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Effects of electromagnetic fields produced by radiotelevision broadcasting stations on the immune system of women

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Abstract

The object of this study was to investigate the immune system of 19 women with a mean age of 35 years, for at least 2 years (mean = 13 years) exposed to electromagnetic fields (ELMFs) induced by radiotelevision broadcasting stations in their residential area. In September 1999, the ELMFs (with range 500 KHz–3 GHz) in the balconies of the homes of the women were (mean ± S.D.) 4.3 ± 1.4 V/m. Forty-seven women of similar age, smoking habits and atopy composed the control group, with a nearby resident ELMF exposure of < 1.8 V/m. Blood lead and urinary *trans–trans* muconic acid (a metabolite of benzene), markers of exposure to urban traffic, were higher in the control women. The ELMF exposed group showed a statistically significant reduction of blood NK $CD16^+$-$CD56^+$, cytotoxic $CD3^-$-$CD8^+$, B and NK activated $CD3^-$–HLA-DR^+ and $CD3^-$-$CD25^+$ lymphocytes. 'In vitro' production of IL-2 and interferon-γ (INF-γ) by peripheral blood mononuclear cells (PBMC) of the ELMF exposed group, incubated either with or without phytohaemoagglutinin (PHA), was significantly lower; the 'in vitro' production of IL-2 was significantly correlated with blood $CD16^+$-$CD56^+$ lymphocytes. The stimulation index (S.I.) of blastogenesis (ratio between cell proliferation with and without PHA) of PBMC of ELMF exposed women was lower than that of the control subjects. The S.I. of blastogenesis of the ELMF exposed group (but not blood NK lymphocytes and the 'in vitro' production of IL-2 and INF-γ by PBMC) was significantly correlated with the ELMF levels. Blood lead and

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urinary *trans–trans* muconic acid were barely correlated with immune parameters: the urinary metabolite of benzene of the control group was only correlated with $CD16^+$-$CD56^+$ cells indicating a slight effect of traffic on the immune system. In conclusion, this study demonstrates that high frequency ELMFs reduce cytotoxic activity in the peripheral blood of women without a dose–response effect. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Electromagnetic field; Immune system; Lymphocyte subpopulations; Cytokines; Traffic

1. Introduction

Several studies have reported that electromagnetic fields (ELMFs) modify the human genome and induce malignancies (Theriault, 1996). An increased incidence of cancer has been found in the area surrounding a radio and television transmitter in Great Britain (Dolk et al., 1997a). On the other hand, these results have not been confirmed by investigations performed on populations resident near other power transmitters in the same country (Dolk et al., 1997b). Although there is concern for the risk of cancer, until now, a clear relation between ELMF exposure and the incidence of neoplasms has not been demonstrated as the studies showed either contradictory results or the presence of confounding factors (Theriault, 1996; Knave, 2000). It is clear that more research on ELMF-exposed populations is needed to get satisfactory epidemiological study results or to demonstrate an involvement of ELMFs in mechanisms which may induce cancer.

Several studies report that the immune system exerts defence mechanisms against cancer: natural killer (NK) cell activity is reduced in patients with neoplastic diseases (Pross and Lotzova, 1993); cytotoxic activity against neoplastic cells is an important prognostic factor in patients with resected lung carcinoma (Fujisawa and Yamaguchi, 1997); modern therapy of cancer includes treatment with lymphokines such as interleukin (IL) 2 and interferon (INF) α and γ (Kamamura et al., 1998). For this reason, it may not be excluded that ELMFs may increase the incidence of cancer by impairing immune defences.

It was shown that ELMFs modify calcium fluxes in the membranes of immune cells of humans acting on the release of tromboxane B_2 and IL-1 (Conti et al., 1985). Moreover, peripheral blood mononuclear cells (PBMC) of humans exposed 'in vitro' to low frequency ELMFs showed changes in [^{3}H]thymidine incorporation following 'in vitro' stimulation by mitogens (Conti et al., 1983, 1986). Recently, PBMC were exposed for 24, 48 and 72 h to an ELMF with repetition frequency of 50 Hz and field intensity of 1.5 mT (Di Gioacchino et al., 2000). The ELMF exposure influenced $CD3^+$, $CD4^+$, $CD8^+$ and $CD16^+$ surface marker expression and/or localisation. Moreover, DNA $CD4^+$ expression strongly increased in the exposed cells.

