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Complex Effects of Long-Term 50 Hz Magnetic Field Exposure In Vivo on Immune Functions in Female Sprague-Dawley Rats Depend on Duration of Exposure

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In previous studies we have demonstrated that 50 Hz, 100 μT magnetic field (MF) exposure of female Sprague-Dawley rats for 13 weeks significantly enhances the development and growth of mammary tumors in a breast cancer model. The present study was designed to test the hypothesis that, at least in part, the tumor (co)promoting effect of MF exposure is due to MF effects on the immune surveillance system, which is of critical importance in protecting an organism against the development and growth of tumors. For this purpose, female Sprague-Dawley rats of the same age as in the mammary tumor experiments were continuously exposed for different periods (2, 4, 8, and 13 weeks) to a 50 Hz, 100 μT MF. Control groups were sham-exposed simultaneously. Following the different exposure periods, splenic lymphocytes were cultured and the proliferative responses to the T-cell-selective mitogen concanavalin A (Con A) and the B-cell-selective pokeweed mitogen (PWM) were determined. Furthermore, the production of interleukin-1 (IL-1) was determined in the splenocyte cultures. The mitogenic responsiveness of T cells was markedly enhanced after 2 weeks of MF exposure, suggesting a co-mitogenic action of MF. A significant, but less marked increase in T-cell mitogenesis was seen after 4 weeks of MF exposure, whereas no difference from sham controls was determined after 8 weeks, indicating adaptation or tolerance to this effect of MF exposure. Following 13 weeks of MF exposure, a significant decrease in the mitogenic responsiveness of lymphocytes to Con A was obtained. This triphasic alteration in T-cell function (i.e., activation, tolerance, and suppression) during prolonged MF exposure resembles alterations observed during chronic administration of mild stressors, substantiating the hypothesis that cells respond to MF in the same way as they do to other environmental stresses. In contrast to T cells, the mitogenic responsiveness of B cells and IL-1 production of PWM-stimulated cells were not altered during MF exposure. The data demonstrate that MF in vivo exposure of female rats induces complex effects on the mitogenic responsiveness of T cells, which may lead to impaired immune surveillance after long-term exposure. *Bioelectromagnetics 19:259–270, 1998.*

Key words: immune system; electromagnetic fields; T lymphocytes; cancer

INTRODUCTION

Magnetic fields (MF), such as those produced in the generation, distribution, and consumption of electric power, are widely encountered in daily life. In view of the increasing number of epidemiological studies indicating a weak association between occupational or residential exposure to time-varying 50 or 60 Hz MF and the increased risk of different types of cancer [Bates, 1991; Aldrich et al., 1992; Szmigielski, 1993; Savitz and Ahlbom, 1994; Savitz, 1995], experimental work relevant to the identification of possible mechanisms is needed. We have recently reported that long-term exposure of female Sprague-Dawley rats to 50 Hz MF accelerates the devel-

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opment and growth of mammary cancers in the 7,12-dimethylbenz(a)anthracene (DMBA) model of breast cancer, indicating that MF acts as a tumor promoter or copromoter [Löscher et al., 1993; Baum et al., 1995; Mevissen et al., 1996a]. Interestingly, a marked suppression of T-lymphocyte proliferative capacity was seen at the end of the 13-week MF exposure period, suggesting that immune system depression may be involved in the increased breast cancer growth observed in MF-exposed rats [Mevissen et al., 1996a].

The immune system is a highly complex system that provides an effective surveillance mechanism against induction and growth of neoplastic cells [Urban and Schreiber, 1988]. MF exposure has repeatedly been suggested to affect the immune system, but data in this respect are equivocal. For instance, some experiments found that MF exposure acts as a proliferative stimulus on lymphocytes, whereas others found suppression or no effect [cf. Walleczek, 1992; Adey, 1993; Stevens, 1993; Löscher and Mevissen, 1994; Liburdy and Löscher, 1997].

Most of these studies used short-term in vitro MF exposure of lymphocytes, which raises the possibility that effects of MF exposure involving humoral factors or effects that are only expressed after prolonged exposure are missed. Indeed, in view of the accumulating evidence that MF exposure interferes with the production and function of melatonin [Reiter, 1993; Liburdy and Löscher, 1997], a pineal hormone that exerts stimulatory effects on the immune system [Maestroni, 1993; Nelson et al., 1995; Skwarlo-Sonta, 1996], indirect effects of MF on immune system function via melatonin would be missed in in vitro assays. In this respect, it is important to notice that MF effects on melatonin production appear to depend on the duration of exposure, suggesting a cumulative effect of MF on pineal function [Selmaoui and Touitou, 1995].

These considerations prompted us to undertake a study in which we exposed female Sprague-Dawley rats for different durations in a 50 Hz MF, followed by determination of proliferative capacity of T and B lymphocytes using primary cultures of splenic lymphocytes. We also measured interleukin-1 (IL-1), an immunoregulatory molecule critical in development of normal immune responses [Kuby, 1992], after long-term MF exposure, because recent studies in sheep have indicated that diminished IL-1 activity may result from long-term MF exposure [Freed et al., 1993].

MATERIALS AND METHODS

Animals

Female Sprague-Dawley outbred rats, 44–47 days old (body weight around 160 g), were obtained from Charles River (Extertal, Germany) and were acclimatized for 5–10 days in one of the animal rooms in the Department of Pharmacology before being used for the MF experiment. The strain and age of the rats and the exposure conditions of the MF experiment were the same as previously used by us in studies on carcinogenic effects of MF exposure in the DMBA breast cancer model [Löscher et al., 1993; Baum et al., 1995; Mevissen et al., 1996a,b].

Magnetic Field Exposure

Our protocol for MF exposure was meant to follow as closely as possible the protocol outline of the National Toxicology Program of the National Institute of Environmental Health Sciences of the National Institutes of Health (Research Triangle Park, NC) for prechronic toxicity evaluation of alternating MF.

