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FOLLOW-UP STUDY ON THE IMMUNE RESPONSE TO LOW FREQUENCY ELECTROMAGNETIC FIELDS IN MEN AND WOMEN WORKING IN A MUSEUM

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Seven women and eight men, exposed to low frequency (50 Hz) electromagnetic fields (EMFs) in a museum for 20 hours a week, were investigated in the years 1999 and 2005; the mean EMF exposure in the working place was 1.7 μT and 1.1 μT, respectively. In the first investigation, the EMF-exposed men showed reduced blood NK lymphocytes in relation to controls, while EMF-exposed women presented reduced PHA-stimulated IFN-γ release from peripheral blood mononuclear cells (PBMC). In the year 2005, blood cytotoxic activity, state and trait anxiety (STAI I and II, respectively) and occupational stress were also investigated. The scores of STAI I and II of the control women were slightly higher than those of the control men. EMF-exposed men showed higher occupational stress but normal immune parameters. EMF-exposed women showed, in relation to controls, lower PHA-stimulated IFN-γ release from PBMC and reduced blood cytotoxic activity/$CD45^+$-$CD16^+$-56^+ NK lymphocytes (but not per ml of blood). One of the women exposed to EMFs, who worked night shift, showed marked lymphopenia with very low NK lymphocytes and reduced IFN-γ release; these immune parameters returned to normal following a change of work site. This study suggests that low frequency EMFs affect the immune functions of women more than those of men. Moreover, the determination of immune parameters seems to be a useful marker of the health effects of exposure to EMFs.

In technologically developed countries, there is concern about the effects of electromagnetic fields (EMFs) on human health. The nature of the interaction with the biological material depends on the frequency or wavelength of the source (1). Moreover, the response of human cells to EMFs depends on their excitability. With regard to this, it is known that environmental EMFs interact in particular with metabolic activities of brain and immune cells (1).

Peripheral blood mononuclear cell (PBMC) stimulation is a model for the study of *in vitro* blastogenesis, proliferation, transcription and translocation of many proteins. Using this method, it was shown that low frequency EMFs modify calcium fluxes in the membranes acting on the release of thromboxane B_2 and interleukin-1 (2). PBMC of humans exposed *in vitro* to low frequency EMFs showed inhibited proliferative response to mitogens (2-5), changes in lymphocyte metabolism and surface marker expression (6). On the other hand, other studies on human PBMC exposed to 50 or 60 EMFs did not demonstrate changes in NK and lymphokine activated killer (LAK) activities or cytokine production (7).

Mice chronically exposed to 50 Hz EMFs, in the same conditions as humans, showed reduced total lymphocytes, leukocytes, polymorphonuclear neutrophil, CD4 and NK cells (8). Subchronic exposure with scalar flux density to 60 Hz EMFs suppressed NK cell activity in both young and mature mice without increasing the incidence of neoplasia (9).

After the first study on the incidence of child leukemia induced by electricity (10), several epidemiological investigations did not highlight association between residential exposure to low frequency EMFs and leukemia (11). However, pooled analysis of data from epidemiological studies on the risk of childhood leukemia

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was the basis for the classification of 50 or 60 Hz EMFs as possible carcinogens by the International Agency for Research on Cancer (IARC); however, the IARC observed that there was no scientific demonstration of cancer induced by static or high frequency EMFs (12).

Men and women employed in a museum, exposed to 50 Hz EMFs were studied in the year 2000; immune parameters of the men were unaltered with the exception of decreased blood lymphocyte NK, while the women showed reduced INF-γ release from PBMC (13). Sixty electric company workers showed a negative correlation between exposure to EMFs and blood ornithine decarboxylase activity and NK cells; the alteration of these immune parameter was stronger among workers with reduced melatonin production (14). With regard to this, the immune and the neuroendocrine systems seem to exert integrated responses: it has been found that immunological activation induces stress-like behavioral and neurochemical changes (15-16). Moreover, environmental stress may affect the activity of cytokines, peptide hormones, neurotransmitters and receptor ligands localized in both the immune and nervous systems ((15-16).

The aim of this follow-up study is to re-examine the museum employees exposed to EMFs (13). Following the hypothesis of an integrated neuro-immune response to environmental stimuli, we investigated not only immune parameters, but also the levels of anxiety and occupational stress.

MATERIALS AND METHODS

Employees in a museum of Central Italy were exposed for 20 hours a week to low frequency (50 Hz) EMFs for eight years. They were employed to survey the museum through the monitors installed in three rooms where electric cables were also located. The work sites were improved in 2004 with reduction of exposure to EMFs. In October 1999 and in October 2005, the EMFs of the three rooms were monitored using the following instruments (Wandel & Golterman, Germany):

a) probes for electric fields (range 100 KHz-3GHz),
b) EMR –300 monitor,
c) wood supports to insulate the probes.

