COMBINED EFFECTS OF ELECTROMAGNETIC FIELDS ON IMMUNE AND NERVOUS RESPONSES

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In technologically developed countries, there is concern about hazards from electromagnetic fields (EMFs). Several studies have reported that immune and neuroendocrine systems exert an integrated response to EMF exposure. The aim of this review is to summarize the results of studies on the effect of low and high frequency EMF on immune and neuroendocrine systems on which our research group has been working for several years.

Exposure to electromagnetic fields (EMFs) occurs in life and work environments. EMFs produced by geomagnetic and atmospheric activities are used by species of insects, birds and reptiles for spatial orientation and migration (1). Anthropogenic EMFs are produced by therapeutic and diagnostic equipment (e.g. magnetic resonance imaging), wires transporting electricity, electric motors (frequency of 50 or 60 Hz), radiotelevision stations (KHz and MHz), radars and cellular telephones (MHz and GHz) (2).

When evaluating the possible connection between biological effects and an electromagnetic source, it is necessary to consider the different regions of the electromagnetic spectrum. The nature of the interaction with the biological material depends on frequency or wavelength of the source (2). Moreover, the response of human cells to EMFs depends on their excitability. It is known that environmental EMFs interact in particular with metabolic activities of brain and immune cells (2). In this review, we examine the results of studies on the effects of ELMFs on immune and neuroendocrine systems which may exert integrated responses to stimulations (3-6).

Integrated nervous and immune mechanisms. It has been found that immunological activation induces stress-like behavioural and neurochemical changes (3-4). Environmental stress may affect the activity of cytokines, peptide hormones, neurotransmitters and receptor ligands localized in both immune and nervous systems (5-6). Brief experimentally induced mental stress was shown to enhance blood natural killer (NK) cell mobilization and activity (7-10) and to reduce blood $CD3^+$ lymphocytes (9-10). The increased number of blood NK lymphocytes enhanced by stress and state (temporary) anxiety may be aimed at protecting the organism during acutely noxious situations (11).

Blood lymphokine activated activity (LAK), depending on NK and T cytotoxic lymphocytes activated by IL-2, is reduced in subjects with a poor lifestyle and/or mental instability (12); moreover, blood NK lymphocytes and/or cytotoxic activity are reduced in subjects with depressive disorders (13) and in subjects with anxiety and low emotional stability (14).

In vitro studies. 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 (15). PBMC of humans exposed *in vitro* to low frequency EMFs showed inhibited proliferative response to mitogens (16-18) and changes in lymphocyte metabolism and surface marker expression (19): EMF exposure influenced $CD4^+$, $CD14^+$ and $CD16^+$ expression and/or localisation; when the $CD4^+$ protein generation was studied, an indication of protein production was found. On the other hand, other studies on human PBMC exposed to 50 or 60 EMFs did not demonstrate changes in NK and LAK activities or cytokine production (20). Uniform EMFs applied to neural tissue modulated neuronal excitability with a stable threshold value. A lower threshold was induced by changes in osmolarity of the extracellular volume, in conditions simulating those of epilepsy (21).

Experimental studies on animals. Static magnetic fields, locally applied to different brain areas of rats modified the immune response depending on the time of exposure and the region of the brain exposed (22). Mice chronically exposed to 50 Hz EMFs, in the same conditions as humans, showed reduced total lymphocytes, leukocytes, polimorphonuclear neutrophil, CD4 and NK cells (23). 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 (24). A series of experiments on EMF exposed animals, demonstrated changes in behaviour patterns. These in-

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clude deficits in spatial learning (25), locomotor circling, rearing and conditioned taste aversion (26), time spent in sleeping and exploration activities (27).

Long-term exposure of newborn rabbits, rats and mice to 50 or 60 Hz EMFs induced changes in the morphology of nerve and neuroglial cells which seemed reversible (28). Rats exposed for 8 months to 50 Hz EMFs showed increased norepinephrine levels in the pineal gland and changes in the opioid system in frontal and parietal cortex and hippocampus (29). Moreover, increased incidence of sudden death was found in epileptic rats (but not in humans) exposed to nocturnal magnetic fields simulating changes in geomagnetic activity (30).

Studies on humans. In the presence of EMF, electric charges and currents in neurons undergo an interaction with EMF forces more than in other human cells. The level of EMFs which may induce symptoms in epileptic patients has not yet been established. However, occurrence of epileptic paroxism was found during repetitive transcranial magnetic stimulation treatment (31). Depressive symptoms and headaches in relation to the proximity of residence to electric transmission lines is object of public controversy (32-33). It has recently been clearly demonstrated in several studies that both motor and sensory cortex of migraine patients are hyper excitable, where a relevant role is played by the inefficiency of inhibitory circuits (34- 36).