The exposure system, which was built by the Department of High Voltage Engineering (Technical University, Braunschweig, Germany) and has been described in detail recently [Baum et al., 1995], consisted of six identical exposure chambers with four square coils each. The arrangement of the coils strictly followed the design of Merritt et al. [1983], with 26 turns on the outer coils and 11 turns on the inner coils. The length of the sides of the coils was 1 m. The gauge of the copper wire was 1.5 mm^2. The exposure chambers were made of Trovidur, which is a special hard PVC. The exposure chambers were placed on wooden posts to avoid vibration. Separate wooden posts were used for the cages inside the coils so that the animal cages were not connected to the same mounting as the exposure chambers. The temperature inside the cages was not altered by the negligible heating produced by the current flow in the coils.

Each exposure chamber had a place for four cages (two levels with two cages each) with 9–10 animals/cage (see below). Three of the exposure chambers were energized from a standard 220-V outlet and a step-down transformer, providing the necessary voltage of 8 V. The other three exposure chambers were used with no induced 50-Hz MF for sham-control. The room with the exposure chambers had dimensions of 7.32 m $\times$ 4.52 m. The three energized exposure chambers stood side by side on one side of the room, whereas the three sham (control) exposure chambers stood on the other side with a distance of about 5 m between energized chambers and controls. No difference between exposure and sham coils regarding noise, vibration, temperature, or light was evident. During the experiment, the experimentators were not aware which exposure chambers were energized and which served as sham control.

The current in the wires of the energized exposure coils was 2.14 A, which resulted in a horizontally polarized magnetic 50 Hz field with a flux density of 100 μT (i.e., 1 Gauss) rms. The nonenergized exposure coils used for sham-exposure received a stray MF field from the energized coils that was calculated to be about 0.1 μT in the volume of the sham exposure chambers. This value was subsequently verified by MF measurements using an EMDEX meter (Electric Field Measurement, West Stockbridge, MA, USA).

24 h measurements of the 50 Hz MF field over the volume of the sham exposure chambers with all coils turned off yielded values of 0.03–0.04 μT, which was due to the ambient 50 Hz MF field in the laboratory. The static earth MF was measured with a Bell 610 Gaussmeter. The component parallel to the exposure field was 16 μT, and the component perpendicular to the exposure field was 36 μT, that is, the generated 50 Hz field was horizontal and parallel to the horizontal component of the earth's North/South MF. The electric field in the exposure chambers was measured with the EMDEX instrument together with an M115EB handle (Electric Field Measurements); ambient values varied from 17 to 58 V/m and were not significantly increased when the exposure chambers were energized.

The exposure parameters described above and several additional parameters important for the exposure conditions were validated and verified in November 1996 by a physicist from another institution not involved in the present study. In addition to verification of the values given above by the use of other instruments, 24 h measurements showed that, at the conditions of the experiment, the MF exposure system produced a stable flux density of 100 μT and a stable frequency of 50 Hz with negligible harmonics.

For the animal experiments, the rats were randomly divided into groups of nine animals. At the onset of the MF experiment, when the rats were about 52–54 days of age, the groups were brought into the room with the exposure chambers and placed in their home cages inside the exposure chambers; MF exposure was then started (flux density 100 μT; frequency 50 Hz, 24 h/day 7 days a week). The sham controls were placed in identical exposure chambers without MF.

Rats were housed 9/cage within the exposure chambers; the 39 × 55 × 22 cm cages were made of acrylic, as were the feeding dishes, water bottles, and cage lids. The rats were housed under controlled conditions of temperature (23–24 °C), humidity (about 50%), and light (12 h dark/light cycle; light off at 6 p.m.). Food (Altromin standard rat diet) and water were available ad libitum. Light intensity produced by the artificial white light in the room with the exposure system varied between 16 and 35 lux (measured by a luxmeter in the exposure chambers). In the dark period, the room was weakly illuminated by dim red light, which led to a light intensity of below 1 lux (measured in the exposure chambers). In this respect, it is important to note that dim red light exposure at night, which itself does not inhibit pineal melatonin production, seems to be a necessary predisposing factor for MF to inhibit the melatonin-forming ability of the mammalian pineal gland [Reiter, 1993].

Animals were weighed once a week; cage cleaning was done 3 times a week, and cage rotation in the exposure chambers was done once a week. The 50 Hz MF in the exposure chambers was measured once a week with an EMDEX meter by a person not involved in the animal experimentation. Thus, all persons involved in handling of animals and subsequent determinations were not aware which groups of rats were MF exposed or sham exposed, i.e., the study was done ''blind.''

After 2, 4, 8, and 13 weeks of MF or sham exposure, one MF-exposed and one sham-exposed group of 9 rats/exposure period were sacrificed for determinations of immune parameters. Some experiments, i.e., the 8 and 13 week exposures, were repeated in additional groups of rats, to determine whether the MF effects were reproducible. All rats were sacrificed between 8 and 9 a.m. to minimize the bias of circadian variations in immune parameters.

T-Cell Activation

Methods of lymphocyte preparation and stimulation of proliferation by the T-cell-selective mitogen concanavalin A (Con A) have been described in detail previously [Szamel et al., 1986]. In short, after sacrifice of animals, the spleen was immediately removed and placed into ice-cold sterile tissue culture medium (RPMI-1640, supplemented with L-glutamine [Life Technologies, Eggenstein, Germany]). The fibrous capsule was removed, and the spleen was cut into small pieces, and macerated by a glass Potter-Elvehjem homogenizer to release lymphocytes. Tissue remnants were removed by filtering cells through nylon wool. The cell suspension was centrifuged at 600*g* for 10 min, and the residue was resuspended in 4 ml RPMI-1640. The number of viable cells was determined by a counting chamber in dilutions of this cell suspension, using the trypan blue exclusion test.

