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Electromagnetic Field Effects on Cells of the Immune System: The Role of Calcium Signalling

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Electromagnetic Field Effects on Cells of the Immune System: The Role of Calcium Signalling [1]

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Footnotes

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[3] Abbreviations: EMF, electromagnetic field; ELF, extremely low frequency; HPBL, human peripheral blood lymphocytes; Con A, Concanavalin A; PHA, Phytohemagglutinin; $[Ca^{2+}]_i$, cytosolic free calcium concentration; J, electrical current density; E, electric field intensity; B, magnetic flux density; NK cells, natural killer cells.

[4] abstract # E-3-2: Combined DC/AC Magnetic Fields Alter Ca^{2+} Metabolism in Activated Rat Thymocytes. J. Walleczek and R.P. Liburdy. 12th Annual Meeting of the Bioelectromagnetics Society, June 1990, San Antonio, Texas.

Abstract

During the past decade considerable evidence has accumulated demonstrating that exposures of cells of the immune system to relatively weak extremely-low-frequency (ELF) electromagnetic fields (< 300 Hz) can elicit cellular changes which might be relevant to in-vivo immune activity. A similar responsiveness to nonionizing electromagnetic energy in this frequency range has also been documented for tissues of the neuroendocrine and musculoskeletal system. However, knowledge about the underlying biological mechanisms by which weak fields induce cellular changes is still very limited. It is generally believed that the cell membrane and Ca^{2+} regulated activity is involved in bioactive ELF field-coupling to living systems. This article begins with a short review of the current state of knowledge concerning the effects of nonthermal levels of ELF electromagnetic fields on the biochemistry and activity of immune cells, and then closely examines new results which suggest a role for Ca^{2+} in the induction of these cellular field effects. Based on these findings it is proposed that membrane-mediated Ca^{2+} signalling processes are involved in the mediation of field effects on the immune system.

Keywords: Immune System, Signal Transduction, Calcium, Cellular Mechanisms, ELF Electromagnetic Fields

The biological effects of low-energy electromagnetic fields (EMFs) [3] have fascinated scientists for a long time. But it is only during the past decade that the study of EMF interactions with whole organisms or isolated cells has become an increasingly recognized area of research within the biological sciences. The main reasons for this development are: (i) an increasing number of experimental findings are reported each year and possible theoretical explanations of weak EMF bioeffects begin to emerge (for reviews see 1-9); (ii) evidence has accumulated demonstrating the efficacy of low-energy, ELF-pulsed magnetic fields in medical therapy, especially in non-union bone fracture healing, confirming that under certain conditions nonionizing electromagnetic energy can influence physiological processes in organisms (10, 11); (iii) from a public health point of view, there is now a growing demand for the study of possible adverse health effects from interactions between the human body and EMFs, for example, generated by 50/60-Hz high-voltage transmission lines, video display terminals, electric blankets or clinical NMR-imaging procedures. Such investigations appear to be particularly called for since epidemiologists found that a significant correlation possibly exists between the exposure of humans to weak EMFs and an elevated risk for developing certain leukemias and other cancers (12, 13). However, a cause-effect relationship between EMF exposure and increased cancer risk in principle cannot be established from such work alone. Therefore, in order to assess the relevance of the epidemiological findings,

understanding how weak EMFs can interact with biological activity under controlled laboratory conditions is critical.

In light of the epidemiological results, it is worthwhile to review the current state of knowledge regarding the effects of ELF EMF signals on the immune system. Since the immune system functions as the body's main protective mechanism against invasion by pathogens and against tumor formation and growth, EMF-induced changes in immune cell biochemistry could directly affect the organism's immune response in either a negative or positive manner. The physiological significance of the epidemiological results as well as of the reported immunological EMF effects, however, cannot be fully evaluated until (i) there are convincing results from whole organism-exposure studies which can be directly related to the in-vitro evidence, and (ii) until the biological mechanisms by which weak EMF may interact with cells of the immune system begin to be understood. With regard to in-vivo effects, evidence already exists that nonthermal EMF exposures can modify blood leukocyte counts (e.g. 14, 15), inflammatory responses (e.g. 16, 17), and the weight of lymphoid organs (e.g. 18). In none of the cited in-vivo studies, however, ELF EMFs as weak as those encountered in the normal human environment have been employed. With respect to the underlying molecular mechanisms of interaction between the immune system and EMFs one can only speculate. Nevertheless, there is new evidence from several independent research groups suggesting that Ca^{2+} signals play a key role in the mediation of EMF effects in cells of the immune system.