'In vitro' cell proliferation of PBMC of subjects exposed to ELMFs produced by an Italian TV broadcasting station was different from that of the control group (Giuliani et al., 1996). Moreover, cytotoxicity tests showed a significant reduction of NK activity when PBMC of the exposed group were re-irradiated 'in vitro' by radiofrequencies (639.25 MHz, 12 V/m average, 50 Hz amplitude modulated or an ELMF of 50 Hz, 0.67 mT). We studied immune parameters of men and women employed in a museum who were exposed to an ELMF (range 0.2–3.6 μT and 40–120 V/m) induced by 50 Hz electricity for 20 h a week (Boscolo et al., 2001). Men working in the museum showed, in relation to the control subjects, a statistically significant reduction of both number and percentage of NK $CD16^+$-$CD56^+$ and NK and B $CD3^-$-$CD25^+$ lymphocyte subsets; women showed a significant reduction in the percentage of $CD3^-$-$CD25^+$ lymphocytes and a slight reduction of $CD16^+$–56^+ lymphocytes. They also showed significantly lower levels of INF-γ in serum or produced 'in vitro' by PBMC, both spontaneously and stimulated by PHA, while they showed no significant changes of serum and 'in vitro' produced IL-4, as well as of blastogenesis of PBMC. These results were similar to the reduced NK and lymphokine activated activity found in subjects with poor lifestyle (Morimoto et al., 1999,

2001) or with a psychological behaviour characterised by difficulty in expressing feelings (Dewaraja et al., 1997).

The purpose of this study was to investigate effects of ELMFs induced by radiotelevison broadcasting stations on the immune system of the ELMF exposed population.

2. Subjects and methods

Women were investigated because they remained in their homes on a hill near several radiotelevision broadcasting stations for a longer period (<12 h a day) than men. This hill, San Silvestro, inhabited by approximately 2000 people, faces the Adriatic Sea 2 km away from Pescara, a town in Central Italy. During October 1997, the higher ELMF levels determined in San Silvestro by the Italian Istituto Superiore Prevenzione Sicurezza e Lavoro (ISPESL) and by the regional environmental protection agency (ARTA), were ranging from 11 to 40 V/m and from 0.5 to 4.0 W/m^2 (Vignati et al., 1997); in the same locality in September 1999, the ELMFs determined by ARTA ranged from 10 to 25 V/m. In the period 1997–1999, the values of ELMFs in the nearby towns of Pescara and Chieti (where the control group was resident), determined by ISPESL and ARTA, were <1.8 V/m. The two towns (with a distance of 15 km) are enclosed between the Adriatic Sea and the Appennino Mountains. In this area, high levels of traffic are inside Pescara and Chieti and in high-speed motorways connecting these towns and those nearby. However, the toxic compounds produced by traffic and those emitted in lesser amounts by factories are almost uniformly distributed in the environment because of climatic and geographic conditions; the population show only slight differences in blood trace elements, including lead, mainly derived from tetraethyl and tetramethyl lead added to gasoline (Boscolo et al., 1993).

In September 1999, the values of ELMFs induced by the radiotelevision broadcasting stations were determined in the balconies and inside the homes of the women resident in San Silvestro recruited for this study (in addition to the values above reported determined in the areas with high ELMF exposure). The following instruments (PMM, Milano, Italia) were utilised:

1. probes for electric fields BA-01 (range 500 KHz–3 GHz; f.s. 120 V/m);
2. probes for electric fields BA-05 (range 10 MHz–1 GHz; f.s. 30 V/m);
3. monitor 8051 with optic repeater OR-01;
4. cables with optic fibres to control the instruments at a distance (to avoid interference of the operators on ELMF levels); and
5. wood supports to insulate the probes.

Nineteen women with a mean age of 35 years (range 22–49 years) resident in San Silvestro at least from 1997 (mean period of residence = 13 years) were investigated. Most of them were housewives; six (31.5%) were smokers (less than 10 cigarettes a day); seven (36.8%) were atopics: six had suffered from seasonal asthma and/or rhinitis with serum IgE >100 IU/ml (CAPFeia, Pharmacia, Uppsala, Sweden) and one of them, with 92 IU/ml of serum IgE, suffered from seasonal rhinitis with positive skin test to inhalant allergens.

