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NONLINEAR DETERMINISM IN THE IMMUNE SYSTEM. IN VIVO INFLUENCE OF ELECTROMAGNETIC FIELDS ON DIFFERENT FUNCTIONS OF MURINE LYMPHOCYTE SUBPOPULATIONS*

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ABSTRACT

Animal studies of the effects of low-frequency electromagnetic fields (EMFs) on the immune system appear inconsistent, and recent evidence indicates that inconspicuous experimental problems are not responsible. We hypothesized that the inconsistencies resulted from use of linear methods and models to study inherently nonlinear input-output relationships. Using a novel analytical method, we found that exposure of mice to 5 G, 60 Hz, for 1–105 days in 6 independent experiments consistently affected a broad panel of immune variables when and only when the reaction of the immune system was modeled to allow the possibility of nonlinearity in the relationship between the field and the immune

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variables. It was possible to mimic the pattern observed in the immune data by sampling from a known chaotic system, suggesting the possibility that the observed pattern was the result of intrinsic nonlinear regulatory mechanisms in the immune system. Overall, the results suggested that lymphoid sub-populations were vulnerable to the physiological consequences of EMF transduction, that it may never be possible to predict specific changes in particular immune-system variables, and that the underlying behavior of the immune system (that which occurs in the absence of specific inputs) may be governed by laws that manifest extreme sensitivity to prior states.

INTRODUCTION

Man-made electromagnetic fields (EMFs) having a broad range of frequencies and intensities are prevalent in the general and workplace environments [1–5]. Some reports suggested that chronic EMF exposure leads to adverse consequences [6] but other studies did not, and the public-health significance of environmental-strength fields is largely unresolved [7–9].

The possibility that the immune system might somehow be a target for low-frequency EMFs was examined in animal studies, but the results presented an unclear picture of how the immune system might be affected. Decreases in T-cell and B-cell subpopulations in rats were seen after 42 days' exposure to magnetic fields of 0.2–1.0 G [10]. Decreases and increases in T cells occurred in baboons exposed for 42 days to 60-Hz electric and magnetic fields of 6–30 kV/m, 0.5–1.0 G, respectively [11]. Mice exposed intermittently over 5 days to 1200 G exhibited increased NK cytotoxic activity [12], but mice exposed for 147 days to 20 G showed no changes in number or function of NK cells [13], and exposure of mice to 0.02–10 G for 28–90 days led to inconsistent increases in NK-cell function [14]. T-cell proliferative capacity in rats increased following 14–28 days' exposure to 1000 mG, but not after 56 days' exposure [15]. Intermittent exposure for up to 24 hours to 100 mG had no effect on lymphocyte subpopulations in male volunteers [16]. One explanation for the various inconsistencies was that they arose from a variety of inconspicuous experimental problems that, if corrected, would lead to a clearly discernible pattern. However, greater efforts at remedying perceived experimental difficulties did not result in a proportionate reduction in variability [8, 17, 18]. Many other physiological stimuli also appear to produce inconsistent effects on the immune system [19–23].

We approached the problem of inconsistency in the EMF studies by considering what factors might make real changes in the immune system appear to be inconsistent. We observed that in essentially all the studies it was assumed that any real response to a field would be governed by a linear law, and that inter-subject measurement differences made under identical

conditions were due solely to stochastic processes. However, the immune system is a self-organizing, spatially and temporally complex system that contains numerous nonlinear mechanisms [24–28]. It was therefore reasonable to anticipate that changes in the immune system associated with certain kinds of stimuli might also display nonlinearity when evaluated in relation to the stimuli. The study of the outputs of nonlinear systems requires specialized forms of analysis [29, 30]. Otherwise, partly or even perfectly deterministic outputs can appear to be stochastic. These considerations suggested to us that the inconsistencies observed in the EMF studies might have resulted from the use of linear models and methods of statistical analysis to study what were inherently nonlinear input/output relationships. More particularly, we thought it possible that the use of a linear approach to analyze these studies could have caused some of them to appear negative, thereby obscuring a discernible inter-study pattern.

We previously described a novel statistical procedure that [1] was capable of revealing EMF-induced effects on the immune system without the need to resort to the assumption of linearity, and [2] could also be modified to include that assumption. We used the procedure to show that low-frequency magnetic fields consistently altered lymphoid phenotype via nonlinear processes [31]. The present study was undertaken to determine whether field exposure produced nonlinear changes under other experimental conditions, as would be expected based on the initial study. We tested the specific hypothesis that exposure for 1–105 days to power-frequency magnetic fields of 5 G would result in changes in the immune system that were nonlinearly related to the applied field.

MATERIALS AND METHODS

Experimental Design

In earlier EMF bioeffects studies of the effect of fields on the immune system it was generally assumed that any real effect would be proportional to the field, unidirectional, and would occur more or less consistently in a particular variable. Sometimes the assumptions were explicit, but more often they were implicit in the statistical procedures utilized. In contrast, we assumed that: (1) a true deterministic response could be either an increase or a decrease, depending on the animal; and (2) the particular variable affected by the field could be *a priori* undeterminable. A roulette wheel exemplifies the latter idea. An input (releasing the ball) always results in an output (ball in a slot) but the particular slot is not predictable [32].

To characterize the immune system, we measured a total of 20 standard immune variables in each animal (see below). We recognized that the large-sample mean of a set of measurements of any particular variable in field-exposed mice that each exhibited the assumed behavior would be similar to

the mean of the controls even if the field produced a deterministic response in every exposed animal. In other words, if the input/output relation were as we hypothesized, an effect of the EMF would not be observed by comparing means in large samples because oppositely-directed changes would be averaged away. A single small sample might reveal the putative effect as a consequence of incomplete averaging, but statistical tests on small samples generally lack statistical power. To overcome the latter problem, we developed a novel statistical procedure that was suitable for inferring the kind of change that we envisioned.

The likelihood approach allows differences in means from replicate series of exposed and control groups to be combined to test an overall statistical hypothesis [33]. The log-likelihood ratio of the t statistic for a t test between an exposed and control group is

$$l = 2N \ln\left[1 + \frac{1}{2N-2} t^2\right], \tag{1}$$

where N is the number of animals in each group. The asymptotic distribution of l is chi-square with 1 degree of freedom [34]. Using a Monte Carlo procedure, it can be shown that the distribution of l for $N \geq 5$ is approximately chi-square. For k pairs, the overall values of the test statistic, L, is $L = \sum_1^k l_i$, which also approximately follows the chi-square distribution, with k degrees of freedom under the hypothesis of no treatment effect. Because L is sensitive to the difference between the exposed and control groups but not to the direction of the difference, L is suitable for testing a single overall hypothesis regarding occurrence of EMF-induced change in the k replicates.

In preliminary studies, we evaluated the use of the log-likelihood ratio for the F test for differences in variance. We found an unacceptably high number of false positive results, and ultimately determined that they arose because of the statistical properties of the variance. In particular, because the variance of sample variance is always greater than the variance of the sample mean. Consequently, differences in variances were not incorporated into our statistical design.

