or reference Jurkat IL-2 (BRMP, NIH, Bethesda, MD). After 24 h of culture, 0.5 μCi [^{3}H]TdR was added to each well for 18 h. Results on [^{3}H]TdR incorporation were calculated by probit analysis for each supernatant vs standards, and are expressed as reference units/ml.

2.4. *IL-2 receptor expression and T-activated lymphocytes*

PBLs from aged subjectes were cultured and stimulated with the optimal dose of PHA (1 μl/ml) as described above. After exposure to PEMFs for 18 h cells were harvested for fluorescence-activated cell sorter (FACS) analysis (by a FACSTAR, Becton-Dickinson FACS Division, Mountain View, USA), washed twice in cold PBS with 5% fetal calf serum (FCS), resuspended at 10^6 cells/ml in 100 μl PBS containing FCS and supplemented with 10 μl monoclonal antibody (MoAb). Double fluorescence labelling was performed by simultaneous incubation with phycoerythrin (PE)- and fluorescein (FITC)-labelled MoAbs. The following MoAbs from Becton-Dickinson (Oxnard, USA) were used: (i) CD 25, i.e. anti-interleukin-2 Receptor MAb (anti-TAC); (ii) CD 3 viz. anti-T lymphocytes (Leu 4); (iii) anti-HLA

Table 1

IL-2 concentration and [^{3}H]TdR incorporation in PHA-stimulated PEMF-exposed lymphocytes from 4 aged subjects

		[^{3}H]TdR incorporation		IL-2 production			
				24 h		48 h	
Subject	PHA (μl/ml)	Control	Exposed to PEMFs	Control	Exposed to PEMFs	Control	Exposed to PEMFs
A	0	455	309	<0.5	<0.5	< 0.5	< 0.5
	1	14191	37418	0.5	2	8	2
	10	2049	3456	0.5	0.5	< 0.5	<0.5
B	0	140	198	<0.5	<0.5	< 0.5	2
	1	43233	73120	8	5	15	0.5
	10	3384	14808	1	0.5	15	0.5
C	0	200	182	<0.5	<0.5	< 0.5	< 0.5
	1	121775	126918	6	2.5	12	9
	10	18094	30027	0.7	0.5	20	1.5
D	0	476	299	<0.5	<0.5	< 3	< 0.5
	1	46375	49155	2	4	7	10
	10	3556	4529	5	0.8	8	8

Values of [^{3}H]TdR incorporation are expressed in cpm; SE values are not reported since they never exceeded 5%. Data regarding the IL-2 concentrations are expressed in reference units/ml. See text for details

DR positive lymphocytes; this MAb recognizes B lymphocytes and T activated cells [14–17]. After incubation for 30 min at 4°C, cells were washed twice, resuspended in PBS without FCS and analyzed using a cytofluorimeter with a laser source which made it possible to excite FITC and PE simultaneously at a wavelength of 488 nm, and to separate the emitted light of FITC and of PE into two separate photomultipliers. A minimum of 10000 cells from each sample was analyzed.

2.5. *Characteristics of PEMFs and exposure conditions*

Microtitre plates were placed between a pair of Helmoltz coils (maintained parallel to the plates) powered by a pulse generator (Igea, Carpi, Italy). The same signal as used in a previous study was employed [7]. Briefly, the pulse duration was about 2 ms and the repetition rate 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 magnetic field being of the order of 1 T/s. The induced voltage, as detected with a coil probe comprising 50 turns (diameter 0.5 cm), was about 2 mV. Taking into account the size of each well and its position with respect to the magnetic flux lines, the induced electric field inside each well was in the plane parallel to the microtitre plate surface, and the maximum field (maximum internal radius) was estimated as 0.02 mV/cm [18]. Control cultures were maintained in the same incubator at a distance where no electromagnetic field was detectable using the above coil. Cultures were exposed for different time periods, viz. 18 h for evaluation of IL-2 receptor-positive cells and percentage of T-activated lymphocytes, and 24 and 48 h for studying the production of IL-2. No thermal effect was produced by the exposure system used, as measured either by a precision thermometer placed in a water bath close to the microplates or by direct assessment of the temperature within the microwells using a specially designed thermoresistor.

