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Combined Effects of Traffic and Electromagnetic Fields on the Immune System of Fertile Atopic Women

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Abstract: Object of this preliminary study was the immune response to high or low frequency electromagnetic fields (ELMF) of non-atopic and atopic fertile women with uniform exposure to toxic compounds produced by traffic. Women were divided in group A (non-atopic, non-exposed to ELMF); B (atopic, non-exposed to ELMF); C (non-atopic, exposed to ELMF); D (atopic, exposed to ELMF). *"In vitro"* cell proliferation of peripheral blood mononuclear cells (PBMC) of atopic women (groups B and D) stimulated by phytohaemoglutinin (PHA) was reduced. The ELMF exposed women (groups C and D) showed lower levels of blood NK CD16+-CD56+ lymphocyte subpopulations and of *"in vitro"* production of interferon-γ (both spontaneously and in presence of PHA) by PBMC, suggesting that ELMF reduces blood cytotoxic activity. Serum IgE of the atopic women exposed to ELMF (group D) was higher than that of the other groups. Linear discriminant analysis including serum zinc and copper (essential enzymes for immune functions), blood lead and urinary trans-trans muconic acid, a metabolite of benzene (markers of exposure to traffic) and key parameters of immune functions (CD16+-CD56+ lymphocyte subset, serum IgE, interferon-γ produced by PBMC in presence of PHA, stimulation index of blastogenesis) showed absence of significant difference between groups A and C and a marked separation of groups B and D. This datum suggests that ELMF have a greater influence on atopic women exposed to traffic than on non-atopic ones.

Keywords: Electromagnetic field, Atopy, Fertile woman, Trace elements, Lymphocytes sub-population, Interferon-γ, Blastogenesis, Exposure to traffic, Basic statistics, Multivariate analysis

Introduction

Exposure to toxic agents of an urban environment was found to modify the immune response. In particular, lead (Pb) exposure, produced by combustion of alkylated Pb compounds of gasoline, seemed to increase production of IgE in atopic men enhancing the incidence of allergic symptoms[1)]. This datum confirmed those of previous investigations performed "*in vitro*" or on experimental animals showing that Pb modulates immune activities stimulating the Th2 "humoral" immune response and inhibiting the Th1 "cell mediated" response[2, 3)].

Experimental studies demonstrated that both low and high frequency electromagnetic fields (ELMF) may influence the immune system. It was shown that ELMF modify calcium fluxes in membranes of immune cells of humans with effects on the release of thromboxane B_2 and interleukin (IL) 1[4)]. Moreover, peripheral mononuclear blood cells (PBMC) of humans exposed "*in vitro*" to low frequency ELMF showed changes in cell proliferation tests following stimulation with mitogens[5, 6)]; the ELMF induced immune modifications were greatly influenced by differences in the experimental

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conditions including stimulation by mitogens. Baboons exposed for six weeks to ELMF induced by 60 Hz electricity displayed changes in blood lymphocyte subsets as well as reduction of IL-2 receptor expression and of proliferative response to pokeweed mitogen[7]; this pattern of immune response was not suggestive of an exposure-related effect. On the other hand, subchronic exposure of mice to 60 Hz ELMF failed to demonstrate significant immune alterations[8].

Radiofrequency emissions of a TV broadcasting station, producing high frequency amplitude modulated ELMF, were found to affect the immune system of the exposed population[9]. "*In vitro*" cell proliferation tests performed with PBMC of the exposed subjects were modified compared to those of a control group. Moreover, citotoxicity tests performed with PBMC re-exposed "*in vitro*" to high frequencies ELMF showed reduction of "natural killer" (NK) activity.

A study of our group also found reduction of NK lymphocytes in peripheral blood of men and women exposed to low frequency ELMF along with decreased serum interferon (INF) γ and INF-γ produced "*in vitro*" by PBMC either spontaneously or in the presence of phytohaemoglutinin (PHA)[10].