The control group, in which blood lead, serum IgE and lymphocyte subsets were determined, was composed of 47 women with a mean age of 35 years (range 21–49 years). This group included another subset of 17 women with a mean age of 34 years (range 22–44 years), in which urinary *trans–trans* muconic acid and 'in vitro' lymphoproliferation and production of cytokines by PBMC was also measured. The control group of 47 subjects comprised 17 atopics (36.1%) and 14 smokers (29.8%); the control group of 17 subjects was also composed by six atopics (35.3%) and five smokers (29.4%). The diagnosis of atopy was made considering the levels of serum IgE, history of allergic disease and skin test to inhalant allergens. Therefore, the ELMF exposed and the control groups of 47 and 17 women showed similar age, atopy and smoking habit, while the control groups, resident in the above reported area of Pescara and Chieti, showed a higher exposure to traffic. The exposure to ELMFs produced by electric appliances in the homes (Mader and Peralta,

1992) of all the women recruited in this study was not considered.

Clinical assessment included physical examination and standard routine blood analyses. Pregnant women, those taking drugs or suffering from diseases were not recruited for the investigation. Atopic women in the active phase of allergic disease treated with systemic drugs (steroids and/or antihistamines) were also excluded.

Informed consent was obtained from the recruited subjects according to a procedure approved by the 'Ethic committee' of the University 'G. D'Annunzio' of Chieti. Blood and urine samples were collected in plastic cryovials (Nalgene, International PBI, Milano, Italy) at 08.00 h, with a standard procedure (Sabbioni et al., 1992) in order to determine blood lead and urinary *trans–trans* muconic acid (a metabolite of benzene), markers of exposure to traffic (Boscolo et al., 2000). Blood lead was determined by an atomic absorption spectrophotometer Perkin-Elmer 4100 ZL (Sabbioni et al., 1992). A further analytical quality control on 10% of samples was carried out in two different laboratories. Urinary *trans–trans* muconic acid was analysed by HPLC (Ducos et al., 1990, 1992).

Fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-conjugated antibodies (Becton-Dickinson, San Jose, CA, USA) were used to determine lymphocyte subsets. The antibodies were CD4-CD45RO [to evaluate helper $CD4^+$-$CD45RO^+$ 'memory' and $CD4^+$-$CD45RO^-$ 'naive or virgin' lymphocytes (Male et al., 1996)], CD3-CD8, CD16-CD56 (NK cells), CD19 (B lymphocytes), CD3-HLADR and CD3-CD25 (both activated NK and B lymphocytes). Two-colour flow-cytometry analysis was performed by FACscan (Becton-Dickinson, San Jose, CA, USA) (Fleiscer et al., 1988). Serum IgE was measured by ELISA (Brostoff et al., 1991).

For determining the production of cytokines, PBMC were incubated for 24 h at 37°C in 5% CO_2-humidified atmosphere in polypropylene tubes (Falcon, Italy) with or without 20 μg/ml of phytohaemoagglutinin (PHA) (Difco). At the end of the incubation period, cell-free supernatants were harvested and stored at −20°C until assay of IL-2, IL-4, IL-5 and INF-γ (Benfer-Scheller, Key-Stone Laboratories, USA) with or without PHA by ELISA (Fridas et al., 1996).

The blastogenesis (proliferation) of PBMC was also determined 'in vitro' according to Conti et al. (1983, 1986). Blastogenesis was determined as the stimulation index (S.I.), which is the rate between [^{3}H]thymidine incorporation by PBMC in the presence of PHA and without PHA, in the last 6 h of incubation after a 48-h incubation period.

Statistical analysis of the data was performed with Statistica, Release 4.5. Kolmogorov–Smirnov tests showed that most of the results concerning immune parameters did not conform to the normal distribution. Therefore, non-parametric methods were used for all simple descriptive statistics.

3. Results

ELMFs with range 500 KHz–3 GHz, determined during the mornings of September 1999 in the balconies of the homes of 17 out of the 19 investigated women resident in San Silvestro hill were (mean ± S.D.) 4.3 ± 1.4 V/m. ELMFs, within the same range of 500 KHz–3 GHz, determined inside the home, were approximately 30–50% lower than outside. ELMF values showed slight modifications during the 24-h. The levels of ELMFs of the homes of the recruited women were lower than those (ranging from 10 to 24 V/m reported in Section 2) determined in another area of San Silvestro. Only one ELMF exposed woman showed values of ELMF exposure (1.2 V/m outside her home) similar to that of the control group (< 1.8 V/m).