The occurrence of changes in the immune system due to exposure to 60-Hz magnetic fields was assessed on the basis of whether $L > \chi^2_{k,0.05}$, with $k = 3$ and $N = 5$. The null hypothesis was that the mean of the exposed mice in the first replicate was equal to the mean of the corresponding control, and, the means in the second replicate were equal, and, the means in the third replicate were equal. We chose a group size of 5 because preliminary studies suggested it was large enough to characterize a population, but small enough to permit the large number of planned measurements on each animal. We chose $k = 3$ because it was the minimum number of replicates that could reasonably be expected to capture the anticipated variability. However, many other choices of k and N were possible. Controls included sham-exposed mice

paired with each exposed group, and two experiments in which both groups were sham-exposed. The occurrence of consistent change in a specific immune variable (which would suggest the applicability of a linear relationship between the field and consequent changes in the immune system was evaluated by combining the individual measurements in the 3 replicates prior to analysis ($L > \chi^2_{1,0.05}$, with $N = 15$). The null hypothesis in this case was that the mean of the 3 exposed groups was equal to that of the combined control groups. Under the conditions of our study, this procedure had a statistical power equivalent to performing a t test on the combined data. The results of the L test with 1 degree of freedom and the t test were identical; for simplicity, only the results with the L test are described below.

L for small samples ($N = 5$) is not precisely chi-square but we showed that the error was immaterial (see Discussion below). The binomial theorem was used to assess whether the observed number of statistically significant L values in a particular experiment could reasonably be attributed to chance.

More than 3600 individual measurements of 20 different immune variables were made. Of this total, about 15 measurements differed by more than 5 standard deviations from their respective means. The outliers were included in the analysis because we had no objective basis to exclude them. Their inclusion had no effect on the interpretation of the data. MathCad (MathSoft, Cambridge, MA) was used for all computations.

Exposure System

Magnetic fields were produced using an arrangement of four square coils [35], with construction details generally as specified elsewhere [36]. The outer and inner coils in each 4-coil consisted of 85 and 35 turns respectively of 12-gauge magnet wire. Each coil was dipped in epoxy to minimize potential effects due to vibration, and wrapped with grounded metal shielding to eliminate the possibility of electric-field effects. The shield was interrupted to prevent magnetic fields due to eddy currents. Each four-coil accommodated 4 cages on 2 shelves. Four sets of four-coils arranged in an octapole configuration [36] constituted an exposure unit.

The units were designed using commercial software (MF3D, ERM Inc., Pittsburgh, PA) to produce homogeneous magnetic fields ($\pm 5\%$) throughout the region occupied by the mice, with a negligible fringing field. The predicted homogeneity and an absence of fringing field beyond 2 m from the unit were verified by direct measurements (Bartington MAG-03, GMW, Redwood City, CA).

Four identical units were built; two were used to produce magnetic fields, and the others were short-circuited and used to house the control mice. The exposure and control units were separated by more than 5 m in a room in the institutional animal-care facility that was chosen because of its low

ambient 60-Hz magnetic fields and uniform geomagnetic field. The ambient 60-Hz field at the locations of the control units averaged 4 mG and was never higher than 7 mG.

The exposure units were energized by power supplies consisting of an isolation transformer, autotransformer, and series capacitors, and were operated in series resonance at 60 Hz to eliminate powerline harmonics. Fourier analysis of the coil currents showed that the strongest harmonics were 50 dB below the fundamental. The power supplies were rack-mounted in solid copper boxes to minimize the magnetic fields created by eddy currents caused by the transformers.

The exposure room was continuously maintained under temperature and humidity control with an unvarying light-dark cycle (12/12). Room air was replaced 15 times/hour with fresh air. Sentinel mice were negative for the presence of hepatitis and Sendai virus. A virtual instrument (Labview, National Instruments, Austin, TX) was created to continuously monitor and record room temperature, coil current, magnetic field, and current harmonic content.

Animals

Female C57BL/6 mice (Jackson Laboratories, Bar Harbor, MN), 6 weeks old at arrival, were rested a minimum of 2 and a maximum of 5 weeks before use; they were housed 5 per cage in accordance with applicable guidelines [37]. The mice were randomly assigned to the exposed or control groups; the mean initial weights differed by less than 5%. The immediate environment of the mice was totally non-metallic and included a polystyrene cage with micro-barrier filter top. The water bottle (glass sipper) was placed inside the cage to minimize differences in electrical potential between the mice and the water. Food pellets were placed in an accessible area of the bottle holder. The animal cages sat on plastic shelves whose indentations automatically centered the cages in the homogeneous portion of the magnetic field. The shelves were wall-mounted to prevent vibrational coupling between the coils and the cages. After commencing an experiment, the mice were exposed continuously, except for the time needed to service the cages (about 1 h/week). To avoid potential confounding effects due to switching transients, the magnetic field was never changed during the time the mice were exposed.

In 6 independent experiments, mice were exposed to 5 G, 60 Hz, for 1–105 days. The field strength was chosen because it is near the largest that would ordinarily be encountered in the environment. The frequency was chosen to be that of the North American electrical power system. To evaluate the reliability of the *L* procedure, 2 sham experiments were performed in which all mice received the control treatment. In each experiment, 3

replicates were evaluated, each consisting of 5 exposed and 5 control mice. The 10 mice in a particular pair were sacrificed (cervical dislocation) on the same morning, and the minimum time between sacrifice of any 2 pairs was 1 week.

Immune Measurements

Flow Cytometry

Spleen and thymus cells were obtained by gently dispersing the organs between glass slides, and bone-marrow cells were obtained by removing and flushing both femurs with phosphate-buffered saline (PBS). The cells were counted (Z1, Coulter, Hialeah, FL) and then resuspended at 10^7 cell/mL in staining buffer (PBS, 2% fetal bovine serum, 1 g/L sodium azide), and populations of interest were identified by two-color flow cytometric analysis using fluorescein isothyocyanate and phycoerythrin (Epics Profile II, Coulter, Hialeah, FL). Cell populations were enumerated by staining with PK 136, for the NK1.1 antigen on NK cells of the B6 mouse, GK1.5, 2.43, 2C11 and anti-Thy1.2 for the respective CD4, CD8, CD3, and CD90 antigens on *T* cells, and anti-IgM, anti-IgD, and anti-CD45 for antigens on B cells. Antibodies were purified from hybridomas (ATCC, Rockville, MD) or purchased (Pharmingen, San Diego, CA; Southern, Birmingham, AL). To prevent nonspecific binding, the cells were incubated with 50 μL of the appropriately diluted anti-Fc receptor antibody.

Assays

Cytotoxic T lymphocytes (CTL) were generated in a one-way mixed lymphocyte culture (MLC) by co-culturing B6 spleen cells and gamma-irradiated A/J spleen stimulator for 5 days. Proliferation was quantified (after 3 days' incubation) using a commercial proliferation assay (CellTiter96 AQ, Promega, Madison, WI). The result was expressed as the ratio of absorption units obtained from stimulated and unstimulated culture (stimulation index). The lytic activity of spleen NK cells was enhanced by culturing spleen cells for 18 h in medium containing IL-2 (800 units/mL) [38].