People exposed to ELMF are generally also exposed to toxic compounds present in the environment. The object of this study was to ascertain the combined effects of ELMF and toxic compounds produced by traffic on the immune response. In particular, the subjects investigated were divided into non-atopic and atopic in order to evaluate the influence of atopia (predisposition to suffer from allergic diseases[11]) on the immune response. For this purpose, a multivariate statistical analysis, including immune parameters, bio-markers of exposure to vehicular traffic as well as serum zinc (Zn) and copper (Cu) (essential enzymes involved in several immune activities[12]), was performed.

Subjects and Methods

Twenty eight women in the fertile period (10 atopic and 18 non-atopic) exposed to ELMF were recruited. Nine women (3 of them atopic) were occupationally exposed to low frequency ELMF and the others (7 of them atopic) were exposed to ELMF induced by radiofrequencies.

Eight women were employed in a museum in Chieti, a town of Central Italy. Their task, for about 20 hours a week in the last two years, was surveillance of premises through monitors in a room (surface about 200 m^2). An electric cable with 360 volt, for the distribution of 50 Hz electricity in the building, was located about 2 meters on the rear of the working places. Levels of ELMF in the room were measured by a EFA-3 EMR instrument (Wandel Golterman). Values (V/m and μT) were obtained with 9 determinations (lasting 60 seconds) at a distance of at least 10 meters. The range of exposure to ELMF was 0.2–3.6 μT and 40–120 V/m. A 9th woman working at monitors, with similar occupational exposure of those exposed to ELMF in the museum, was recruited in the study.

The other 19 women in the fertile period exposed to ELMF were resident, during the last two years, in the area of San Silvestro (a hill inhabited by about 2000 people, 2 km from Pescara and 12 from Chieti). The levels of exposure to ELMF produced by radio-television transmitters in this locality were measured by Italian ISPESL in collaboration with the PMIP (a local environmental protection agency) of Pescara in the period August-October 1997 using different methods and instruments, as stated in technical reports[12]. Moreover, other determinations of the ELMF were performed by the PMIP of Pescara near the residences of the 19 investigated women in the period September-October 1999. The levels of ELMF in October 1997 were ranging from 11 to 40 V/m and 0.5 e 4.0 W/m^2, while those determined in October 1999 were ranging from 6 to 25 V/m. The values of ELMF in the hill of San Silvestro in October 1997 with all the radiotelivision transmitters out of work were about 1.2–1.8 V/m.

The two groups of women, exposed to low or high frequency ELMF, showed a similar age (35 years) and smoking habit (only few were smokers). Moreover, they were not occupationally exposed to toxic agents.

Other 17 women in the fertile period (9 non-atopics and 8 non-symptomatic atopics) were recruited among the white-collar staff and doctors of the University "G. D'Annunzio" of Chieti occupationally not exposed to noxious agents. These women were resident in urban or suburban areas of Pescara and Chieti with low levels of ELMF (not exceeding 4 V/m in the period 1997–1999). They presented age and smoking habit similar to the ELMF exposed women.

The exposure to ELMF produced by electric appliances[13, 14] in the homes of all the women recruited in this preliminary study was not considered.

In the area of Chieti and Pescara, bordered between the Adriatic sea and the Appennino mountains, with low air pollution produced by factories, the levels of toxic compounds produced by vehicular traffic are almost uniform. The population residing in this area present uniform values of trace elements including blood Pb (mainly deriving by alkylated Pb compunds of gasoline[15]). The mean age of all the investigated women was 35 years (range 19–49 years). The mean age of the non-atopic control women (group A)

was 34 years, that of atopic control women (group B) was 33 years, that of the non-atopic women exposed to ELMF (group C) was 37 years and that of the atopic women exposed to ELMF (group D) was 34 years. Most of the investigated subjects were non-smokers. The percentage of smoking subjects was similar in the four groups.