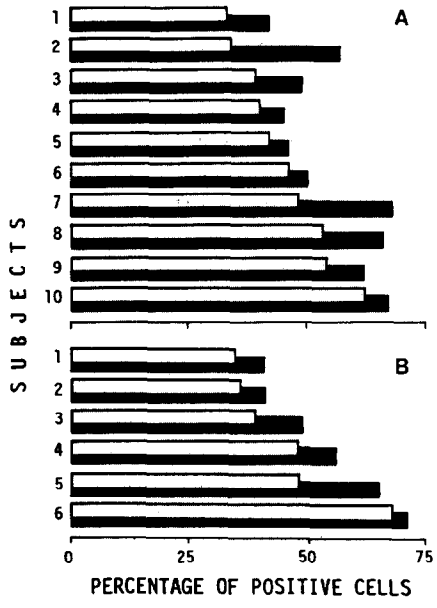

Fig.1. Exposure to PEMFs increase the percentage of IL-2 receptor (IL-2R)-positive (A) and T-activated lymphocytes (B) in PHA-stimulated cultures from aged subjects. Open bars: unexposed cells; filled bars, PEMF-exposed cells; each bar refers to one subject. Statistical analysis was performed by paired Student's *t*-test: percentage of IL-2R-positive cells (mean ± SE): unexposed = 45.1 ± 2.9; PEMF-exposed = 55.2 ± 3.1; $p < 0.001$; percentage of T-activated (CD3+ HLA-DR+) lymphocytes (mean ± SE): unexposed = 45.6 ± 4.6; PEMF-exposed = 53.8 ± 5.0; $p < 0.01$.

3. RESULTS

As one of the major effects of PEMFs was observed in lymphocytes from old people whose reduced proliferation is known to be caused, at least in part, by diminished production and utilization of IL-2, we measured this growth factor in unexposed and PEMF-exposed cultures of lymphocytes from old donors, stimulated with optimal and supraoptimal doses of PHA. The results reported in table 1 suggest that PEMFs did not increase IL-2 production. In fact, the IL-2 concentration at 24 h was similar in PEMF-exposed and unexposed cultures. However, a marked decrease in IL-2 at the 48th hour was observed but only in those cultures which responded to PEMF exposure with increased [^{3}H]TdR incorporation, i.e. subjects A (PHA 1), B (PHA 1, 10) and C (PHA 10). As the data in table 1 suggest that PEMFs could increase lymphocyte proliferation by increasing utilization of IL-2, we considered it to be worthwhile to determine the expression of IL-2 receptor on PEMF-exposed lymphocytes. Fig. 1A shows that expression of IL-2 receptor on lymphocyte cell membranes, as assessed by cytofluorimetric analysis with CD MAb, was markedly increased in PEMF-exposed lymphocytes, this effect being evident in 10 out of 10 aged subjects. In 6 of these subjects the percentage of activated T lymphocytes was also assessed by counting the percentage of CD3 positive and anti-HLA DR positive cells. The data reported in fig. 1B show that in 5 out of 6 cases a marked increase in percentage of activated T lymphocytes was observed after PEMF exposure.

4. DISCUSSION

Here, one of the possible mechanisms of action of PEMFs was investigated. Several studies on proliferation of human lymphocytes had suggested that one of the critical steps involved was expres-

sion of the receptor for a critical lymphocyte growth factor, such as IL-2 [19–23]. The fact that PEMFs were particularly effective in lymphocytes from old people, whose impaired proliferation has been ascribed either to defective production of IL-2 or to defective expression of high-affinity IL-2 receptors [24,25], supports this hypothesis. Even if the MoAb used in the present study were unable to discriminate between low- and high-affinity IL-2 receptors [26,27], these data, together with those reported in table 1, would suggest that functional (high-affinity) receptors might be involved in the PEMF-induced augmentation of IL-2 utilization.

The possibility of the involvement of other growth factors for T lymphocytes besides IL-2 cannot be excluded and deserves further attention.

We are currently studying the effects of PEMFs at the molecular level to ascertain whether the expression of mRNAs for IL-2 and IL-2 receptor is increased by PEMF exposure as would be predicted on the basis of the present data.

The interest of this work for the assessment of risk factors associated with environmental exposure to PEMF fields similar to those used in the present study should also be considered.

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