^{51}Cr-labeled target cells were combined with various numbers of effector cells to give a range of effector-to-target (E:T) ratios (2:1, 3:1, 6:1, 13:1, 25:1, 50:1, and 100:1) that were each assayed in triplicate. The specific lysis (SL) was calculated as: $SL = (E - S)/(M - S)$, where E and S were respectively the counts per minute released in the presence of the effector cells and the presence of medium alone, and M was the maximum value (determined by lysing the target cells with acetic acid). For determination of NK-cell

cytotoxicity, YAC-1 and P815 cells were used as positive and negative targets, respectively. YAC-1 and IL-4 cells were used as the respective positive and negative targets for the CTL generated in the MLC. For simplicity, the results are expressed in terms of a single pre-determined E:T ratio for each assay.

RESULTS

Twenty immune variables were measured in each of 5 mice exposed to 5 G for 105 days and in each of 5 sham-exposed control mice, and the mean $\pm$ standard deviation for each immune variable was determined. Using Eq. (1), l was calculated for each of the 20 comparisons between the 2 groups. The entire procedure was performed two additional times, totaling 3 replicates, and 20 L values were computed by summing the corresponding constituent values of l (Table 1). We tested hypotheses concerning the occurrence of field-induced change by using the replicate means to assess whether L exceeded the critical value ($P < 0.05$) of the chi-square distribution with 3 degrees of freedom, and found 4 statistically significant differences (Table 1).

The experiment was performed 5 additional times, corresponding to exposures of 1, 5, 10, 21, and 49 days. After 49 days, statistically significant differences were found in 4 immune variables (Table 2). The results from the experiments using shorter exposure durations were similar. After 1 or 5 days there were 4 significant differences, after 10 days there were 3 differences, and after 21 days 5 such differences were found (detailed data not shown, but see Fig. 1).

To explore the possibility that the relatively large number of significant differences were somehow a by-product of our novel statistical procedure, the experiment was performed two additional times, but with no field applied to the putatively exposed group in either of the sham studies. The sham exposure lasted 21 days in one case and 75 days in the other. The durations were chosen for convenience; the numerical values are unimportant because the purpose of the experiments was to ascertain the frequency of false-positive results produced by our statistical procedure. We found one significant difference in each experiment (data not shown). The statistically significant differences observed in all field and sham experiments are summarized in Table 3, and the cumulative frequency of the significant differences is a function of L as shown in Fig. 1.

To examine the ability of linear statistical analysis to detect the deterministic effect of the field, in each experiment, L was computed directly from the 15 exposed and 15 control mice, and evaluated based on the chi-square distribution with 1 degree of freedom. The procedure produced no significant differences in any instances where effects were noted initially (Table 3).

Table 1. Immune Parameters (Mean ± SD) in Mice Exposed in 3 Replicates to 5 G, 60 Hz, for 105 Days

	Replicate	E	C	*l*	*L*
Cellularity (no. of cells$\times 10^7$):					
Spleen	1	10.66 ± 0.60	11.78 ± 1.85	1.892	
	2	11.48 ± 2.09	11.40 ± 0.89	0.008	1.917
	3	12.10 ± 1.34	12.00 ± 0.89	0.017	
Thymus	1	3.36 ± 0.82	3.76 ± 1.07	0.533	
	2	5.46 ± 2.57	3.70 ± 0.49	2.487	3.029
	3	4.64 ± 0.64	4.70 ± 1.51	0.009	
Bone Marrow	1	3.40 ± 0.44	3.28 ± 0.31	0.309	
	2	3.34 ± 0.92	3.28 ± 0.48	0.021	0.676
	3	3.40 ± 1.08	3.07 ± 0.15	0.346	
Distribution (%):					
Spleen:					
CD45...	1	3.25 ± 0.96	4.00 ± 1.22	1.202	
	2	2.40 ± 0.55	2.40 ± 0.89	0	1.219
	3	2.40 ± 0.89	2.33 ± 0.58	0.017	
IgM +	1	54.25 ± 4.03	54.60 ± 2.30	0.035	
	2	54.40 ± 2.97	54.80 ± 1.64	0.087	4.701
	3	64.20 ± 3.19	60.00 ± 1.00	4.579	
IgM + IgD −	1	20.50 ± 4.51	21.40 ± 2.88	0.144	
	2	20.80 ± 1.79	20.20 ± 1.92	0.321	0.677
	3	12.60 ± 2.07	12.00 ± 2.00	0.212	
IgM + IgD + ...	1	40.00 ± 2.45	40.60 ± 2.88	0.140	
	2	42.40 ± 1.34	41.80 ± 0.84	0.863	4.398
	3	52.60 ± 2.19	49.33 ± 3.06	3.395	
CD90 + CD3 + ...	1	10.25 ± 2.63	9.20 ± 1.64	0.672	
	2	9.40 ± 1.14	9.60 ± 1.14	0.095	4.988
	3	14.00 ± 3.39	18.33 ± 1.53	4.221	
NK1.1...	1	3.70 ± 0.66	2.76 ± 0.68	4.357	
	2	2.84 ± 0.15	2.94 ± 0.39	0.349	*9.705
	3	4.16 ± 0.24	3.47 ± 0.64	4.999	
Marrow:					
CD45	1	12.80 ± 2.78	10.80 ± 2.17	1.837	
	2	9.80 ± 1.92	7.80 ± 1.79	3.093	5.712
	3	10.80 ± 2.17	9.67 ± 1.53	0.782	
IgM +	1	11.40 ± 2.51	11.80 ± 1.30	0.124	
	2	15.80 ± 1.48	14.20 ± 1.92	2.400	6.292
	3	13.20 ± 2.28	16.67 ± 2.89	3.768	
IgM + IgD −	1	9.20 ± 3.42	9.40 ± 2.51	0.014	
	2	11.60 ± 2.51	9.80 ± 2.95	1.266	3.134
	3	8.60 ± 5.77	13.00 ± 1.73	1.854	
IgM + IgD +	1	5.00 ± 2.00	5.60 ± 1.14	0.416	
	2	8.40 ± 0.89	7.80 ± 2.05	0.440	3.568
	3	7.40 ± 1.95	9.67 ± 2.08	2.712	
Thymus:					
CD90 + CD3 + ...	1	8.00 ± 1.58	7.00 ± 0.71	1.892	
	2	10.00 ± 5.10	13.40 ± 0.89	2.38	4.519
	3	11.40 ± 3.05	12.33 ± 2.89	0.239	

(continued)

Table 1. Continued

	Replicate	E	C	l	L
CD4 + CD8 −	1	12.40 ± 1.52	15.20 ± 1.64	6.831	
	2	12.00 ± 1.87	13.00 ± 1.58	0.991	*8.054
	3	8.20 ± 2.95	9.00 ± 1.73	0.232	
CD4 − CD8 +	1	2.20 ± 0.45	1.40 ± 0.55	5.878	
	2	1.80 ± 0.45	2.60 ± 0.55	5.878	*11.936
	3	1.40 ± 1.14	1.67 ± 0.58	0.180	
CD4 + CD8 +	1	77.40 ± 1.52	75.60 ± 2.61	2.008	
	2	79.60 ± 1.52	77.20 ± 2.78	3.075	5.296
	3	86.80 ± 4.21	85.67 ± 3.06	0.213	
Function:					
SI (ratio of cell numbers)					
	1	1.97 ± 0.28	2.12 ± 0.40	0.544	
	2	1.93 ± 0.56	2.17 ± 0.46	0.636	1.457
	3	1.89 ± 0.61	2.10 ± 0.69	0.277	
CTLa (%)	1	72.60 ± 9.07	67.40 ± 11.01	0.798	
	2	77.00 ± 5.57	77.80 ± 5.45	0.066	5.392
	3	67.20 ± 8.08	78.33 ± 4.73	4.528	
NKa (%)	1	17.60 ± 3.85	20.00 ± 1.58	1.890	
	2	13.00 ± 1.58	15.40 ± 3.78	1.941	*12.026
	3	38.00 ± 3.08	31.33 ± 2.08	8.195	

SI, Stimulation index. CTLa, Cytotoxic T lymphocyte assay (E:T, 13:1). NKa, Natural killer cell cytotoxic assay (E:T, 25:1).
*$p < 0.05$.