Women with evident clinical history of allergic symptoms (asthma and rhinitis, rhinitis, asthma and dermatitis and dermatitis and/or urticaria) were considered atopic.

Clinical assessment included physical examination and standard routine blood analyses[16]. Women in condition of pregnancy, taking drugs or suffering from diseases, were not recruited for the investigation. Atopic symptomatic women suffering from allergic diseases in treatment with drugs were also excluded from the study.

Informed consent was obtained from the investigated subjects according to a procedure approved by the "Ethic committee" of the University. Blood and urine samples of the examined subjects were collected in plastic cryovials (Nalgene, International PBI, Milano, Italy) at 8 a.m., with a standard procedure for avoiding contamination[16]. Blood Pb, serum Cu and Zn were determined by the atomic absorption spectrophotometers Perkin Elmer 4100 ZL and Varian 300Z[16]. A further analytical quality control on 10% of samples was carried out in different laboratories. Urinary trans-trans muconic acid, a metabolite of benzene, was analysed by HPLC[17, 18].

Fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-conjugated anti-bodies (Becton-Dic-kinson, 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[19]), CD3-CD8, CD16-CD56 (NK cells), CD19 (B lymphocytes). Two-colour flow-cytometry analysis was performed by FACscan (Becton-Dickinson, San Jose', CA, USA)[20]. Serum IgE, and INF-γ(Benfer-Scheller, Key-stone laboratories, USA) were determined by ELISA[21]. Determination of "*in vitro*" production of IL-4 and INF-γ (with or without PHA) PBMC was also made[21].

The blastogenesis (proliferation) of PBMC was also determined "*in vitro*" according to Conti *et al.*[5, 6]. Blastogenesis was determined as stimulation index (S.I.) which is the rate between ^{3}H thymidine incorporation by PBMC in presence of PHA and without PHA in the incubation liquid.

Computations for basic and multivariate statistics were performed with Statistica, Release 4.5.

Kolmogorov-Smirnov tests showed that most of the results concerning lymphocyte subsets, expressed as number of cells/μl, were not conformed with the normal distribution. Therefore the non-parametric methods (Kruskall-Wallis test) was used to test the statistical differences among groups under study. Median and 50% range corresponding to the 25th–75th percentiles were used for simple descriptive statistics.

Results

Blood Pb and urinary trans-trans muconic acid, a metabolite of benzene (both biomarkers of exposure to traffic in Italy) of the four groups of fertile women did not present significant differences (Table 1).

Serum Zn and Cu of the four groups also did not show differences (Table 1).

Total blood lymphocytes and CD3+, "virgin" CD4+-CD45RO-, CD3+-CD8 and CD19+ lymphocytes did not present significant differences among the groups, while "memory" CD4+-CD45RO+ lymphocytes of the ELMF exposed women (groups C and D) were slightly more elevated than those of the groups A and B not exposed to ELMF

Table 1. Blood lead, serum zinc and copper and urinary trans-trans muconic acid of non-atopic women not exposed to ELMF (A), of atopic women not exposed to ELMF (B), of non-atopic women exposed to ELMF (C) and of atopic women exposed to ELMF (D)

	A (Non-atopic not exposed to ELMF)		B (atopic not exposed to ELMF)		C (non-atopic exposed to ELMF)		D (atopic exposed to ELMF)		
	median	25th–75th perc.	median	25th–75th perc.	median	25th–75th perc.	median	25th–75th perc.	p-level*
Blood lead (μg/l)	52	44–60	57	51–75	54	42–65	46	32–60	0.8705
Serum zinc (μg/l)	850	850–875	900	900–900	870	850–945	900	800–950	0.5694
Serum copper (μg/l)	1,325	1,250–1,450	1,400	1,400–1,462	1,390	1,161–1,587	1,325	1,200–1,475	0.7617
Urinary trans-trans muconic acid (μg/l)	40	25–88	33	19–102	23	8–39	14	8–63	0.4516

*Kruskall-Wallis test.