Using a dilution of 2×10^6 cells/ml, lymphocytes were then cultured in RPMI-1640 supplemented with 10% fetal calf serum (Boehringer-Mannheim, Germany) in 96 well microtiter plates in a total volume of 200 μl of RPMI-1640 (supplemented with penicillin/

streptomycin), either with or without adding 1 μg Con A (which resulted in optimal stimulation [Szamel et al., 1986]). Four samples per spleen were cultured with and 4 samples without Con A (Biochrom, Berlin, Germany; dissolved in phosphate-buffered saline [PBS] and added in a volume of 20 μl). In some experiments, melatonin (final concentration 1–100 nM) or combinations of melatonin and Con A were added in addition to adding Con A alone. Melatonin was freshly dissolved in ethanol, diluted with PBS to the intended concentration, and added in a volume of 20 μl. (Adding the same high dilution of ethanol without melatonin had no effect on T cell proliferation.) In all experiments, including those in which melatonin and Con A were added, final culture volume was 200 μl/well.

After 50 h at 37 °C with 5% CO_2, 1 μCi of [^{3}H]thymidine was added (specific activity 5 Ci/mmol; Amersham, Braunschweig, Germany), and the cells were further incubated for 3 h. Lymphocytes were then harvested with an automatic cell harvester (Scatron, Flow Laboratories) on glass fiber filters. Radioactivity was measured in a scintillation cocktail by liquid scintillation counting. Values of the 4 samples/spleen preparations were averaged, and the mean was used for calculation of group data. Samples that did not show a clear response to Con A (i.e., no stimulated proliferation) were omitted from further evaluation.

B-Cell Activation

Preparation of splenic lymphocytes was the same as described for T cells, but the B-cell-selective pokeweed mitogen (PWM; Biochrom) was added to the wells (1 μg/well) instead of Con A, to distinguish B and T lymphocytes in culture. All subsequent procedures were as described above.

Determination of IL-1 Activity

The cytokine IL-1 is a product of activated B cells and numerous other cell types. It has a range of activities, including a costimulation of T lymphocytes [Gery et al., 1972]. The amount of IL-1 produced by the above-described splenocyte cultures was quantified in the culture supernatants before and after PWM stimulation in a proliferation assay [Orencole and Dinarello, 1989]. In brief, the level of IL-1 was estimated through its ability to stimulate the proliferation of the murine D10.G4.1 helper T-cell line. Serial twofold dilutions of 50 μl aliquots of the supernatants were cultured with D10.G4.1 cells (2×10^4/well) for 24–48 h at 37 °C with 5% CO_2 in triplicates. A murine recombinant IL-1 standard (Sigma; 10 ng/ml) was run in addition, prior to each measurement. Analytical verification of the response of the cells to IL-1 present in the dilution series was performed by a colorimetric assay.

The determination used the ability of metabolically active D10.G4.1 cells to support the formation of dark blue crystals from the redox-sensitive dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) via dehydrogenase [Mosmann, 1983]. After the crystals were dissolved with an organic solvent, the optical density was measured automatically using an enzyme-linked immunosorbent assay (ELISA) microtiter plate reader. For each sample the dilution step that supported a 50% response of the respective cell line, was determined graphically in a probit scale. An IL-1 concentration of 1 unit/ml was defined as the reciprocal of the dilution of the supernatant that induced half-maximal response of the respective cells. The average amount of IL-1 from the triplicate determinations was used for further calculation of group data. Samples that did not show a typical decay of activity on dilution were omitted from further evaluation. For the interpretation of data from the D10.G4.1 proliferation assay, it must be considered that the D10 cells are also sensitive to a minor degree to secondary stimuli provided by IL-2 and IL-4 [Sierra-Honigmann and Murphy, 1989].

Statistics

All observations were tested for normality using the Kolmogorov-Smirnov test, and subsequent statistical comparisons were done by either parametric or nonparametric tests, i.e., Student's *t* test or, depending on whether data were paired or nonpaired, the *U* test or Wilcoxon signed rank test. All comparisons were done between an MF-exposed group and a concurrent control group, i.e., no multiple comparisons were undertaken.

RESULTS

General Observations

No behavioural differences were observed between exposed and sham-exposed animals during the 3 month period of exposure. Furthermore, as reported previously for chronic exposure of female Sprague-Dawley rats at 100 μT [Löscher et al., 1993; Baum et al., 1995], the body weight gain did not differ significantly between MF-exposed and sham-exposed groups of rats.

Alterations in Number of Splenic Lymphocytes in Response to MF Exposure

As shown in Figure 1, in all MF-exposed groups the number of viable splenic lymphocytes was signifi-