DISCUSSION

In each of two sham experiments we found only one significant difference in 20 tests at $P < 0.05$ (Fig. 1), which was the expected result on the basis of chance (probability of 64%). In the field experiments, the fewest number of significant differences was 3, which occurred after 10 days' exposure to the EMF. The probability that occurred by chance (at the observed pair-wise significance levels) was less than 5%. In all the other experiments the family-wise error rate was also less than 5% (Fig. 1). Thus the sham and EMF experiments, taken together, indicated that the increased frequency of significant differences in the field experiments could not be accounted for by the statistical methodology.

However, neglecting the results of the sham experiments, it could be suggested that the increased number of significant differences in the field experiments were somehow due to the use of the chi-square distribution, because L for small samples ($N = 5$) is not precisely chi-square. It can be shown that small-sample probability is overestimated when $|t| < 2.05$ (ranging from 20% for $t = 0.1$ to 1% for $t = 2.0$), and that otherwise it is

Table 2. Immune Parameters (Mean ± SD) in Mice Exposed in 3 Replicates to 5 G, 60 Hz, for 49 Days

	Replicate	E	C	l	L
Cellularity (no. of cells$\times 10^7$):					
Spleen	1	8.66 ± 1.86	8.84 ± 1.24	0.008	
	2	10.26 ± 0.29	10.02 ± 0.71	0.122	0.599
	3	9.28 ± 0.68	9.84 ± 0.59	0.469	
Thymus	1	4.84 ± 0.49	6.46 ± 0.86	2.871	
	2	4.26 ± 0.42	4.88 ± 0.34	1.552	5.106
	3	4.14 ± 1.12	3.18 ± 0.62	0.683	
Bone Marrow	1	3.92 ± 0.35	4.48 ± 0.22	2.066	
	2	2.40 ± 0.24	2.70 ± 0.11	1.491	5.070
	3	3.56 ± 0.38	2.88 ± 0.46	1.513	
Distribution (%):					
Spleen:					
CD45...	1	4.20 ± 0.20	4.60 ± 0.40	0.952	
	2	6.20 ± 0.20	2.70 ± 0.11	0	1.995
	3	8.20 ± 2.13	10.40 ± 0.98	1.043	
IgM +	1	60.00 ± 1.05	56.40 ± 0.98	5.801	
	2	56.40 ± 3.76	59.40 ± 1.89	0.616	*7.917
	3	9.60 ± 2.58	12.60 ± 1.47	1.200	
IgM + IgD −	1	15.40 ± 0.51	15.20 ± 1.07	0.036	
	2	13.80 ± 0.20	13.20 ± 0.49	1.491	6.217
	3	6.20 ± 1.07	9.20 ± 0.86	4.690	
IgM + IgD + ...	1	46.20 ± 1.32	44.20 ± 0.74	1.984	
	2	48.20 ± 1.24	47.40 ± 0.93	0.327	3.221
	3	5.40 ± 1.29	7.00 ± 1.30	0.910	
CD90 + CD3 + ...	1	11.26 ± 0.92	17.12 ± 9.39	2.160	
	2	14.26 ± 1.59	13.84 ± 2.14	0.154	2.479
	3	0.35 ± 0.11	0.42 ± 0.22	0.165	
NK1.1...	1	3.98 ± 1.05	4.58 ± 0.99	1.024	
	2	2.00 ± 0.54	1.90 ± 0.40	0.136	*15.491
	3	0.26 ± 0.20	1.20 ± 0.37	14.331	
Marrow:					
CD45	1	12.80 ± 0.58	12.00 ± 1.00	0.580	
	2	12.60 ± 0.68	8.60 ± 0.40	14.412	*20.272
	3	6.00 ± 0.45	7.60 ± 0.51	5.280	
IgM +	1	14.40 ± 0.40	13.80 ± 1.53	0.178	
	2	12.00 ± 0.84	12.00 ± 1.41	0	0.332
	3	57.00 ± 0.45	57.80 ± 2.22	0.154	
IgM + IgD −	1	10.00 ± 0.95	10.20 ± 1.77	0.012	
	2	7.80 ± 1.36	8.80 ± 2.33	0.170	0.213
	3	11.20 ± 1.20	11.40 ± 0.40	0.031	
IgM + IgD +	1	7.80 ± 0.58	6.80 ± 0.58	1.688	
	2	5.80 ± 0.70	7.00 ± 0.63	2.877	6.460
	3	45.60 ± 1.12	47.80 ± 1.28	1.895	
Thymus:					
CD90 + CD3 + ...	1	22.60 ± 0.81	24.60 ± 1.21	2.119	
	2	10.40 ± 0.68	10.40 ± 0.75	0	2.937
	3	29.00 ± 8.52	21.40 ± 3.50	0.818	

(continued)

Table 2. Continued

	Replicate	E	C	l	L
CD4 + CD8 −	1	9.20 ± 0.66	11.60 ± 1.03	3.922	
	2	17.60 ± 1.29	17.00 ± 0.45	0.239	7.671
	3	23.20 ± 4.84	14.20 ± 0.80	3.510	
CD4 − CD8 +	1	1.46 ± 0.22	1.82 ± 0.63	1.691	
	2	1.68 ± 1.26	1.54 ± 0.36	0.070	2.576
	3	5.72 ± 7.10	3.00 ± 2.04	0.815	
CD4 + CD8 +	1	84.00 ± 0.63	80.80 ± 1.59	3.613	
	2	75.80 ± 1.74	77.80 ± 0.58	1.380	7.227
	3	62.00 ± 1.08	77.60 ± 2.06	2.234	
Function:					
SI (ratio of cell numbers)					
	1	2.94 ± 0.21	1.96 ± 0.19	9.194	
	2	3.65 ± 0.29	3.07 ± 0.18	3.125	*17.312
	3	2.10 ± 0.07	2.58 ± 0.20	4.993	
CTLa (%)	1	55.40 ± 2.44	57.40 ± 4.16	0.213	
	2	58.80 ± 4.14	53.00 ± 6.50	0.685	1.492
	3	50.00 ± 8.67	56.20 ± 1.83	0.594	
NKa (%)	1	35.20 ± 2.82	35.00 ± 2.59	0.003	
	2	27.60 ± 2.91	19.60 ± 1.57	5.496	5.766
	3	37.40 ± 2.09	36.00 ± 2.17	0.267	

SI, Stimulation index. CTLa, Cytotoxic T lymphocyte assay (E:T, 13:1). NKa, Natural killer cell cytotoxic assay (E:T, 25:1).
*$p < 0.05$.

underestimated. Application of the appropriate correction factor to each l in the study did not alter any of the results. This finding indicated that the large number of significant differences in the EMF experiments could not be attributed to the use of the chi-square distribution, thereby eliminating any need for the laborious task of evaluating L using exact distributions (a total of 8 experiments×3 replicates×20 variables = 480 randomization procedures would have been required).