Table 2. Blood lymphocyte sub-populations of non-atopic women not exposed to ELMF (A), of atopic women not exposed to ELMF (B), of non-atopic women exposed to ELMF and of atopic women exposed to ELMF

	A (Non-atopic not exposed to ELMF)		B (atopic not exposed to ELMF)		C (non-atopic exposed to ELMF)		D (atopic exposed to ELMF)		
	median	25th–75th perc.	median	25th–75th perc.	median	25th–75th perc.	median	25th–75th perc.	p-level*
Lymphocytes /μl	1,800	1,674–2,127	1,861	1,761–2,339	2,112	1,825–2,535	2,082	1,650–2,450	0.4997
CD3+/μl	1,387	1,060–1,584	1,359	1,284–1,623	1,640	1,264–1,863	1,542	1,240–1,892	0.1733
CD4+CD45RO-/μl	261	189–323	305	282–315	279	207–530	294	260–440	0.3616
CD4+-CD45RO+/μl	446	335–562	488	429–581	671	529–755	520	404–763	0.0740
CD3+-CD8+/μl	496	412–725	492	456–661	539	436–632	531	449–851	0.7529
CD16+-CD56+/μl	306	300–328	386	337–441	238	176–331	255	240–398	0.0771
CD19+/μl	152	112–257	220	198–255	215	159–276	192	158–253	0.3368

*Kruskall-Wallis test.

Table 3. Serum IgE and INF-γ and INF-γ produced "*in vitro*" in presence or absence of phytohemoglutinin (PHA) by peripheral blood mononuclear cells (PBMC) of non-atopic women not exposed to ELMF (A), of atopic women not exposed to ELMF (B), of non-atopic women exposed to ELMF (C) and of atopic women exposed to ELMF (D)

	A (Non-atopic not exposed to ELMF)		B (atopic not exposed to ELMF)		C (non-atopic exposed to ELMF)		D (atopic exposed to ELMF)		
	median	25th–75th perc.	median	25th–75th perc.	median	25th–75th perc.	median	25th–75th perc.	p-level*
Serum IgE (IU/l)	13	7–42	13	5–97	28	6–71	108	80–205	0.0325
Serum INF-γ(pg/ml)	540	510–588	446	419–472	349	225–416	459	290–587	0.0266
INF-γ in absence of PHA (pg/ml)	60	33–110	90	72–102	16	3–24	38	21–92	0.0001
INF-γ in presence of PHA (pg/ml)	830	450–2,300	5,295	3,387–6,160	805	130–1,322	1150	645–1,690	0.0148

*Kruskall-Wallis test.

(Table 2). On the other hand, "natural killer" (NK) CD16+-CD56+ lymphocytes of groups C and D were slightly lower than those of groups A and B.

Serum IgE of the group D (atopic women exposed to ELMF) were more elevated than those of the other groups, while serum IgE of group A (non-atopic women not exposed to ELMF) were lower (Table 3). Moreover, serum IgE of atopic groups B and D were slightly more elevated than those of the non-atopic groups A and C, respectively, and serum IgE of the groups C and D exposed to ELMF were slightly more elevated than those of groups A and B, respectively (Table 3).

Serum INF-γ of the group C (non-atopic women exposed to ELMF) was lower than that of the other groups (Table 4). INF-γ produced spontaneously (in absence of PHA) and in presence of PHA "*in vitro*" by PBMC of groups C and D were lower than those of groups A and B, respectively (Table 3).

S.I. of blastogenesis of group C was significantly lower than that of groups A, B and D, while S.I. of blastogenesis of group D was only slightly lower than that of groups A and B (Table 4).

Linear principal component analysis

From a statistical elaboration of the data, it is to be noted that there was a positive correlation only between CD16+-CD56+ lymphocytes and urinary trans-trans muconic acid. All the other correlations were near to zero, in absolute value. Five principal components accounting for 78.41% of the total variation were extracted. Only 3 principal components were considered since the values of their eigenvalues were more than 1.00. On the first principal component, urinary trans-trans muconic acid and CD16+-CD56+ lymphocytes have a major weight with a positive sign of association. On the second principal component, no variable has importance, whereas serum IgE has a significant weight on the third one.