The EMF levels in 1999 and 2000 are reported in Table I.

Recruitment of the subjects and blood collection

Eight women (mean age and range in 2005: 42 and 38-49 years, respectively) and seven men (mean age and range: 45 and 37-58 years) had been working for at least 8 years in the monitoring rooms of the museum. Three men and three women were atopic; two women and three men were smokers (less than 10 cigarettes a day). The diagnosis of atopy was made determining the levels of serum IgE (CAPFeia, Pharmacia, Uppsala, Sweden), history of allergic diseases and skin test to inhalant allergens. The control groups in 2005 consisted of 52 women (mean age 42 years) and 16 men (mean age 45 years) with similar smoking and drinking habits of the EMF exposed groups. The control groups were mainly composed of employees of the University of Chieti and Pescara. A small part of all the recruited men (about 15 %) were habitual drinkers of a small quantity of wine; the women of both the control group and those exposed to EMFs were not habitual drinkers but drank only on special occasions.

Clinical assessment included physical examination and standard routine blood analyses. Subjects taking drugs or suffering from diseases were not recruited for the investigation. Informed consent was obtained from the recruited subjects according to a procedure approved by the "Ethics committee" of the University "G. d'Annunzio" of Chieti and Pescara.

Blood was collected from fertile women during the central part of the menstrual cycle. In the morning between 08:00 and 10.00 h, blood was drawn through the antecubital vein, collected in heparinized tubes kept at room temperature and transported to the laboratory for processing within 1 h from collection.

Determination of lymphocyte subpopulations

CD45-CD3-CD4-CD8 and CD45-CD3-CD16-56-CD19 antibodies (Becton-Dickinson, San Jose, CA, USA) were used. Four-colour flow-cytometry analysis was performed by FACScalibur with an automatic sample processor SP-1 Becton-Dickinson to determine $CD45^+$ cells (all the lymphocytes), T $CD45^+$-$CD3^+$, T helper $CD45^+$-$CD3^+$-$CD4^+$, T cytotoxic $CD45^+$-$CD3^+$-$CD8^+$, $CD45^+$-$CD3^-$-$CD8^+$,NK $CD45^+$-$CD16^+$-56^+ and B $CD45^+$-$CD19^+$ lymphocytes.

Determination of the IFN-γ release from PBMC

Spontaneous and PHA-stimulated IFN-γ release from PBMC was determined according to a previous study (13)

Determination of the cytotoxic activity

Human erythroleukemia K562 cell line was cultured in CRPMI (complete RPMI) -1640 medium (Gibco). Cytotoxicity experiment was performed using Cytotox 96 (Promega, Southampton, UK) according to Allen and Rhuston (17) following the manufacturer's protocol. This assay is based on the release of the cytosol enzyme LDH from cells. The end-point of the LDH assay is an estimation of damage to human erythroleukemia K562 target cells after exposure of the PBMC ($2x10^4$ cells per well), used as effector cells. Cytotoxicity was calculated as % = [effectors and target mix-effectors control spontaneous)/(maximum-spontaneous)] x 100.

Determination of the level of anxiety

The recruited women filled in the questionnaires in a quiet room during the morning of the blood sample collection. State-trait-anxiety inventory (STAI) was used in the scale I, to measure state-anxiety as a temporary and varying condition, and in scale II, to monitor trait-anxiety, as a relatively fixed tendency of the personality (18-19). Occupational stress was monitored by a 10 item test (with a score of 10 for each item) of the Association of American Psychologists.

Statistical analysis

Analysis of the data was performed with Statistica, Release 4.5. Kolmogorov-Smirnov test was used to determine the distribution of the data in order to use parametric or non-parametric methods for descriptive statistics.

Table I. *Values of electromagnetic fields (μT) in the rooms of the museum*

	Working place		Rooms nearby the working place	
	Median	range	median	Range
Year 1999	1.6	0.2-3.6	3.6	0.3-7.2
Year 2005	1.1	0.1-3.4	1.1	0.1-4.7

Table II. *Lymphocyte subpopulations of women and men and exposed to electromagnetic fields.*