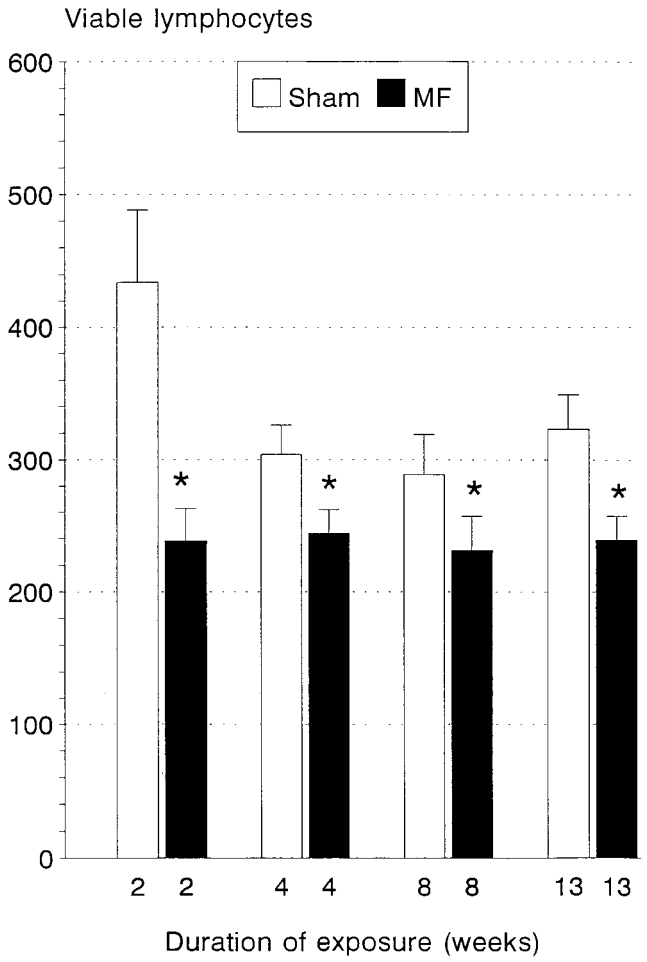


Fig. 1. Effect of different periods of 50-Hz MF exposure (100 μT) on the number of viable splenic lymphocytes in female rats. Data are shown as mean numbers ($\times 10^6$/ml) + SE of 9 rats/exposure period. Control (sham) groups were sham-exposed together with the MF-exposed groups. Significant differences between MF-exposed rats and the concurrent control group are indicated by an asterisk (*P* at least <0.05).

cantly reduced when compared with concurrent sham control. Because the same number of viable lymphocytes (2×10^6/ml) was used for subsequent lymphocyte cultures and mitogen stimulation, this difference in total cell counts had no effect on the MF-induced alterations in lymphocyte proliferation described below.

Alterations in T- and B-Cell Proliferation in Response to MF Exposure

Basal (i.e., not mitogen-stimulated) proliferation rates of splenic lymphocyte cultures did not differ significantly between sham-exposed and MF-exposed groups for any of the exposure durations examined (not illustrated).

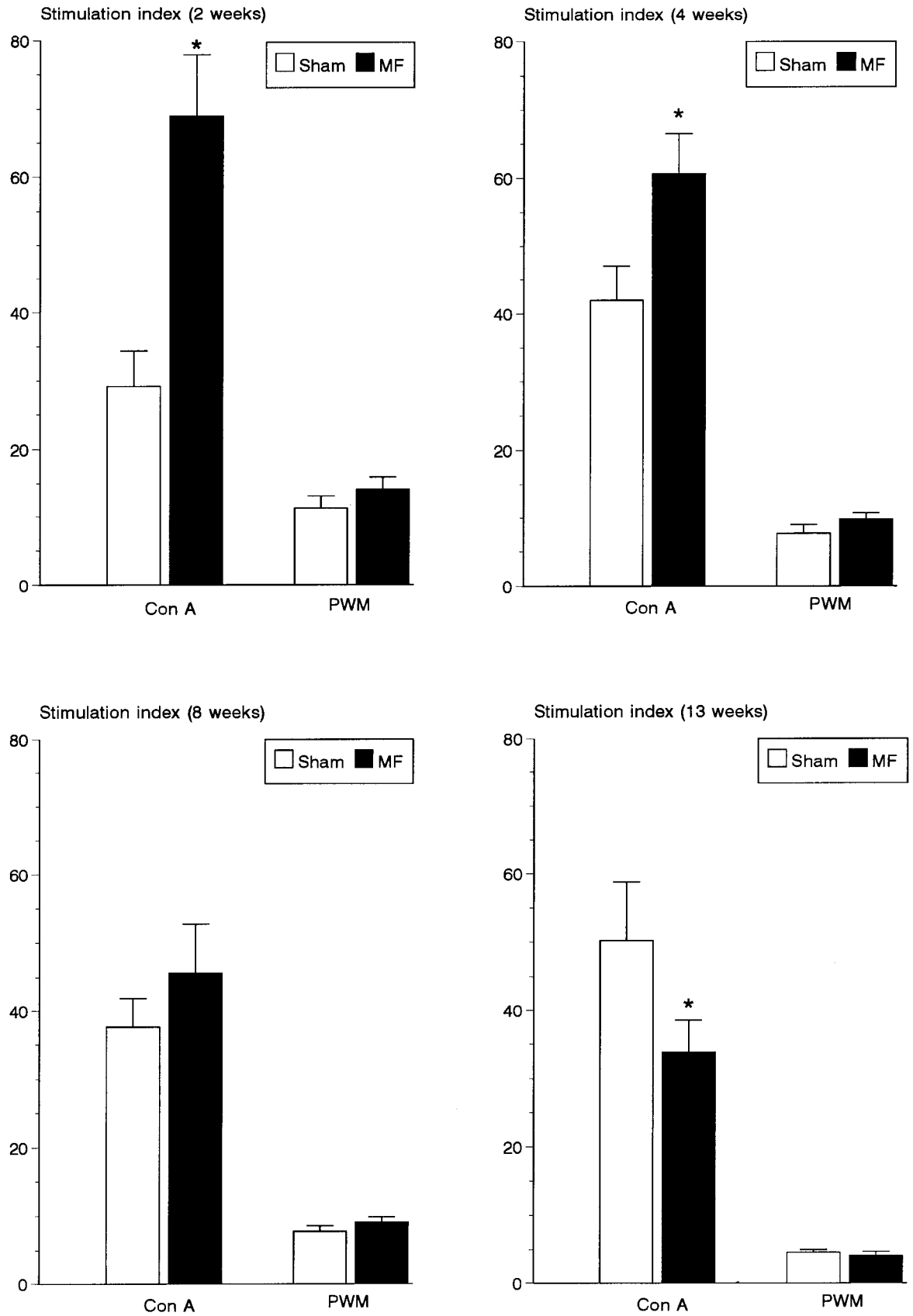
In all groups of MF-exposed or sham-exposed rats, the blastogenesis of splenic lymphocytes was stimulated by the T-cell-selective mitogen Con A, as shown by the incorporation of [^{3}H]thymidine into DNA after culturing lymphocytes with Con A (Fig. 2). However, the extent of stimulation differed significantly among groups. In sham-exposed rats, Con A-induced T-cell proliferation tended to increase with duration of sham exposure, most likely as a result of the increase in age of animals during the 3 months of exposure (Fig. 2). When sham-exposed groups were compared with age-matched MF-exposed groups, significant differences were seen between groups after 2, 4, and 13 weeks of exposure (Fig. 2). Following 2 weeks of MF exposure, there was a marked increase in stimulation index of about 150% above the concurrent sham control. After 4 weeks of MF exposure, there was again a significant increase above concurrent control, but this increase in Con A-induced T-cell proliferation was less marked compared with the 2 week exposure period (Fig. 2).