The values of blood lead and urinary *trans–trans* muconic acid, biomarkers, of exposure to traffic (Boscolo et al., 1999a, 2000) of the women of San Silvestro were lower than those of the control group resident in the nearby area of the towns of Pescara and Chieti (Table 1).

Serum IgE of the ELMF exposed women did not show statistically significant differences from those of the control subjects (Table 2).

ELMF exposed women showed a significant reduction of T 'virgin' $CD4^+$-$CD45RO^-$ lymphocytes and a higher ratio between 'memory' $CD4^+$-

Table 1
Blood metals and urine *trans–trans* muconic acid of women exposed to electromagnetic fields[a]

	Control			Exposed		
	No.	median	25th–75th percentiles	No.	Median	25th–75th percentiles
Blood lead (μg/l)	47	5.9	4.9–7.0	19	4.4	3.7–4.7*
Urinary *trans–trans*-muconic acid (μg/l)	17	42.0	19.1–107.5	19	15.0	9.5–32.5*
Urinary *trans–trans*-muconic acid (μg/g creatinine)	17	33.5	13.2–92.5	19	11.0	6.5–28.0**

[a] Mann–Whitney *U*-test. Statistical significant difference: $*P < 0.05$; $**P < 0.01$.

$CD45RO^+$ and 'virgin' $CD4^+$-$CD45RO^-$ lymphocytes (Table 2). ELMF exposed women showed a reduction in both the number and percentage of total lymphocytes of blood NK $CD16^+$-$CD56^+$ lymphocytes, $CD3^-$-$CD8^+$ cells [$CD8^+$ lymphocytes without the expression of the antigen T $CD3^+$ and NK $CD16^+$ cells (Male et al., 1996)] and of $CD3^-$-$CD25^+$ B and NK activated lymphocytes (Table 2 and Fig. 1). $CD3^-$-$HLA\text{-}DR^+$ B and NK activated lymphocytes of the ELMF exposed group were also significantly lower than those of the control group when determined as the percentage of total lymphocytes (Table 2 and Fig. 1).

In 'vitro' production of di IL-2 and INF-γ by PBMC of the ELMF exposed women was significantly lower than that of the control group either without or with PHA in the incubation liquid (Table 3). On the other hand, there was no difference in the production of IL-4 and IL-5 by PBMC of the ELMF exposed and control women (Table 3).

Table 2
Serum IgE and blood lymphocyte subpopulations of women exposed to electromagnetic fields[a]

Cells/μl	Control (*n* 47)		Exposed (*n* 19)	
	median	25th–75th percentiles	median	25th–75th percentiles
Serum IgE (IU/ml)	25	10–79	41	14–121
Lymphocytes	1999	1780–2605	2050	1700–2545
$CD3^+$	1421	1270–1811	1531	1214–1854
$CD4^+$	884	711–1213	955	684–1152
$CD4^+$-$CD45RO^-$	323	260–477	264	211–347*
$CD4^+$-$CD45RO^+$	562	448–711	673	496–779
$CD4^+$-$CD45RO^+$/$CD4^+$-$CD45RO^-$	1.50	1.01–2.02	2.36	1.99–3.18*
$CD3^+$-$CD8^+$	520	442–726	562	467–674
$CD3^-$-$CD8^+$	133	98–194	93	56–123**
$CD16^+$-$CD56^+$	395	306–489	239	176–293**
$CD19^+$	210	138–291	208	153–251
$HLA\text{-}DR^+$	482	392–601	432	361–561
$CD3^-$–$HLA\text{-}DR^-$	330	263–431	295	250–355
$CD3^+$–$HLA\text{-}DR^+$	124	92–176	131	89–220
$CD25^+$	356	273–496	372	263–444
$CD3^-$-$CD25^-$	95	77–126	68	31–92**
$CD3^+$-$CD25^+$	248	195–351	287	239–386

[a] Mann–Whitney *U*-test. Statistical significant difference: $*P < 0.05$; $**P < 0.01$.