The purpose of this review is to summarize representative findings of ELF EMF exposure studies using immune cells and to discuss new results suggesting that the observed EMF influences can, at least to some extent, be explained by the interaction of an external EMF with transmembrane Ca^{2+} signalling events. The immunological effects of in-vivo exposures to EMFs and the effects of field signals oscillating in the radio and micro wave frequency range will not be addressed. The review begins with an outline of biophysical concepts frequently used in the characterization and description of EMF bioeffects. Next, a brief overview of representative EMF effects on the biochemistry and in-vitro activity of normal and transformed cells of the immune system is given. Then, the role of Ca^{2+} as a possible mediator of EMF influences on the immune system, in particular during cell membrane-mediated signal transduction processes, is evaluated. Finally, selected theoretical approaches which seek to identify the physical basis of nonthermal cellular field effects are mentioned and conclusions are drawn.

BIOPHYSICAL BACKGROUND

Nonthermal Electromagnetic Field Effects

EMFs and radiation in the frequency range from 0 Hz up to several hundred GHz are known to be nonionizing forms of energy, because their quantum energy is too low to cause physico-chemical or biological effects by ionization of molecules. This, for example,

is illustrated by the fact that even relatively strong power frequency (50/60 Hz) EMFs were unable to produce genotoxic effects in lymphocytes (e.g. 19-21), whereas appropiate doses of ionizing radiation routinely produce chromosomal damages in these cells. This is the major reason why the view has prevailed for a long time among biologists and physicists that nonionizing electromagnetic energy in the ELF, low-frequency, radio and microwave frequency range is capable of producing detectable effects only via mechanisms which involve significant heating in nonexcitable cells such as cells of the immune system. Over the past 10 years, however, bioelectromagnetics research has provided strong evidence that nonionizing electromagnetic energy can indeed induce a variety of biological effects not only by thermal interactions but also through interaction mechanisms which do not involve any macroscopic heating (this means that the global temperature rise in the exposed biological sample, due to the EMF influence, is generally less than 0.001°C). Low-intensity field effects, which apparently are not induced by thermal interactions, are referred to in the literature as "athermal" or alternatively "nonthermal" field effects. It should be pointed out that the calculated temperature rises due to EMF-induced Joule heating, over an 1-hour exposure period, for the exposure experiments analyzed here, are much less than 0.001°C and for this reason the field effects are interpreted to be of nonthermal origin.

Biophysical Concepts and Units

In this section concepts and units that are frequently used in bioelectromagnetics research are briefly described in order to give the reader unfamiliar with the terminology some useful background. For a detailed introduction, see the book edited by Polk and Postow (22). These concepts and quantities are described to allow comparison of the field exposure conditions based on parameters such as the applied magnetic flux density, electric field intensity, signal frequency and current density between the different exposure experiments. Briefly, the electric field intensity (E) is given in Volts per meter (V/m), where Volt is the unit of the electric potential. When a biological system (e.g. cell, tissue or whole organism) is exposed to an electric field, the mobile charges in the system will be forced to move in the direction to the induced electric field lines, hence, establishing an electric current measured in Amperes (A). The distribution of this current and the direction of its flow is defined by the current density (J) which is quantified in Amperes per square meter (A/m^2). If a time-varying magnetic field is applied to an electrically conductive material, such as living matter, an electric field is induced in accord with Faraday's Law of Induction. For purely sinusoidal magnetic fields Faraday's Law can be stated in this simple form: $E = \pi r f B$, where r is the radius in meters of a circular conductive path in the magnetic field-exposed object, f is the frequency in Hz and B is the magnetic flux density which defines the intensity of the

magnetic field. The unit of B is Tesla (T). An older unit for B is Gauss (G), where 1 T = 10^4 G. For the experiments described herein, the magnitude of the applied magnetic fields ranges from 0.02 to 22 mT (0.2-220 G). For comparison, B of the static earth magnetic field environment is from 0.02 to 0.07 mT (0.2-0.7 G). The peak magnetic flux densities used in EMF-facilitated bone healing range from 1 to 16 mT (10-160 G; ref. 23) and peak flux density values associated with switched gradient magnetic fields in clinical NMR-imaging systems are in the order of 0.1 to 10 mT (1-100 G).