After 8 weeks of MF exposure, the difference from sham controls was further reduced and no longer significant. When this experiment with 8 weeks of exposure was repeated in other groups of rats, again no significant difference between MF- and sham-exposed groups was obtained. Since the data from the two experiments with 8 weeks of MF exposure did not significantly differ, data of the two experiments were averaged and are shown together in Figure 2.

Following 13 weeks of MF exposure, there was a decrease in the stimulation index of T cells from MF-exposed rats compared with the concurrent sham control. This finding was reproduced in a replicate experiment; since the reduction in T-cell proliferation after 13 weeks of MF exposure did not significantly differ between the two experiments, data from the two experiments were averaged and are shown together in Figure 2. For illustration of this striking dependence of MF-induced alterations in T-cell proliferative capacity from exposure duration, the percent alterations from concurrent sham control are illustrated in Figure 3.

In contrast to the marked changes in Con A-induced T-cell proliferation after different durations of MF exposure, no significant effects were determined for PWM-induced B cell proliferation (Fig. 2).

In some experiments, melatonin was added to splenic lymphocyte cultures from sham-exposed and MF-exposed rats instead of or together with Con A. Melatonin (final concentration in the culture 1–100 nM) did not significantly alter T-cell proliferation in comparison with basal (non-mitogen-stimulated) values (not illustrated). Furthermore, when added together


Stimulation index (2 weeks)
Sham
MF
80
60
40
20
0
*
Con A
PWM
Stimulation index (4 weeks)
Sham
MF
80
60
40
20
0
*
Con A
PWM
Stimulation index (8 weeks)
Sham
MF
80
60
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0
Con A
PWM
Stimulation index (13 weeks)
Sham
MF
80
60
40
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0
*
Con A
PWM

Figure 2.

with Con A to lymphocyte cultures from the 2 and 4 week MF exposure experiments, melatonin did not significantly change the T-cell proliferation obtained with Con A alone (not illustrated). The same was true for data from sham controls, except for one experiment with a relatively high concentration of melatonin (100 nM), in which the stimulation index in response to Con A and melatonin was significantly higher compared with Con A alone (Fig. 4). For comparison, maximum (nocturnal) melatonin plasma levels in female Sprague-Dawley rats of the age used for the present experiments are about 100–200 pg/ml, i.e., about 0.5–1 nM (unpublished data).

IL-1 Activity After Prolonged MF Exposure

Following 13 weeks of MF or sham exposure, IL-1 activity was determined in the splenic lymphocyte cultures also used for the B-cell proliferation experiments shown in Figure 2. In supernatants from non-mitogen-stimulated cultures, IL-1 activity was below the detection limit of the method. In supernatants of the PWM-stimulated cultures, IL-1 activity was not significantly different between sham-exposed and MF-exposed groups (Fig. 5).

DISCUSSION

Although the immune surveillance system has repeatedly been proposed as a potential link in MF effects on carcinogenesis [Walleczek, 1992; Adey, 1993; Stevens, 1993; Löscher and Mevissen, 1994; Liburdy and Löscher, 1997], until now, only few studies have dealt with the in vivo effects of MF exposure on the immune system of rodents or other species. McLean et al. [1991] reported some experimental evidence that 2 mT 60 Hz MF exposure of female SENCAR mice for 21 weeks may effect tumor growth in a skin cancer model by suppressing natural killer (NK) cell activity. Tremblay et al. [1996] found that 2 mT 60 Hz exposure of Fischer F344/N rats for 6 weeks significantly reduced subpopulations of T lymphocytes ($CD5^+$, $CD4^+$, and $CD8^+$) that possess tumoricidal activity. A similar finding was reported by Murthy et al. [1995] following 6 weeks of exposure of male baboons in a combined 60 Hz electric (6 kV/m) and magnetic field (50 μT). Furthermore, the proliferative response of baboon T cells and IL-2 receptor expression were reduced during exposure [Murthy et al., 1995].

We recently reported that 50 μT, 50 Hz MF exposure of female Sprague-Dawley rats for 13 weeks significantly suppressed the mitogenic responsiveness of T cells, an effect that could have been critically involved in the increased growth of mammary cancers in response to DMBA seen in this experiment [Mevissen et al., 1996a]. A similar significant suppression of T-cell proliferative capacity was determined in the present experiments with 13 weeks of MF exposure at 100 μT, thus substantiating our previous finding with 50 μT. The finding of a relative marked suppression of T-cell mitogenesis upon long-term MF exposure suggests that different subpopulations of splenic T cells were affected, which is also indicated by the recent study of Tremblay et al. [1996].