Table 4. Stimulation index (S.I.) (cpm) of blastogenesis of peripheral blood mononuclear cells (PBMC) of non-atopic women not exposed to ELMF (A), of atopic women not exposed to ELMF (B), of non-atopic women exposed to ELMF (C) and of atopic women exposed to ELMF (D)

	A (Non-atopic not exposed to ELMF)		B (atopic not exposed to ELMF)		C (non-atopic exposed to ELMF)		D (atopic exposed to ELMF)		
	median	25th-75th perc.	median	25th-75th perc.	median	25th-75th perc.	median	25th-75th perc.	p-level*
S.I.	700	352–714	527	460–655	378	301–427	520	377–584	0.0301

*Kruskall-Wallis Test.

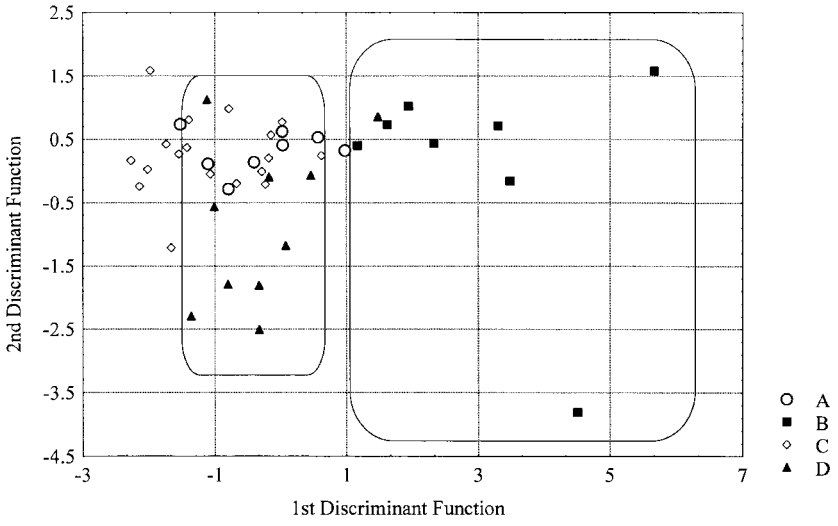


Fig. 1. Distribution of data, expressed as discriminant scores, along the first two eigenvectors regarding non-atopic women not exposed to ELMF (A), atopic women not exposed to ELMF (B), non-atopic women exposed to ELMF (C) and atopic women exposed to ELMF (D).

Data for samples were expressed graphically as a projection of linear principal scores along the first three eigenvector axes. In this scatterplot it is not possible to distinguish the various groups; in fact all samples appear to be mixed. Therefore this statistical technique is not able to differentiate correctly all groups under study.

Linear discriminant analysis

In this multivariate approach three discriminant functions were estimated because the number of the groups in the sample was 4 and 4–1 was the maximum number of eigenvalues of the matrix $W^{-1}B$. The first discriminant eigenvalue (2.2768) had a Wilks Λ value close to zero (0.21), whereas the second (0.2232) and the third (0.1475) had a Λ value of 0.71, 0.87, respectively. The ratio between the within-group sum of squares and the total sum of squares, Wilks' Λ, is a statistics giving the possibility to know if most of the total variability is due to the differences between the group means or to the within group variability. The value of Λ can range between 0 and 1: $\Lambda = 1$ occurs when the two group means are equal, while $\Lambda = 0$ if they differ.

The distribution of data were graphically expressed as discriminant scores along the first two eigenvectors. Figure 1 shows that it is possible to distinguish between the group B and the group D. In fact, groups B and D are well distinct being located in the opposite quadrant in relation with the first discriminant function axes. Furthermore, the centroids of these two groups are equally distinct.