At first glance it might appear that the dependent nature of the immune variables measured in this study might weaken the family-wise error rates for the 6 EMF experiments which, as mentioned above, were all less than 5%. Actually, however, the opposite was true. Under our statistical design, assessment of the existence of a deterministic effect depended only on the number of significant differences, not on the absence of correlations. Any correlations that might have existed between immune variables simply made it more likely that we could detect an effect of the field on the immune system. Therefore, considering the results of the sham studies, any correlations that might have existed among the immune variables could not have produced

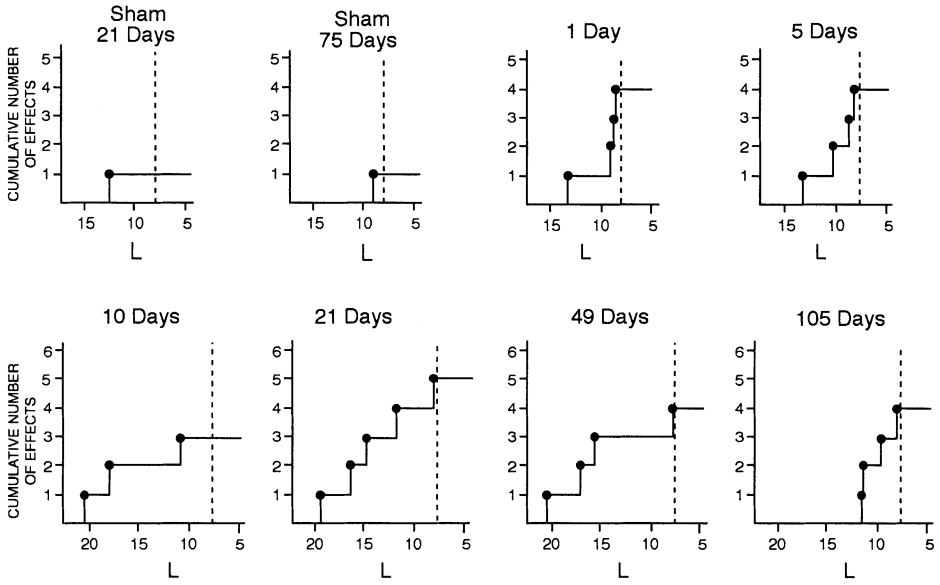

Figure 1. Cumulative frequency of immune parameters (out of 20) that were significantly affected by EMF exposure for the duration indicated, as a function of the magnitude of the test statistic. Regions beyond the dotted line indicate $P < 0.05$ ($L > 7.83$).

false positive results. On the basis of all the mentioned considerations, we conclude that the observed rate of significant comparisons in the exposed mice above that of the controls reliably indicated that the EMFs were transduced into biological signals which ultimately affected the immune system.

When the data was analyzed on the basis of a linear model (L computed directly from the 15 exposed and 15 control mice, based on the chi-square distribution with one degree of freedom), no significant differences were found in any instance where effects were noted initially (Fig. 1). Since the field affected the immune system in a way that could not be explained on the basis of a linear model, we conclude that the response of the immune system to the EMFs was governed by nonlinear dynamical laws.

The overall impact of the field on the immune system as judged by the number of significant differences did not differ materially from the results found previously following exposure to 1 G [31]. The absence of a proportional relationship between field strength and its effect on the immune system indicated that the interaction that led to the immune changes was a trigger phenomenon like, for example, sensory transduction, rather than a process that was driven energetically, like the interaction of ionizing radiation and hard tissue. No clear pattern was seen regarding the particular variables impacted by the field, but thymus cellularity and NK-cell cytotoxicity were the more frequently affected variables.

Table 3. *L* Values for Statistically Significant Comparisons Involving Immune Parameters in Mice Exposed to 60-Hz Magnetic Fields or to Control Conditions (The Mice Were Exposed in a Series of 3 Replicates, Each Consisting of 5 Exposed and 5 Control Mice. The *L* Value of all Pair-Wise Comparisons for Which $L > \chi^2_{3,0.05} (L > 7.8)$ is listed.)

	Duration of Magnetic Field Exposure (Days)						Duration of Sham Exposure (Days)	
	1	5	10	21	49	105	21	75
Cellularity:								
Spleen	—	—	—	14.6	—	—	—	—
Thymus	8.9	8.2	—	19.6	—	—	—	—
Bone Marrow	—	—	—	—	—	—	—	—
Distribution:								
Spleen:							—	—
CD45...	—	—	—	—	—	—	—	—
IgM +	—	—	—	—	7.9	—	—	—
IgM + IgD −	8.2	—	—	16.3	—	—	—	—
IgM + IgD + ...	—	—	—	—	—	—	—	—
CD90 + CD3 + ...	—	—	—	8.0	—	—	—	—
NK1.1...	—	10.4	—	—	15.5	9.7	—	—
Marrow:								
CD45	—	—	—	11.9	20.3	—	—	—
IgM +	—	—	—	—	—	—	—	—
IgM + IgD −	—	—	—	—	—	—	—	—
IgM + IgD +	—	13.5	—	—	—	—	—	—
Thymus:								
CD90 + CD3 + ...	—	—	—	—	—	—	—	—
CD4 + CD8 −	8.7	—	20.4	—	—	8.0	—	—
CD4 − CD8 +	—	—	—	—	—	11.9	—	8.9
CD4 + CD8 +	—	7.9	18.1	—	—	—	—	—
Function:								
SI	—	—	—	—	17.3	—	—	—
CTLa (%)	13.0	—	—	—	—	—	12.5*	—
NKa (%)	—	NM	10.9*	—	—	12.0	—	—

*, Evaluated using only 2 replicates; *L* was adjusted to the equivalent 3-replicate value. SI, Stimulation index. CTLa, Cytotoxic T lymphocyte assay (E:T, 13:1). NKa, Natural killer cell cytotoxic assay (E:T, 25:1).