Activation of T lymphocytes is a pivotal event in the generation of immune responses to most antigens, including tumor antigens [Szamel and Resch, 1995], and appears to be important for antitumor activity and its immune regulation [Urban and Schreiber, 1988]. Previous studies with the DMBA model of breast cancer have shown that immune system depression, including reduced splenocyte proliferative response to the T-cell selective mitogen Con A, facilitates the rate of tumor growth [Gallo et al., 1993]. Since Con A-induced stimulation of lymphocytes is thought to reflect the proliferative response of T lymphocytes to antigens, including tumor antigens [Roitt et al., 1987], the previous and present finding of reduced proliferative capacity of T cells after long-term MF exposure could be critically involved in the enhanced development and growth of DMBA-induced mammary cancers observed in our previous studies with flux densities of 50–100 μT [Löscher et al., 1993; Baum et al., 1995; Mevissen et

Fig. 2. Splenic lymphocyte blastogenesis in response to the T-cell selective mitogen Con A and the B-cell selective mitogen PWM. Splenocyte proliferative capacity was evaluated after different periods of 50-Hz MF exposure (100 μT) or sham exposure of female rats. T-cell proliferative capacity (T-cell activation) was calculated as stimulation index, i.e., by dividing the radioactivity of incorporated [^{3}H]thymidine of Con A-stimulated lymphocytes by the respective radioactivity of nonstimulated lymphocytes. The mitogen responsiveness of B cells was calculated in the same way by using PWM instead of Con A. Data are shown as mean values (+SE) of splenocytes from 9 rats/group (2 weeks and 4 weeks) or 18 rats/group (8 weeks and 13 weeks), respectively. The star indicates that the T-cell stimulation index of MF-exposed rats was significantly (P at least <0.05) different from stimulation index in the concurrent sham-exposed control group.

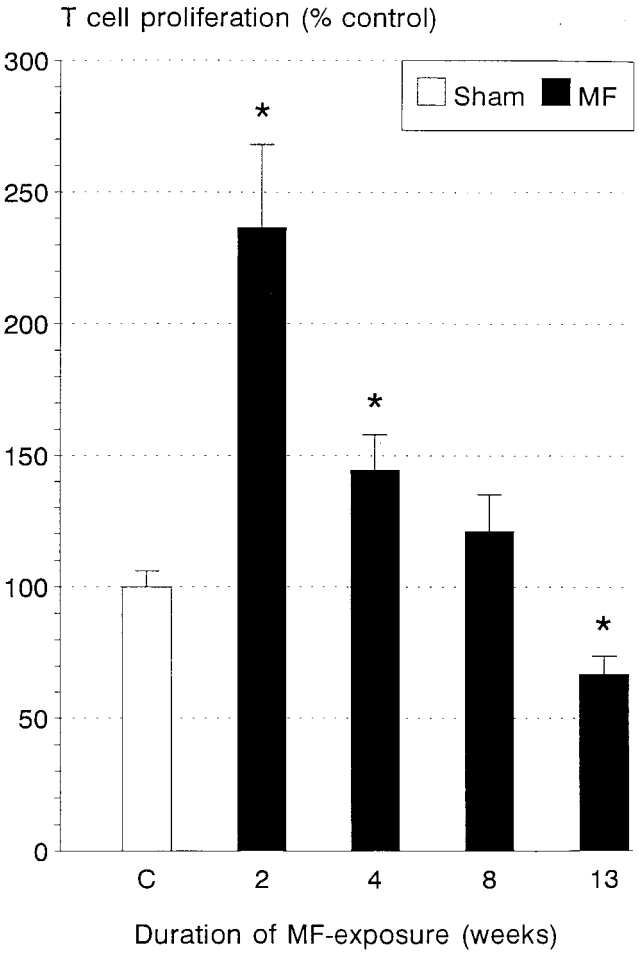

Fig. 3. Mitogen responsiveness of splenic T cells after different periods of 50-Hz MF exposure (100 μT) in female rats. Proliferation in response to Con A of splenocytes from MF-exposed groups is shown as average percent (+SE) of the concurrent sham-exposed control group (controls are set at 100%). Significant difference from the concurrent control is indicated by an asterisk (*P* at least <0.05). For more details see Figure 2.

al., 1996a]. Furthermore, the present finding that the number of lymphocytes, which are responsible for adaptive immunity, was significantly decreased throughout the period of MF exposure would suggest that MF exposure could potentially stimulate tumor growth by suppressing adaptive immune surveillance mechanisms, particularly T-lymphocyte populations, as recently shown by Tremblay et al. [1996].

However, unexpectedly, shorter MF exposure periods of 2–4 weeks resulted in a significant enhancement of Con A-induced T-cell proliferation, while no significant effect of MF exposure was seen at an intermediate exposure duration of 8 weeks. To our knowledge, this is the first study demonstrating that effects of in vivo MF exposure on immune system performance critically depend on the duration of exposure. Relatively short exposures induced a co-mitogenic effect, i.e., an increase in Con A-stimulated T-cell proliferation, and long-term exposures induced suppression of T-cell proliferative capacity. Interestingly, the present observations from short-term in vivo MF exposure are in line with several studies on in vitro MF exposures that indicated an activation/proliferation of lymphocytes [cf. Liburdy, 1995; Liburdy and Löscher, 1997]. One explanation of enhanced mitogen-induced T-cell proliferation in response to in vitro or in vivo MF exposure could be increased calcium influx, which is