Discussion

Both values of blood and urinary Pb may be considered biomarkers of exposure to traffic in Central Italy where about 40% of cars are still using gasoline containing 0.15% of

alkylated Pb compounds[1, 22, 23]. Moreover, the urinary levels of trans-trans muconic acid are considered biomarker of exposure to benzene since they were found correlated with those of the exposure to this compounds[17, 18, 24]. However, urinary trans-trans muconic acid may not be used as biomarker for workers exposed to both benzene and toluene[24, 25].

Blastogenesis induced by mitogens is today considered a non-specific immune test. However, an impared lymphocyte transformation in response to antigens may be considered a sensitive indicator of disorders in immune functions[11]. The reduced blastogenesis of PBMC of the ELMF exposed fertile women is in agreement with data of previous studies on proliferation response to mitogens of baboons exposed to low frequency ELMF[7] and with results of investigations on the proliferation of PBMC of humans exposed "*in vivo*" to ELMF[5, 6] or of studies on PBMC of humans exposed "*in vivo*" to radiofrequencies[9].

The slight increase of blood "memory" CD4+-CD45RO+ lymphocytes in the ELMF exposed fertile women may be related to a stimulation of "virgin" CD4+-CD45RO- lymphocytes to mature into "memory" lymphocytes.

This study demonstrates a reduction of CD16+-CD56+ NK lymphocyte subsets in both non-atopic and atopic fertile women exposed to ELMF in relation to those not exposed to ELMF. This result is correlated with the reduced production of INF-γ by PBMC of the women exposed to ELMF in relation to the non exposed women. It is known that INF-γ, produced by blood T and NK lymphocyte, may activate the same NK lymphocytes with an "autocrine loop"[19]. Also for this reason, it is considered a marker of the "cell-mediated" immune response and of the Th1 response which protects from infections and neoplasms[19]. Therefore, the above reported results, in agreement with those of previous studies[9], suggest that ELMF induces a reduction of cytotoxic activity in the peripheral blood of fertile women. However it remains to be demonstrated if a similar effect is present in post-menopausal women.

The slight increase of blood T CD45RO+ lymphocytes in the ELMF exposed women may be related to a stimulation of CD4+-CD45RO+ "virgin" lymphocytes which mature into helper "memory" CD4+-CD45RO+ lymphocytes.

It is known that atopy enhances the Th2 "humoral" response stimulating production of IgE[19]. However, in this study, there were higher levels of serum IgE not only in the groups B and D of atopic women exposed or not exposed to ELMF, in relation to the non-atopic groups A and C, but also there was an increase of serum IgE in the groups C and D of non-atopic and atopic women exposed to ELMF. This suggests that ELMF may stimulate the Th2 immune response, in both non-atopic and atopic fertile women.

In this study on the combined effects of exposure to traffic and ELMF on the immune system, we selected key parameters for multivariate statistical analysis; blood Pb and urinary trans-trans muconic acid are biomarkers of exposure to traffic[1, 17, 18, 22–25]; Zn and Cu are involved as essential elements in several metabolic functions of immune cells[11, 23]; blood CD16+-CD56+ lymphocyte subsets and the production of INF-γ by PBMC are related to blood cytotoxic activity[20]; serum IgE is a marker of atopy[11] and blastogenesis is a traditional marker of immune response[5, 6]. For this reason, the results obtained elaborating key parameters by multivariate statistical analysis can be considered a sensitive test of the combined effects of toxic compounds produced by traffic and ELMF on immune functions. Statistical analysis showed an evident separation between atopic fertile women exposed to compounds produced by traffic but not exposed to ELMF (group B) and those exposed to ELMF (group D). This result, which demonstrates a different influence of ELMF on non-atopic fertile women exposed to the toxic compounds of traffic and the non-atopic ones, suggests that exposure to toxic compounds may increase the effects of ELMF in the atopic subjects more than in those non-atopic.

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