We concluded that the input/output characteristics of the immune system were nonlinear when the input was a magnetic field. It is worthwhile to consider the implications of this conclusion with regard to the system's intrinsic regulatory mechanisms. A convenient way to do this is to focus on the differential equations that govern the system. We obviously cannot identify them with the specificity needed to predict immune-system behavior, but we can be certain the equations exist because the system's behavior is

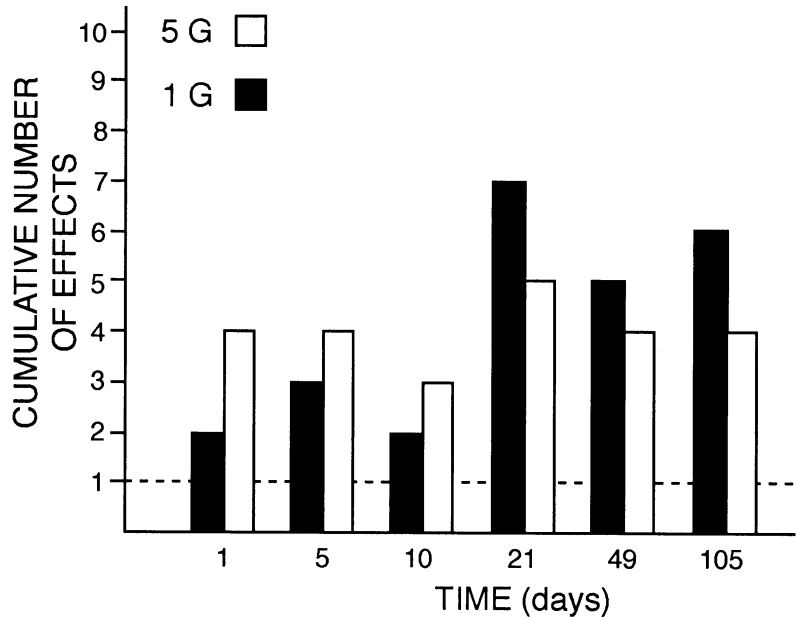

Figure 2. Number of immune parameters (out of 20) that were significantly affected by EMF exposure as a function of exposure time. Results from a previous study are presented for comparison [31]. The dotted line indicates the results from the positive-control experiments (one significant difference in each of 2 sham experiments).

always lawful. The state variables in the equations would include (among others) those measured in this study, and a solution would specify the time evolution of the state variables. What would be the nature of a solution? It would appear as a bounded irregular time series (see Fig. 3A for a qualitative illustration) and one possibility is that it could be fully described by a linear stochastic model [39]. In such a model, the output is partially predictable only for a particular period into the future that can be characterized by the autocorrelation function. Thereafter, any prediction would be no better than a guess. In this model the many possible immune variables would surely be correlated, but their coupling would be random. In the alternative model, namely one in which the governing equations were nonlinear, a change in one variable could produce a deterministic effect on other immune variables. Equations that were originally derived to model the weather [40] provide a simple example of this form of fundamental coupling (Fig. 3B).

The potential attractiveness of a nonlinear model for the time series of the output of the immune system (as distinct from the nonlinearity in the input/output relations demonstrated above) is that it has recently become clear that systems governed by coupled equations can display some remarkable behaviors. One example, known as sensitivity to initial conditions, is illustrated in Fig. 3C in the context of the Lorenz system.

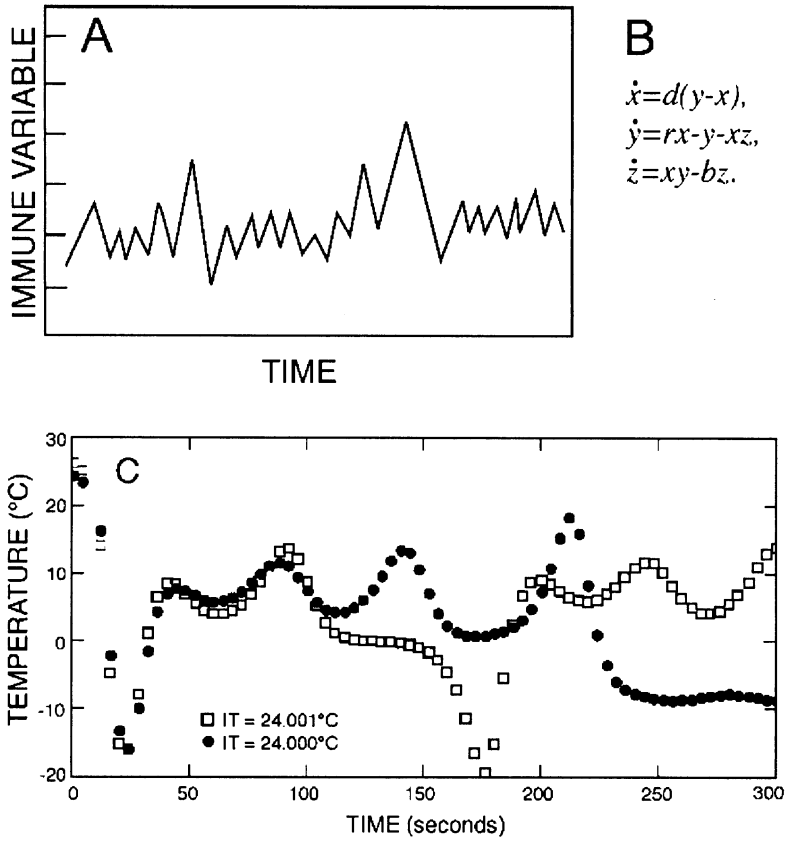

Figure 3. A, Hypothetical time course of an arbitrary immune variable. If the variable could be measured as a time series, it presumably would vary between an upper and lower bound in an apparent irregular pattern. B, Lorenz differential equations governing fluid convection [40]. The state variables are related to fluid velocity (x) and temperature (y and z). d, r, and b are parameters. For some values of the parameters the system exhibits extreme sensitivity to earlier states (chaos). C, Moving averages for temperature in the Lorenz system, demonstrating chaos. A small change in temperature caused the system to evolve along a completely different trajectory such that observers at say $t = 250$ seconds would record widely different average temperatures. IT, initial temperature ($N = 5$ for each point).

The parameters in this example were chosen so that the system was in the chaotic mode, and the evolution of one of the state variables (temperature) was calculated over 300 seconds for initial values that differed by less than 1 part in 10^4. The example shows that chaotic systems can follow completely different paths as a result of extremely small perturbations. If the variables in the immune system were coupled nonlinearly, that could explain the nonlinearity in the input/output relationships which was observed in this study. To see that this could be the case, we will now show that sampling from the system shown in Fig. 3C and analyzing the data using the L statistic can lead to results that mimic those found in our study.

Three replicates ($N = 5$ in each) were formed by random sampling and the two time series were compared to determine whether $L > \chi^2_{3,0.05}$. A typical result is shown in Table 4. The procedure correctly recognized that the two time series actually differed ($L = 9.01, P < 0.05$). When the 15 individual samples were combined to compute whether $L > \chi^2_{1,0.05}$, no effect was found ($L = 2.45, P > 0.05$). The point of this example is that sampling from a chaotic system can mimic the pattern of the immune data. This is some evidence that the intrinsic laws governing the immune system are chaotic. However, the alternative possibility, namely that the laws are linear cannot be excluded because, in theory, sampling from a linear stochastic system could also mimic the pattern of results observed in the immune data. In either case, as mentioned above, this study shows that the input/output relationships in the immune system are nonlinear in the case where the input is a power-frequency magnetic field.