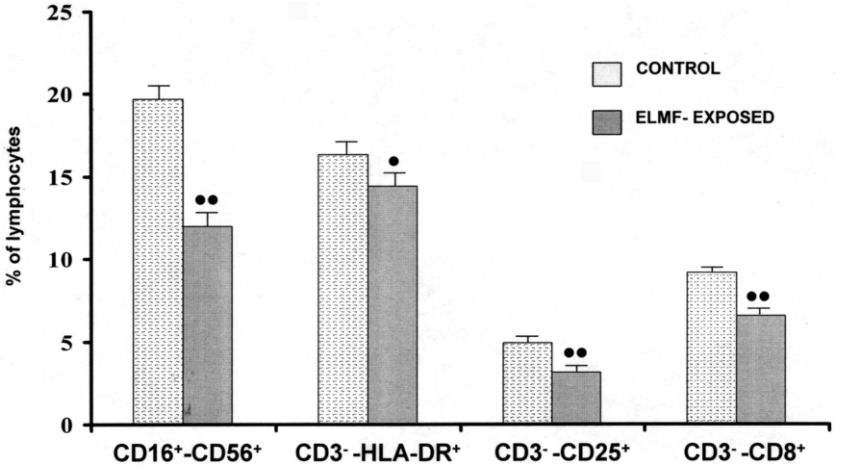

Fig. 1. Blood $CD16^{+}$-$CD56^{+}$, $CD3^{-}$-HLA-DR^{+}, $CD3^{-}$-$CD25^{+}$ and $CD3^{-}$-$CD8^{-}$ lymphocyte subsets (expressed as percentage of total lymphocytes) of women exposed to electromagnetic fields. Values are expressed as mean ± S.E. Mann–Whitney *U*-test (control women: $n = 47$; ELMF exposed women: $n = 19$). Statistical significant difference: $^{\bullet}P < 0.05$; $^{\bullet\bullet}P < 0.01$.

The S.I. of blastogenesis (proliferation) of PBMC, determined using PHA, of the ELMF exposed group was significantly lower than that of the control group (Table 4). Notwithstanding this result, there was a significant correlation between the values of ELMFs determined from the balconies of 17 homes of the women resident in San Silvestro and their S.I. of blastogenesis. The curve which better interpolates data regarding the relation between V/m and S.I. is illustrated in Fig. 2: it is a logarithmic curve having the following equation: $y = 16.944x^{0.553}$ with $R^2 = 0.5519$. On the other hand, there was no significant correlation between the values of ELMFs determined in the homes of the women resident in San Silvestro and their lymphocyte subsets or production of cytokines by their PBMC.

Blood lymphocytes $CD16^{+}$-$CD56^{+}$ (reported as percentage of total lymphocytes) of all the examined women showed a positive linear correlation with the values of IL-2 produced 'in vitro' by PBMC either incubated in absence of PHA ($n = 36$, $R = 0.557$, $P < 0.001$) or with PHA ($n = 36$, $R = 0.416$, $P < 0.02$). Moreover, INF-γ produced 'in vitro' by PBMC with PHA was significantly correlated with the IL-2 produced 'in vitro' by

Table 3
'In vitro' production of cytokines by peripheral blood mononuclear cells (PBMC) incubated with or without phytohaemoagglutinin (PHA) of women exposed to electromagnetic fields[a]

Cytokines (pg/ml)	Control (*n* 17)		Exposed (*n* 19)	
	median	25th–75th percentiles	median	25th–75th percentiles
IL-2 without PHA	17.70	5.50–38.70	1.57	1.29–2.50**
IL-2 with PHA	55.30	17.01–163.20	6.70	3.23–32.80*
IL-4 without PHA	1.70	1.29–3.01	2.11	1.5–2.59
IL-4 with PHA	3.30	1.60–5.80	3.31	2.35–5.40
IL-5 without PHA	1.28	0.85–1.98	1.90	1.10–3.27
IL-5 with PHA	12.50	6.95–18.10	11.40	6.20–37.25
INF-γ without PHA	0.81	0.49–1.11	n.d.	
INF-γ with PHA	27.50	8.30–51.50	7.60	1.03–15.65*

[a] Mann–Whitney *U*-test. Statistical significant difference: $^{*}P < 0.002$; $^{**}P < 0.001$; n.d. = non-determinable.