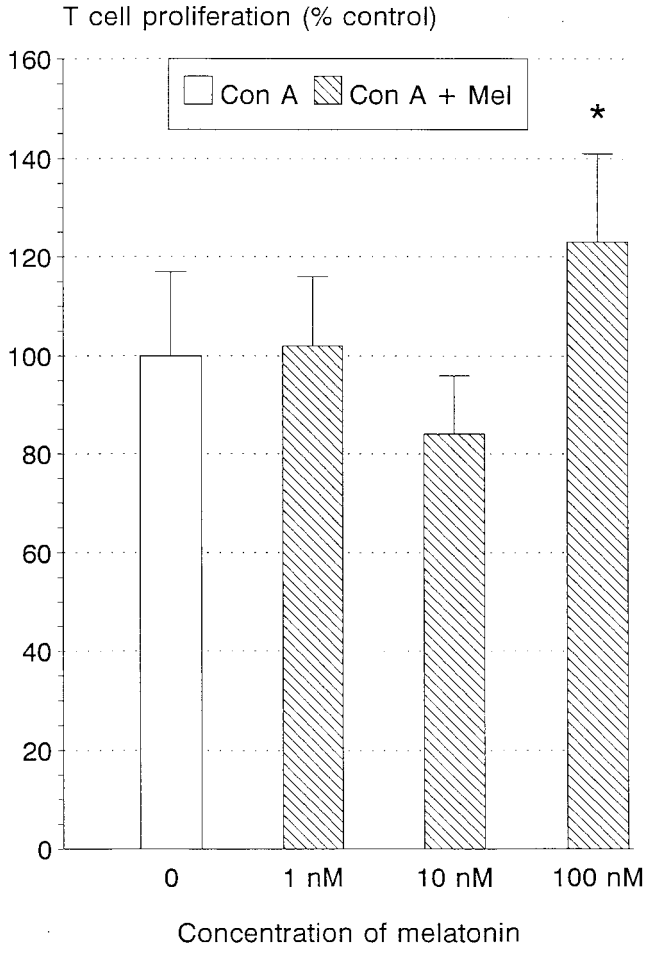

Fig. 4. Effect of melatonin on mitogenic responsiveness of splenic T cells from female rats. T-cell proliferation was induced by Con A in either the absence or presence of different concentrations of melatonin. Data in the presence of melatonin are shown as mean (+SE; $n = 9$) percentage of the concurrent control without melatonin (which is set at 100%). The 0 column (i.e., for Con A without melatonin) is the mean (+SE) of three control groups ($n = 9$ per group). Data are from three separate experiments in sham-exposed rats.

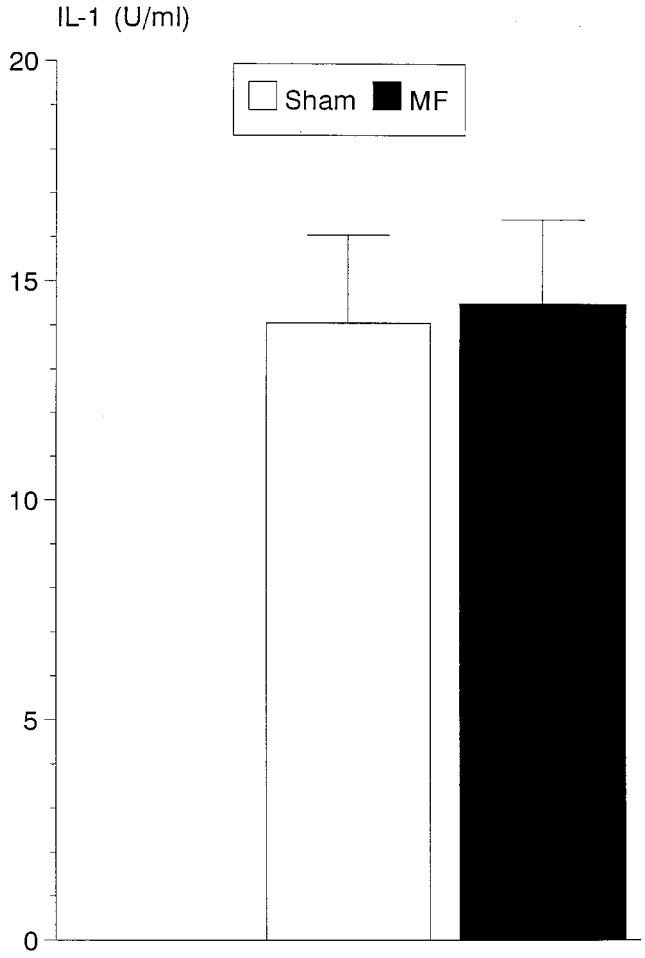

Fig. 5. IL-1 activity determined in supernatants from PWM-stimulated splenocytes of MF-exposed (50-Hz, 100 μT) or sham-exposed female rats. Duration of exposure was 13 weeks. Data are shown as means +SE of 9 rats/group.

one of the initial events in T-cell activation [Liburdy, 1995]. Indeed, Walleczek and Liburdy [1990] showed that, while nonactivated rat thymocytes were unresponsive to MF exposure, the MF induced an increase in thymocyte calcium uptake of 50–100% in the presence of Con A. This observation would be consistent with the present finding that MF exposure does not alter basal T-cell proliferation but enhances mitogenic responsiveness of T cells, at least after exposure periods of up to 4 weeks.

In addition to direct effects of MF on lymphocytes, e.g., via changes in calcium influx, another explanation for alterations in proliferation of activated T cells during MF exposure might be the ''melatonin hypothesis'' [Stevens, 1993]. There is increasing evidence from laboratory studies that exposure to static or alternating MF can suppress pineal function and thereby reduce circulating concentrations of melatonin, an effect that may explain many of the biological effects of MF exposure [Reiter, 1993]. In rats, including female rats of the strain and age used in the present study, prolonged exposure to 50 Hz MF in the μT range has been shown to reduce pineal and/or plasma melatonin levels significantly [Kato et al., 1993; Löscher et al., 1994; Selmaoui and Toutiou, 1995; Mevissen et al., 1996b].

Although melatonin is generally thought to enhance humoral and cell-mediated immune functions [Maestroni, 1993; Nelson et al., 1995; Skwarlo-Sonta, 1996], evidence in this respect is not unequivocal. For instance, melatonin has been reported to reduce Con A-stimulated proliferation of mouse splenocytes and thymocytes at a high concentration (200 μM) in vitro [Persengiev and Kyurkchiev, 1993], possibly via an effect on melatonin receptors recently identified on lymphocytes [cf. Skwarlo-Sonta, 1996]. Thus, based on these in vitro data suggesting an inhibitory action of melatonin on stimulated T-cell proliferation, reduction of melatonin levels by MF exposure could be involved in the increased proliferation of Con A-stimulated T cells observed in the present experiments after 2 and 4 weeks of exposure.