How can our finding that the effects of EMFs on specific variables in the immune system were inconsistent be reconciled with the established role of the immune system as a reliable sensory and effector organ? It is reasonable to view evolution by natural selection as the process responsible for the generally faithful correspondence between stimuli and responses found in the immune system. Progressively more reliable sensory and effector systems facilitated acquisition of a selective advantage. In the absence of natural selection, however, consistency in response to a stimulus might not come about. EMFs were not present during evolution, and therefore a mechanism capable of producing a predictable response to them would not have served any evolutionary purpose. The sensitivity of biological organisms to fields could thus be a result of a vulnerability in the specific designs of the body's sensory or regulatory systems that were selected by nature. An argument

Table 4. Comparison of Chaotic Systems Defined by the Lorenz Equations [40]

Rep. No.	24°C	26°C	l	L
1	5.78 ± 10.69	5.34 ± 10.96	0.07	9.01*
2	7.34 ± 2.20	6.48 ± 3.83	3.08	
3	1.76 ± 8.45	4.91 ± 8.03	5.86	

The parameters were chosen so that the system operated in the chaotic mode ($\sigma = 16, b = 4, r = 45.92$), and the equations were solved using a fourth-order Runge–Kutta method with a time step of 0.0125 seconds. Two sets of three replicates were formed ($N = 5$ from each set in each replicate) from the transient solution of one of the three state variables (see Fig. 3). The two sets of samples were obtained from systems having initial conditions $x_0 = 20, y_0 = 10, z_0 = 24$ and $x_0 = 20, y_0 = 10, z_0 = 26$, respectively. L was statistically significant ($L = 9.01, P < 0.05$). No difference was found when L was computed from the 15 values without regard to replicate structure ($L = 2.45, P > 0.05$).
*$P < 0.05$.

against this view is that physiological systems do respond in predictable ways to novel environments as, for example, space flight.

It may be useful to speculate on the possible immunological significance of our approach and our results. We showed that magnetic fields of the type studied consistently produced a deterministic effect on the immune system in the sense that the average values of some of a set of immune variables differed depending on whether or not the field was applied (Fig. 1). This result necessarily implied the occurrence of transduction of the field, either at the locus of a specific detector as in sensory transduction, or as a result of the kind of vulnerability discussed above. In either case, fundamental biophysical considerations indicate that the energy of interaction between the field and biological tissue was too low to allow us to imagine that the transduction process was governed by linear dynamical laws [41]. Consequently, some form of "nonlinearity" is required to explain our observations. The term is perhaps unfortunate because it suggests to some the same error as calling physiology "the study of non-immunological biological systems." Nevertheless, no clearer term is presently in use to convey the idea that something more than a traditional linear approach is needed to explain our results.

To consider any such alternative, it is necessary to resort in principle or practice to a mathematical description of the behavior of the immune system. Most past attempts have done so explicitly, by postulating equations, solving them, and fitting the solutions to data by adjusting constants [26, 42–44]. This results in a compact mathematical description of time-dependent change in a population or of the average of a sample as the model system under study moves towards its final state (its "attractor"). However, even complex mathematical equations greatly oversimplify the immune system, with the result that they do not lead to reliable information about the future behavior of individual systems. The ability to do so is the validating property of true equations of motion.

Our approach, in contrast, incorporated nonlinearity as a qualitative characteristic of a dynamic model of the response of the immune system to EMFs. We showed that determinism in the data could not have been found but for the nonlinear characteristic of the model (which we assume mirrors the nonlinear characteristic of the immune system). Our approach differed from the past nonlinear approaches in several salient respects. First, no attempt was made to divine equations that governed the immune system, although they were assumed to exist, in principle. Second, no attempt was made to predict precisely how the immune system would respond to the field. Rather, the goal was to discriminate between two sets of data without making any assumptions regarding how the data sets might differ. The absence of such an assumption differed from traditional practice, which is usually based on the assumption that any difference between data sets would be related only linearly to the factor that caused the difference (in our case, a field).

We think that these considerations show that the immune system is more complex than previously perceived because, in addition to its recognized role

of detecting and responding to pathogenic agents, they show that the immune system is intrinsically susceptible to fields. The physiological consequences of that susceptibility remain to be ascertained.

Finally, the question of the possible biophysical mechanism responsible for the interaction of EMFs with biological receptors has been considered by others [45–47] and was not addressed here. The further question of the biological significance of the type of change reported here was similarly not addressed in our experimental design.

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REFERENCES

1. Boivin, W.S.; Boyd, S.M.; Coletta, J.A.; Neunaber, L.M. Measurement of Radiofrequency Electromagnetic Fields in and Around Ambulances. Biomed. Instrum. Technol. **1997**, *31*, 145–154.
2. Lanera, D.; Zapotosky, J.E.; Colby, J.A. Study of Magnetic Fields from Power-Frequency Current on Water Lines. Bioelectromagnetics **1997**, *18*, 307–316.
3. Bracken, T.D.; Rankin, R.F.; Senior, R.S.; Alldredge, J.R.; Sussman, S.S. Magnetic Field Exposure Among Utility Workers. Bioelectromagnetics **1995**, *16*, 216–226.
4. Kaune, W.T.; Miller, M.C.; Linet, M.S.; Hatch, E.E.; Kleinerman, R.A.; Wacholder, S.; Mohr, A.H.; Tarone, R.E.; Haines, C. Children's Exposure to Magnetic Fields Produced by U.S. Television Sets Used for Viewing Programs and Playing Video Games. Bioelectromagnetics **2000**, *21*, 214–227.
5. Mantiply, E.D.; Pohl, K.R.; Poppell, S.W.; Murphy, J.A. Summary of Measured Radiofrequency Electric and Magnetic Fields (10 kHz to 30 GHz) in the General and Work Environment. Bioelectromagnetics **1997**, *18*, 563–577.
6. Marino, A.A. Electromagnetic Fields, Cancer, and the Theory of Neuroendocrine-Related Promotion. Bioelectrochem. Bioenerg. **1993**, *29*, 255–276.
7. Evaluation of the Potential Carcinogenicity of Electromagnetic Fields. United States Environmental Protection Agency, 1990.
8. Portier, C.J.; Wolfe, M.S., Eds. Assessment of Health Effects from Exposure to Power-line Frequency Electric and Magnetic Fields: NIH, 1998.
9. Repacholi, M.H. Low-level Exposure to Radiofrequency Electromagnetic Fields: Health Effects and Research Needs. Bioelectromagnetics **1998**, *19*, 1–19.
10. Tremblay, L.; Houde, M.; Mercier, G.; Gagnon, J.; Mandeville, R. Bioelectromagnetics **1996**, *17*, 373–383.
11. Murthy, K.K.; Rogers, W.R.; Smith, H.D. Initial Studies of the Effects of Combined 60-Hz Electric and Magnetic Field Exposure on the Immune System of Non-Human Primates. Bioelectromagnetics Suppl. **1995**, *3*, 93–102.