Cells/l	Control		EMF exposed	
	median	25^{th}-75^{th} percentiles	median	25^{th}-75^{th} percentiles
Women				
$CD45^+$ (lymphocytes)	1640	1453-2015	1727	1512-2136
$CD45^+$-$CD3^+$	1210	1044-1480	1206	1077-1614
$CD45^+$-$CD3^+$-$CD4^+$	728	568-860	766	615-871
$CD45^+$-$CD3^+$-$CD8^+$	461	378-579	438	298-570
$CD45^+$-$CD16^+$-$CD56^+$	219	168-277	332	263-346
$CD45^+$-$CD19^+$	199	147-250	167	134-271
Men				
$CD45^+$ (lymphocytes)	2015	1665-2281	1650	1342-1743
$CD45^+$-$CD3^+$	1289	1028-1728	1024	877-1162
$CD45^+$-$CD3^+$-$CD4^+$	792	670-1018	692	551-696
$CD45^+$-$CD3^+$-$CD8^+$	458	307-683	309	294-406
$CD45^+$-$CD16^+$-$CD56^+$	313	225-448	217	195-258
$CD45^+$-$CD19^+$	230	161-311	206	136-296

RESULTS

The men and women exposed to EMFs did not show changes of lymphocyte subpopulations in relation to the control groups (Table II). Although there were no significant alterations, the $CD45^+$-$CD16^+$-56^+ subsets of the control women were slightly lower than those of the women exposed to EMFs and the $CD45^+$-$CD3^+$-$CD8^+$ of all the women were slightly higher than those of the men.

Blood cytotoxic activity of the women exposed to EMFs expressed per $CD45^+$-$CD16^+$-56^+ NK lymphocytes (but not per ml of blood or number of blood lymphocytes) was significantly lower than that of the controls, while blood cytotoxic activity of the EMF exposed men was unaltered (Table III).

The INF-γ release from PBMC of the women exposed to EMFs was significantly reduced in relation to that of the controls, while the INF-γ release from PBMC of the men exposed to EMFs did not show any significant decrease (Fig. 1).

One of the women exposed to EMFs, the only one working night shifts with her husband, showed marked lymphopenia with very low blood NK $CD45^+$-$CD16^+$-56^+ and $CD45^+$-$CD8^+$ lymphocytes, reduced IFN- release-γ and symptoms of astenia; the immune parameters returned to normal following the change of work site.

The scores of STAI I and STAI II of the control women were slightly higher than those of the control men, while this difference was not evident between the scores of EMF exposed women and men (Table IV). The STAI I and II of the EMF exposed men and women were similar.

Moreover, the values of occupational stress of the men exposed to EMFs were higher than those of the control men and of control and E MF exposed women (Table IV).

The score of STAI I and STAI II of the control women showed a close linear correlation (No. 52, $p = 0.505$, $p < 0.001$). The scores of STAI I were negatively correlated with the cytotoxic activity expressed per number of blood

Table III. *Blood cytotoxic activity of of women and men and exposed to electromagnetic fields.*

%	Control		EMF exposed	
	median	25^{th}-75^{th} percentiles	median	25^{th}-75^{th} percentiles
Women				
Units of cytotoxic activity/ ml of blood	19.6	15.9-23.0	13.8	7.1-21.0
Units of cytotoxic activity/ $CD45^+$cells of ml of blood x 10^3	12.0	10.2-15.8	8.3	5.0-12.7
Units of cytotoxic activity/$CD45^+$-$CD16^+$-56^+ cells of blood x 10^3	107.2	62.3-147.5	48.5	34.5-75.1*
Men				
Units of cytotoxic activity/ ml of blood	20.7	11.4-29.8	19.4	18.7-23.8
Units of cytotoxic activity/ $CD45^+$cells of ml of blood x 10^3	9.5	5.7-14.1	10.9	8.9-20.3
Units of cytotoxic activity/$CD45^+$-$CD16^+$-56^+ cells of blood x 10^3	70.1	43.0-105.5	83.2	69.8-98.7

*Mann-Whitney U-test.; Statistical significant difference: *p<0.05*

Table IV. *STAI I, STAI II and occupational stress of women and men and exposed to electro-magnetic fields.*

%	Control (n 8)		EMF exposed	
	median	25^{th}-75^{th} percentiles	median	25^{th}-75^{th} percentiles
Women				
STAI I	41.0	33.0-44.0	36	32.5-43.7
STAI II	40.0	34.0-43.0	38.0	33.7-42.2
Occupational stress	43.5	28.2-56.7	37.0.7	29.0-58.2
Men				
STAI I	36.0	33.7-39.2	39.0	29.5-43.5
STAI II	37.5	33.0-38.2	38.0	32.5-41.0
Occupational stress	35.0	28.5-43.0	56.0	52.5-62*

*Mann-Whitney U-test.; Statistical significant difference: *p<0.05*

NK $CD45^+$-$CD16^+$-$CD56^+$ cells (but not expressed per ml of blood or number of lymphocytes). The values of STAI II of the controls were negatively correlated with cytotoxic activity per ml of blood, number of lymphocytes, and cytotoxic activity per number of NK $CD45^+$-$CD16^+$-$CD56^+$ cells (Table V). It is also interesting to observe that there was a positive linear correlation between the cytotoxic activity/ml of blood of the control group and the IFN-γ release from PBMC of both control women and women exposed to EMFs (No. 27, r = 544, $p < 0.01$).