Table 4
Stimulation index (S.I.)[a] of blastogenesis (proliferation) of peripheral blood mononuclear cells (PBMC) of women exposed to electromagnetic fields[b]

	Control (n 17)		Exposed (n 19)	
	median	25th–75th percentiles	median	25th–75th percentiles
Simulation index (S.I.)	54.5	37.6–71.4	36.8	32.2–43.2*

[a] S.I. is the rate between [^{3}H]thymidine incorporation by PBMC in presence of phytohaemoagglutinin (PHA) and without PHA.
[b] Mann–Whitney U-test. Statistical significant difference: $^*P < 0.05$.

PBMC both incubated in absence of PHA ($n = 36$, $R = 0.674$, $P < 0.0001$) and with PHA ($n = 36$, $R = 0.410$, $P < 0.02$).

Blood lead and urinary *trans–trans* muconic acid did not show a significant correlation with immune parameters with the exception of the correlation between urinary *trans–trans* muconic acid (reported as μg/g creatinine) and $CD16^+$-$CD56^+$ lymphocytes ($n = 17$, $R = 0.627$, $P < 0.01$) and $CD4^+$-$CD45RO^-$ 'virgin' lymphocytes ($n = 17$, $R = 0.625$, $P < 0.01$) of the control group. For this reason, a correlation analysis between ELMF values of exposure and immune parameters (including $CD16^+$-$CD56^+$, $CD3^-$-HLA-DR^+, $CD3^-$-$CD25^+$, $CD3^-$-$CD8^+$ cells, S.I. of blastogenesis of PBMC and production of INF-γ and IL-2 by PBMC) was performed with adjustment for urinary *trans–trans* muconic acid: all these statistical analyses were not significant.

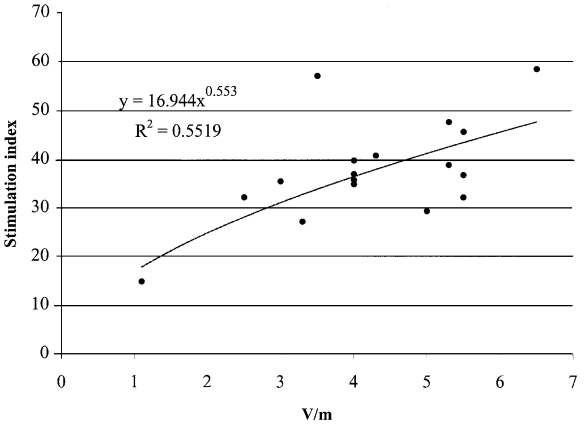

Fig. 2. Linear correlation between the values of electromagnetic fields produced by radiotelevision broadcasting stations on the balconies of the homes of women and their stimulation index (S.I.) of blastogenesis (proliferation) of peripheral blood mononuclear cells (PBMC). Pearson's correlation coefficient: $n = 17$, $R = 0.608$; $P < 0.01$.

4. Discussion

The women exposed to ELMFs produced by radiotelevision broadcasting stations showed a reduction of NK $CD16^+$-$CD56^+$ and cytotoxic $CD3^-$-$CD8^+$ cells and of NK and B $CD3^-$-HLA-DR^+ and $CD3^-$-$CD25^+$ activated lymphocytes in the peripheral blood along with a significant reduction of IL-2 and INF-γ produced 'in vitro' by PBMC. None of these immune parameters showed a dose–response effect with the ELMF values determined outside the homes of the investigated women.

Blood NK $CD16^+$-$CD56^+$ lymphocytes of all the examined subjects were significantly correlated with the values of IL-2 and INF-γ produced 'in vitro' by PBMC. It is known that IL-2 and INF-γ, produced by activated T lymphocytes, exert a potent effect on macrophages and a synergistic action with that of other cytokines [e.g. TNF-α (Male et al., 1996)]. It is also known that INF-γ produced by both T and NK lymphocytes, may activate NK lymphocytes with an 'autocrine loop' (Male et al., 1996). For this reason, lower levels of blood NK cells and of the 'in vitro' production of IL-2 and INF-γ by PBMC of the ELMF exposed women are an expression of low cytotoxic activity in the peripheral blood.