12. de Seze, R.; Bouthet, C.; Tuffet, S.; Deschaux, P.; Caristan, A.; Moreau, J.M.; Veyret, B. Effects of Time-Varying Uniform Magnetic Fields on Natural Killer Cell Activity and Antibody Response in Mice. Bioelectromagnetics **1993**, *14*, 405–412.
13. McLean, J.R.; Stuchley, M.A.; Mitchell, R.E.; Wilkinson, D.; Yang, H.; Goddard, M.; Lecuyer, D.W.; Schunk, M.; Callary, E.; Morrison, D. Cancer Promotion in a Mouse-Skin Model by a 60-Hz Magnetic Field: II. Tumor Development and Immune Response. Bioelectromagnetics **1991**, *12*, 273–287.
14. House, R.V.; Ratazczak, H.V.; Gauger, J.R.; Johnson, T.R.; Thomas, P.T.; McCormick, D.L. Immune Function and Host Defense in Rodents Exposed to 60-Hz Magnetic Fields. Fund. Appl. Toxicol. **1996**, *34*, 228–239.
15. Mevissen, M.; Loscher, W.; Szamel, M. Effect of 50-Hz Magnetic Field in Vivo Exposure on the Immune System of Female Sprague-Dawley Rats (Abstract). Bioelectromagnetics Soc. **1996**, 230.
16. Selmaoui, B.; Bogdan, A.; Auzeby, A.; Lambrozo, J.; Touitou, Y. Acute Exposure to 50-Hz Magnetic Field does not Affect Hematologic or Immunologic Functions in Healthy Young Men: A Circadian Study. Bioelectromagnetics **1996**, *17*, 364–372.
17. Berman, E.; Chacon, L.; House, D.; Koch, B.A.; Koch, W.E.; Leal, J.; Løvtrup, S.; Mantiply, E.; Martin, A.H.; Martucci, G.I.; Mild, K.H.; Monahan, J.C.; Sandström, M.; Shamsaifar, K.; Tell, R.; Trillo, M.A.; Ubeda, A.; Wagner, P. Development of Chicken Embryos in a Pulsed Magnetic Field. Bioelectromagnetics **1990**, *11*, 169–187.
18. National Institute of Environmental Health Sciences. EMF Science Review Symposium: Clinical and In Vivo Laboratory Findings. 1998.
19. Dhabhar, F.S.; McEwen, B.S. Stress-Induced Enhancement of Antigen-Specific Cell-Mediated Immunity. J. Immunol. **1996**, *156*, 2608–2615.
20. Miller, A.H. Neuroendocrine and Immune System Interactions in Stress and Depression. Psychiatric Clin. N. Am. **1998**, *21*, 443–463.
21. Pruett, S.B.; Ensley, D.K.; Crittenden, P.L. The Role of Chemical-Induced Stress Responses in Immunosuppression: A Review of Quantitative Associations and Cause-Effect Relationships Between Chemical-Induced Stress Responses and Immunosuppression. J. Toxicol. Environ. Health **1993**, *39*, 163–192.
22. Glaser, R.; Kiecolt-Glaser, J.K. Stress-Associated Immune Modulation: Relevance to Viral Infections and Chronic Fatigue Syndrome. Am. J. Med. **1998**, *105*, 35S–42S.
23. Barbour, S.E.; Nakashima, Szang, J.-B.; Tangada, S.; Hahn, C.-L.; Schenkein, H.A.; Tew, J.G. Tobacco and Smoking: Environmental Factors That Modify the Host Response (Immune System) and have an Impact on Periodontal Health. Crit. Rev. Oral Baol. Med. **1997**, *8*, 437–460.
24. Husband, A.J. The Immune System and Integrated Homeostasis. Immunol. Cell Biol. **1995**, *73*, 377–382.
25. Schweitzer, N.; Anderson, R. Helminths, Immunology and Equations. Immunol. Today **1991**, *12*, A76–A81.
26. Anderson, R.M. The Croonian Lecture, 1994. Populations, Infectious Disease and Immunity: A Very Nonlinear World. Phil. Trans. R. Soc. Lond. B Biol. Sci. **1994**, *346*, 457–505.

27. Harada, K.; Ikegami, T. Evolution of Specificity in an Immune Network. J. Theor. Biol. **2000**, *203*, 439–449.
28. Nowak, M.A.; May, R.M. *Virus Dynamics: Mathematical Principles of Immunology and Virology*. Oxford: Oxford University Press, 2000.
29. Ruelle, D. *Chance and Chaos*. Princeton, NJ: Princeton University Press, 1991.
30. Skinner, J.E.; Molnar, M.; Vybiral, T.; Mitra, M. Application of Chaos Theory to Biology and Medicine. Integrative Physiological and Behavioral Science **1992**, *27*, 39–53.
31. Marino, A.A.; Wolcott, R.M.; Chervenak, R.; Jourd'heuil, F.; Nilsen, E.; Frilot, C. Nonlinear Response of the Immune System to Power-Frequency Magnetic Fields. Am. J. Physiol. Regulatory Integrative Comp. Physiol. **2000**, *279*, R761–R768.
32. Ford, J. How Random is a Coin Toss? Physics Today **1983**, April, 40–47.
33. Anderson, T.W. *An Introduction to Multivariate Statistical Analysis*. New York: Wiley, 1984.
34. Flury, B. *A First Course in Multivariate Statistics*. New York: Springer-Verlag, 1997.
35. Merritt, R.; Purcell, C.; Stroink, G. Uniform Magnetic Field Produced by 3, 4, and 5 Square Coils. Rev. Scientific Instr. **1983**, *54*, 879–882.
36. Wilson, B.W.; Caputa, K.; Stuchly, M.A.; Saffer, J.D.; Davis, K.C.; Washam, C.E.; Washam, L.G.; Washam, G.R.; Wilson, M.A. Design and Fabrication of Well Confined Uniform Magnetic Field Exposure Systems. Bioelectromagnetics **1994**, *15*, 563–577.
37. National Research Council. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press, 1996.
38. Wolcott, R.M.; Jennings, S.J.; Chervenak, R. In Utero Exposure to Ethanol Affects Postnatal Development of T- and B-lymphocytes, But Not Natural Killer Cells. Alcohol Clin. Exp. Res. **1995**, *19*, 170–176.
39. Wei, W.W.S. *Time Series Analysis: Univariate and Multivariate Methods*. Redwood City, CA: Addison-Wesley Publishing Co., Inc., 1990.
40. Lorenz, E.N. Deterministic, Nonperiodic Flow. J. Atmos. Sci. **1963**, *20*, 130–141.
41. Astumian, R.D.; Weaver, J.C.; Adair, R.K. Rectification and Signal Averaging of Weak Electric Fields by Biological Cells. Proc. Natl. Acad. Sci. USA **1995**, *92*, 3740–3743.
42. Burke, M.A.; Morel, B.F.; Oriss, T.B.; Bray, J.; McCarthy, S.A.; Morel, P.A. Modeling the Proliferative Response of T Cells to IL-2 and IL-4. Cell. Immunol. **1997**, *178*, 42–52.
43. Antia, R.; Pilyugin, S.S.; Ahmed, R. Models of Immune Memory: On the Role of Cross-Reactive Stimulation, Competition, and Homeostasis in Maintaining Immune Memory. Proc. Natl. Acad. Sci. **1998**, *95*, 14926–14931.
44. Segel, L.A.; Bar-Or, R.L. On the Role of Feedback in Promoting Conflicting Goals of the Adaptive Immune System. J. Immunol. **1999**, *163*, 1342–1349.
45. Liboff, A.R. Cyclotron Resonance in Membrane Transport. In: *Interaction between Electromagnetic Fields and Cells*; Chiabrera, A., Nicolini, C., Schwan, H.P., Eds.; New York: Plenum, 1985; p. 281–296.

46. Blanchard, J.P.; Blackman, C.F. Clarification and Application of An Ion Paramagnetic Resonance Model for Magnetic Field Interactions with Biological Systems. Bioelectromagnetics **1994**, *15*, 217–238.
47. Bezrukov, S.M.; Vodyanoy, I. Signal Transduction Across Alamethicin Ion Channels in the Presence of Noise. Biophys. J. **1997**, *73*, 2456–2464.