ELECTROMAGNETIC FIELD EFFECTS ON CELLS OF THE IMMUNE SYSTEM

Effects From Exposures to Time-Varying Magnetic Fields

To date at least ten laboratories have carried out independent studies with immune cells investigating possible nonthermal effects of time-varying magnetic fields of intensities in the range from 0.1 to 10 mT or more on Ca^{2+} metabolism, RNA transcription or DNA synthesis (19, 20, 24-32). Out of the ten laboratories, nine have published evidence demonstrating nonthermal field effects on (i) intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) or mitogen-dependent $^{45}Ca^{2+}$ uptake, (ii) ^{3}H-uridine uptake or specific gene transcript levels, and (iii) ^{3}H-thymidine uptake or cell cycle kinetics (see Table I). The results from the laboratory that reported the absence of any

detectable field effects (19), however, do not necessarily contradict the positive findings of the other research teams, because these investigators have used magnetic fields which were about 10 to 30-fold weaker in intensity compared to the magnetic fields which were employed in the other studies (compare Table 1; effects on DNA synthesis).

In addition to the studies employing magnetic field intensities of 0.1 mT (1 G) and more (see Table 1), there are at least two reports indicating that ELF magnetic fields even at intensities as low as 0.02 mT could possibly affect lymphocyte $^{45}Ca^{2+}$ uptake (33, 34). Consistent with these data are preliminary results from this laboratory which have been reported in abstract form [4] : it was observed that $^{45}Ca^{2+}$ uptake in Concanavalin A (Con A)-activated rat thymic lymphocytes can be enhanced by 60 % ($p < 0.01$) during an 1-hour exposure to a 0.021-mT, 14.3-Hz magnetic field in the presence of an 0.021 mT static magnetic field. No effect was detected in resting cells. Although these reports together provide evidence that even very weak fields ($B < 0.1$ mT) under certain experimental conditions can affect cellular Ca^{2+} regulation, these results are mentioned for completness only and will not be further discussed because more research is needed before their significance in relationship to other cellular parameters (e.g. cell proliferation) can be evaluated.

Effects From the Application of Electrical Currents

Research on the effects of electrical currents applied over agar-bridges to prevent possible side effects from electrode electrolysis products in the absence of an applied magnetic field on immune cell biochemistry and activity, has progressed more slowly. There are currently only four independent reports available (see Table 2): three of the four reports demonstrate that 60-Hz, sinusoidal electrical currents can modify ornithine decarboxylase activity in field-exposed human lymphoma cells (35) or lymphocyte-mediated cytotoxicity when either the target or the effector cells were field-exposed prior to the cytotoxicity assay (36, 37). In contrast, proliferation of neither phytohemagglutinin (PHA)-activated human peripheral blood lymphocytes (HPBL) nor murine cytotoxic T cells (CTLL-1) was influenced by 60-Hz, sinusoidal electric field intensities of 2.4 and 10 mV/cm respectively (Table 2, refs. 19 and 37).

Magnetic Field Intensity-response Thresholds

The lower intensity-response thresholds for magnetic field effects on cellular Ca^{2+} metabolism and RNA synthesis have not been established yet. But for the EMF effects on the proliferation of HPBL a first observation can be made: exposures of PHA-activated HPBL to 60-Hz, 0.2-mT sinusoidal magnetic fields proved to be ineffective in modifying cell cycle progression rates (19). On the other hand, a 50-Hz sinusoidal magnetic field

with a 25-fold higher intensity, namely 5 mT, significantly increased the proliferation index of PHA-treated HPBL (20). Thus, the field response threshold for altering proliferation rates of HPBL should lie between 0.2 and 5 mT for power frequency (50/60-Hz) magnetic fields and two to three day continuous exposures (compare refs. 19 and 20 in Table 1). In contrast, for nonsinusoidal, ELF-pulsed magnetic fields no lower intensity response treshold has been identified yet: the applied peak magnetic flux densities, ranging from 2.5 to 10 mT, all appeared to be effective modulators of lymphocyte DNA synthesis as measured by ^{3}H-thymidine uptake in mitogen-activated lymphocytes (compare refs. 24-27, 31 in Table 1).

After reviewing the data in Tables 1 and 2, it becomes evident that a variety of important metabolic activities of immune cells can be affected by relatively weak EMFs. It is remarkable that bioactive EMF signals can differ considerably from each other in field intensity, frequency and wave form, and it is apparent that a simple, linear relationship between field exposure parameters and the magnitude or quality of an EMF response cannot be established (compare Table 1): for example, EMFs can act as inhibitors or stimulators of cellular activity, and the occurence of field effects on lymphocytes strongly depends on the biological status of the exposed cells (27, 29, 38) as well as on the applied EMF exposure parameters (e.g 31, 39) indicating the involvement of complex interaction mechanisms.