Melatonin is a hormone that is mainly secreted by the pineal gland during the dark phase of the light-dark cycle with a supposed oncostatic action (37). Reduced 6-sulfatoxymelatonin excretion was observed in groups of womem resident near high power lines (38) and in workers using cellular telephones occupationally exposed to electricity (39). However, these results were not confirmed by other investigations (37, 40-41). The effects of the use of mobile phones on thyroid function was evaluated in 2598 employees (men and women) divided into three groups on the basis of personal use of a mobile phone (42). The group of employees with more prolonged conversation time showed lower levels of serum TSH. The authors were unable to establish whether these results were determined by EMF exposure from mobile phones or by the stress of using these instruments.

After the first study on the incidence of child leukemia induced by electricity (43), several epidemiological investigations did not evidence association between residential exposure to EMF and leukemia (44). However, pooled analysis of data from epidemiological studies on the risk of childhood leukemia was the basis for the classification of 50 or 60 Hz EMF as possible carcinogens by the International Agency for Research on Cancer (IARC); however, the IARC observed that there was no scientific demonstration for cancer induced by static or high frequency EMFs (45). Recently, higher incidence of childhood and adult leukemia was reported near a power radio transmitter located in the suburbs of Rome (46). No casual implication was drawn by the authors of this report because of the small number of cases and the lack of exposure data. A preliminary study in Korea also showed higher mortality rates for all cancer and leukemia in vicinity of a radio broadcasting station (47). On the other hand, authors who examined the studies reporting high incidence of neoplasias induced by EMF exposure, concluded that these results were inconsistent (48-49).

It was demonstrated that temporary or prolonged EMF exposure of humans modifies blood leukocytes. Patients and volunteers showed decreased % of total blood lymphocytes and cytotoxic T cells and increased helper lymphocytes immediately following magnetitic resonance imaging (50). Radar operators (exposed to EMFs ranging from 390 MHz to 10.96 GHz) also showed reduced blood cytotoxic $CD8^+$ lymphocytes and increased serum IgM (51). A reduced number of $CD4^+$ and $CD8^+$ lymphocytes were reported in non-symptomatic welders exposed to low frequency EMFs (52). 60 electric utility workers showed a negative correlation between EMF exposure and blood ornitine decarboxilase (ODC) activity and NK cells; the alteration of these hematologic parameters was stronger among workers with reduced melatonin production (53).

Men and women employed in a museum, exposed to 50 Hz EMF (range 0.2-3.6 μT) were object of a follow up study (years 2000-2005) (54-55); immune parameters of men were not altered with the exception of decreased blood lymphocyte NK cell in the previous observation, while women showed persistent reduced INF-γ release from PBMC and reduced blood cytotoxic activity/ $CD45^+$-$CD16^+$-56^+ NK cells (but not per ml of blood) in the follow up. In particular, one woman with higher EMF exposure and working night shifts, showed marked lymphopenia and astenia.

We also investigated 19 women exposed to EMFs emitted by radiotelevision broadcasting stations in their residential area in the year 2000 and 12 of them in 2005 (56-57). The EMFs in the balconies of the homes were (mean $\pm$ S.D.) 4.3 ± 1.4 V/m in the first determination and 3.7 ± 1.3 V/m in the follow up, while the residential exposure of the control women, was <2.0 V/m. The EMF exposed group showed in 2000 reduced blood NK lymphocytes as well as PHA stimulated PBMC proliferation and IL-2 and IFN-γ release. In the follow up, state (temporary) and trait (tendency of the personality) anxiety (STAI I and II, respectively) were determined (58). The ratio STAI I/STAI II of the EMF exposed group was lower than that of the control ($p<0.01$). Blood cytotoxic activity/ml of blood and /blood NK cells of the EMF

exposed women was lower ($p<0.01$). In the control group, cytotoxic activity was negatively correlated with STAI II ($p<0.001$). Two out of the 12 exposed women showed very low levels of blood cytotoxic activity and IFN-γ release from PBMC. These results suggest that EMFs affect nervous mechanisms related to anxiety, while their effect on immune functions may be both direct and modulated by nervous mechanisms (5-6).

CONCLUSIONS

By analysing the immune and neuroendocrine response of mice to 5 and 60 GHz for 1-175 days (59), it was hypothesised that EMFs induce changes in both endocrine and immune systems with lows which manifest extreme sensitivity to prior states without dose-effect relationships. In regard to this, in the follow up of our studies on 20 women exposed to EMF induced by electricity or radio- and television frequencies, three women showed altered immune parameters, in one case with temporary symptoms (56). We suggest that the effects of EMFs are more evident in subjects with vulnerable immune and/or nervous system. Occupational stress (42, 54) may also modify the response to EMFs. Moreover, ELMFs may influence the response to toxic agents such as those produced by traffic (60).

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