To examine this possibility, we undertook some in vitro experiments with melatonin. First, in contrast to the experiments of Persengiev and Kyurkchiev [1993] with mouse lymphocytes, melatonin did not reduce Con A-induced T-cell proliferation in rat splenocytes at concentrations near to the physiological range, but rather potentiated the effect of Con A, although only at a relatively high concentration of 100 nM. Second, addition of melatonin did not counteract the increase in Con A-induced T-cell proliferation observed after 2 and 4 weeks of MF exposure. Thus, it appears unlikely that changes in melatonin levels were involved in this observation. However, in view of the cumulative effect of MF exposure on pineal function [Selmaoui and Touitou, 1995], decreased melatonin levels could be involved in the suppression of T-cell responsiveness to Con A observed after long-term MF exposure in the present experiments. Indeed, reduction of circulating melatonin levels by pharmacological or surgical pinealectomy impairs the blastogenic response of splenocytes, particularly T cells, to mitogens, which can be counteracted by administration of melatonin [Maestroni, 1993; Nelson et al., 1995].

Interestingly, the complex alterations in T-cell proliferation during MF exposure seen in the present study resemble stress responses. Thus, since the pioneering experiments from Selye, it is known that initially stress may activate immune functions to a higher than

normal level [Dorian and Garfinkel, 1987; Lysle et al., 1990; O'Leary, 1990; Jain and Stevenson, 1991]. If the stress continues, immune reactivity gradually declines to normal, which can be explained by adaptation, tolerance, or contraction of the T-cell response because of activation-induced apoptosis [Ahmed and Gray, 1996; Mondino et al., 1996]. If the stressful condition persists, immune reactivity declines below normal, leading to decreases in functional immune measures such as reduced proliferative response of T cells to mitogens [Dorian and Garfinkel, 1987; O'Leary, 1990; Khansari et al., 1990; Herbert and Cohen, 1993]. Similar to the present observations of enhanced T-cell mitogenesis after 2–4 weeks of MF exposure, daily restraint stress for up to 33 days increased responses of splenic lymphocytes to Con A in rats [Jain and Stephenson, 1991]. The concomitant reduction in numbers of splenic lymphocytes observed in the present experiments might have been due to redistribution of cells between immune compartments, which has been previously observed under mild stress and is thought to reflect the ability of the immune system to respond to potential or ongoing immune challenge [Dhabhar et al., 1994, 1995].

There is increasing evidence that cells respond to MF exposure in the same way as they do to other environmental stresses [Smith, 1996]. As a consequence, the present findings that, at least in terms of T-cell responses, rats subjected to MF undergo a typical stress response, and that the direction and extent of the response critically depend on the duration of MF exposure, may explain many apparently conflicting results of MF exposure in the literature [Liburdy and Löscher, 1997]. In this respect it is important to note that it has become clear in recent years that a full understanding of T-cell activation, tolerance, and suppression cannot be achieved with reductionist in vitro approaches that separate the individual lymphocyte from its in vivo environment [Mondino et al., 1996]. Besides the antigen signal, T cells require a variety of ''accessory'' signals for optimal activation [Szamel and Resch, 1995]. T cells can also be inactivated at all stages of their development by a variety of endogenous mechanisms, including immunosuppressive cytokines [Mondino et al., 1996], all of which can be subject to change under in vivo MF exposure [Liburdy and Löscher, 1997]. Furthermore, in view of the in vivo effects of melatonin on specific and nonspecific signals involved in T-cell blastogenesis [Maestroni, 1993; Pioli et al., 1993], in vivo suppression of melatonin production by prolonged MF exposure might be important for the stress-like alterations of T cells in response to long-term MF exposure. Indeed, stress-induced immunosuppression is counteracted by melatonin [Maestroni, 1993].

In contrast to the findings with T cells, mitogen-induced proliferation of B cells seemed not to be altered by MF exposure. Furthermore, production of IL-1 by PWM-stimulated lymphocytes was not significantly changed after MF exposure for 13 weeks. Because IL-1 promotes the proliferation of T lymphocytes, activates macrophages, and stimulates the synthesis of other cytokines (e.g., IL-2) involved in growth stimulation of T cells [Kuby, 1992], suppressed production of IL-1 by long-term MF exposure could have been involved in the impaired proliferative mitogen response of T cells observed after long MF exposure. Interestingly, in contrast to the present data in rats, reduced production of IL-1 by mitogen-stimulated B cells has recently been reported in sheep after 2 and 4 months of 60 Hz MF exposure [Freed et al., 1993]. As in the present observations on mitogen susceptibility of T cells after long-term MF exposure, in vivo reduction of melatonin levels by MF could have been involved in these observations in sheep, because melatonin has been shown to increase IL-1 production [Pioli et al., 1993]. Although the present data indicate that changes in IL-1 production of B cells are not involved in the long-term effects of MF exposure on immune system function in rats, this does not exclude the possibility that other cytokines or IL receptor binding or expression are affected by MF exposure, as recently indicated by experiments from in vivo exposure of nonhuman primates [Murthy et al., 1995].

In conclusion, in line with previous observations from in vitro MF exposures [Walleczek, 1992], the present data from in vivo MF exposure indicate that 50 Hz MF in the μT range can act as stimulator or inhibitor of cellular activity. They also show that the occurrence of MF effects on lymphocytes depends strongly on the biological status of the exposed cells as well as on the duration of MF exposure, indicating the involvement of complex interaction mechanisms. As recently proposed on the basis of cellular in vitro studies [Smith, 1996], in vivo MF exposure seems to act as a physical stressor with initial co-mitogenic effects on T lymphocytes, followed by T-cell tolerance and suppression. Because the immune system functions as the body's main protective mechanism against invasion by pathogens and against tumor formation and growth [Kuby, 1992], immunosuppressive effects as observed after long-term in vivo MF exposure in the present study may contribute to the tumor copromoting action of MF exposure reported in some rodent cancer models [Löscher and Mevissen, 1994]. However, more work is needed to understand the biological signifi-

cance of the present findings and to characterize the biological mechanisms involved.

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