The results of this study are in agreement with those of Giuliani et al. (1996), who showed altered cytotoxicity tests in subjects exposed to

ELMFs produced by a TV broadcasting station. This investigation also confirms the data of a previous study of our group on men and women employed in a museum exposed to an ELMF induced by 50 Hz electricity who showed low blood NK $CD16^+$-$CD56^+$ and/or B and NK $CD3^-$-$CD25^+$ lymphocytes and reduced production of INF-γ by PBMC (Boscolo et al., 2001)

The results of this investigation are similar to those of Morimoto et al. (1999, 2001) and Dewaraja et al. (1997) who found low blood NK activity or a reduction of blood NK lymphocytes in subjects with poor lifestyle and with a psychological behaviour characterised by difficulty in expressing feelings. Therefore, we do not exclude that the effects of the ELMFs on the immune system may be, in part, mediated by those on nervous mechanisms which are closely connected with the immune ones (Male et al., 1996; Jankovic, 1992; Szentivayi, 1997). With regard to this, the blood levels of both total and cytotoxic lymphocytes decreased following exposure to magnetic resonance imaging of the brain of volunteers, suggesting that the nervous system may regulate the trafficking of lymphocytes in peripheral blood (Reichard et al., 1996). It was also shown that the circadian biorhythm regulates not only the release and production of neurohormones, but also blood levels of lymphocyte subsets (Boscolo et al., 1999b).

Although blood cytotoxic activity may be modified by lifestyle (Morimoto et al., 1999, 2001), psychological behaviour (Dewaraja et al., 1997) and possibly occupational stress (Boscolo et al., 2001), the monitoring of blood cytotoxic activity and NK lymphocytes may be considered useful as a biomarker of exposure to ELMFs. A more interesting biomarker seems to be the $CD16^+$-$CD56^+$ NK lymphocyte subpopulation, which is significantly correlated with the 'in vitro' production of IL-2 by PBMC.

Blood lead and urinary *trans–trans*-muconic acid, markers of exposure to traffic (Boscolo et al., 2000) of the control subjects, were higher than those of the ELMF exposed. These markers of traffic exposure were not correlated with parameters of cytotoxic activity, with the exception of the correlation between urinary *trans–trans*-muconic acid and $CD16^+$-$CD56^+$ lymphocytes of the control group. We could thus suggest that the difference in the values of $CD16^+$-$CD56^+$ NK lymphocytes, between ELMF exposed and control women may rather depend on a higher effect of the toxic compounds produced by traffic on the control subjects. However, adjustment for urinary *trans–trans*-muconic acid did not show significant correlation between ELMF values and immune parameters (including $CD16^+$-$CD56^+$ lymphocytes). For this reason, an effect of the exposure to traffic on the values of NK cells has to be considered as unimportant.

Urinary *trans–trans*-muconic acid was also positively correlated with $CD4^+$-$CD45RO^-$ 'virgin or naïve' lymphocytes of the control group. Moreover, the control group showed higher blood levels of $CD4^+$-$CD45RO^-$ lymphocytes, as well as of lead, than the ELMF exposed group. Sata et al. (1998) found a correlation between the percentage of 'naïve' lymphocytes and blood lead of male workers with mean blood lead of 19 ug/dl. These data suggest that the values of blood $CD4^+$-$CD45RO^-$ and their ratio with $CD4^+$-$CD45RO^+$ lymphocytes may depend on several factors, including lead and benzene produced by traffic.

ELMF exposed women showed a reduced stimulation of PHA on the proliferation of PBMC in relation to the control subjects with a higher exposure to traffic. We cannot exclude an enhancement of the proliferation of PBMC induced by the toxic compounds of traffic, as in the previous case. On the other hand, the S.I. of blastogenesis of the ELMF exposed women (although lower than that of the control subjects) was positively correlated with the ELMF levels determined outside their homes. Therefore, an effect of ELMFs in stimulating PBMC proliferation may also not be excluded.

A part of the immune parameters investigated in this study, plasma zinc and copper markers of metabolic activities (Prasad, 1988; Harbige, 1996), blood lead and urinary *trans–trans*-muconic acid were determined in women exposed to low and high frequency ELMFs and in control women considering the presence of atopy (Del Signore et al., 2000). Linear discriminant analysis applied to the above mentioned parameters showed no sig-

nificant difference between non-atopic groups exposed or not exposed to ELMFs, whereas there was a marked differentiation between atopic women non-exposed and atopic women ELMF exposed. This suggested that ELMFs have a greater influence on atopic women than on non-atopic ones.

We may conclude that exposure to ELMFs induces a modification of immune parameters in humans. ELMF exposure inhibits a Th1-like cytotoxic immune-response without a dose–response effect, while the enhancement of a Th2-like immune response is not demonstrated. Follow-up studies may be useful to explain the biological importance of the reported results.

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