DISCUSSION

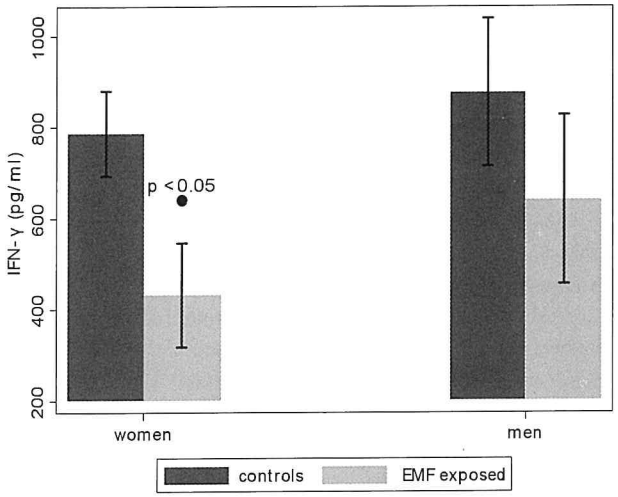
The men exposed to EMFs showed reduced blood NK lymphocytes in the previous study, while in the follow up, there were no alterations of immune parameters. This may depend on the reduction of exposure to EMFs due to improvement of the work sites. The occupational stress of the men exposed to electricity was higher than that of the control men and of all the women. However, occupational stress did not significantly influence either state and trait anxiety (STAI I and II) or the immune parameters.

This study demonstrates that the EMFs produced by electricity affect immune functions more in women than in men. The women exposed to EMFs not only showed reduced blood cytotoxic activity per NK, cells but also lower values of IFN-γ than the controls, both in 2000 and in 2005. A significant correlation was found between blood cytotoxic activity and INF-γ release from PBMC. With regard to this, it is known that the activity of the NK cells is regulated by the INF-γ produced by both T and NK lymphocytes (20).

Table V. *Significant linear correlation of STAI I and STAI II with immune parameters in 52 control women.*

	Vs	r	P
STAI I	Cytolytic activity/ ml blood	-0.173	ns
	Cytolytic activity/CD45$^+$ cells	-0.213	ns
	Cytolytic activity/ CD45$^+$-CD16$^+$-56$^+$ cells (No)	-0.456	<0.002
STAI II	Cytolytic activity /ml blood	-0.459	<0.002
	Cytolytic activity/CD45$^+$ cells	-0.455	<0.002
	Cytolytic activity/ CD45$^+$-CD16$^+$-56$^+$ cells (No.)	-0.505	<0.001

Fig 1. *IFN-γ (pg/ml) releaase from PBMC of EMF exposed women and men.*



Values are mean ± S .D

Both STAI I and STAI II of the control women were negatively correlated with blood cytotoxic activity/number of NK lymphocytes; STAI II (but not STAI I) was also significantly correlated with cytotoxic activity per ml of blood and per number of lymphocytes. These results are in agreement with those demonstrating reduced blood NK lymphocyte and LAK cell activities in subjects with a poor lifestyle and/or mental instability (21), in young adults with depressive disorders (22), and in young subjects with high anxiety and low emotional stability (23). The existence of reciprocal mechanisms of regulation acting between the immune and nervous systems in response to environmental and behavioral signals may in part explain the above reported data (15-16).

A previous study by our group suggests that the effects of the exposure to both high and low frequency EMFs may modify the immune response to toxic agents, such as those produced by traffic (24). A similar effect may also be induced by occupational stress. A group of employees using mobile phones with prolonged conversation time showed lower levels of serum TSH (25). The authors were unable to establish whether these results were determined by exposure to EMFs from mobile phones or by the stress of using these instruments. In the follow up of our study, one woman exposed to EMF, working night shifts in the museum, showed marked altered immune parameters with temporary symptoms. In this case, occupational stress may also have contributed to modify the immune response to EMFs. Therefore, we suggest that the effects of EMFs are more evident in subjects with vulnerable immune and/or nervous systems; this condition may be determined by a genetic predisposition, environmental exposure to toxic agents, lifestyle or stress.

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