CALCIUM SIGNALLING AS A POSSIBLE TARGET OF ELECTROMAGNETIC FIELD ACTION IN IMMUNE CELLS

Electromagnetic Field Interactions With Cell Membrane-mediated Signal Transduction Events

Cells interact with their environment through the cell membrane. Among other functions the cell membrane is responsible for the detection and the subsequent transduction of external biochemical or other signals into the cytoplasmic space. The cell membrane is also considered the primary interaction site of EMF signals with cellular systems, and it has been proposed that an interference of an EMF with membrane-mediated signal detection, transduction or amplification processes may underly many of the biological field effects reported in the literature (2, 40). In particular the mobilization of cellular Ca^{2+} in response to external EMF signals or the interference of an EMF with Ca^{2+} regulatory processes is considered an important target of EMF action.

For lymphocytes, Ca^{2+} mobilization is among the earliest detectable events triggered upon binding of a ligand (e.g. antigen, receptor antibody, mitogenic lectin) to an appropiate receptor structure exposed on the outer cell surface. The cascade of cellular reactions in lymphoid cells subsequent to ligand-receptor interaction is best understood for T cells and has been reviewed extensively (e.g. 41). In short, ligand-induced Ca^{2+} mobilization is reflected by an initial rise in $[Ca^{2+}]_i$ caused by inositol 1,4,5-triphosphate-induced release of Ca^{2+} from

intracellular stores and followed by a sustained receptor-mediated Ca^{2+} influx from the extracellular medium. It is known that a perturbation of these events with chemical agents such as Ca^{2+} channel blockers, Ca^{2+}-specific ionophores, or by lowering the extracellular Ca^{2+} concentration using chelators, can lead to changes in Ca^{2+}-membrane fluxes and to subsequent modifications in cellular activity including cell proliferation, secretion, motility, or cytotoxicity. With regards to EMF effects on the immune system it is proposed here that Ca^{2+} regulation in lymphoid cells could be similarly affected by appropiate EMF signals thereby leading to the reported cellular EMF responses, e.g. on proliferation or cell-mediated cytotoxicity.

Early Evidence for Field Effects on Calcium Regulation in Nonlymphoid Systems

Historically, the effects of nonionizing, weak EMF signals on Ca^{2+} were first demonstrated using nonlymphoid tissues. The pioneering work by Bawin and Adey (42, 43) in the mid-1970s documented that Ca^{2+} efflux from chick brain can be measurably altered by nonthermal exposures to an ELF-modulated radio frequency carrier wave <u>in-vitro</u>; the unmodulated carrier wave was found to have no effect. This early finding was subsequently confirmed by Blackman and coworkers in an extensive series of experiments (e.g 44-46). During the past decade many other studies investigating the direct or indirect role of Ca^{2+} in biological field effects have followed (for reviews see refs. 1-

7). One important notion emerging from these experiments was that ELF signal frequencies below 100 Hz seemed to be most effective in modifying Ca^{2+} regulation, and in some cases field responses appeared to be frequency-specific. For example, EMF signals oscillating around a center frequency of 15 Hz were found to be more powerful modulators of Ca^{2+} fluxes than field signals of higher or lower frequency. An analogous phenomenon was described for the applied field intensity (42-46).

The establishment of strong experimental evidence for the existence of field effects on Ca^{2+} metabolism in different biological systems was probably the main contribution of these early studies. However, the task of determining the physiological significance of EMF-altered Ca^{2+} changes in the various systems is only beginning to be addressed by new research.

Current Evidence for the Role of Calcium in the Mediation of Electromagnetic Field Effects on Lymphocyte DNA Synthesis

Several lines of direct and indirect evidence suggest that EMF-altered Ca^{2+} regulation is an early trigger of field effects in cells of the immune system. First, the findings that correlate EMF-modified Ca^{2+}-mediated signal transduction events with EMF-altered lymphocyte DNA synthesis will be discussed. This is followed by an analysis of field effects in relationship to the kinetics of EMF influences on lymphocyte mitogenesis and on RNA transcription, lymphocyte-mediated cytotoxicity and cell viability within the context of Ca^{2+} homeostasis.

As outlined in the previous sections, evidence exists that weak ELF magnetic fields can modify Ca^{2+} regulation in lymphoid cells (for examples see Table 1). Taking into account the importance of Ca^{2+} in (co)regulating lymphocyte proliferation (see above), it is reasonable to hypothesize that EMF-altered Ca^{2+} regulation might be able to modify lymphocyte DNA synthesis. This view is supported by the results from a number of different experiments. For example, it was shown that EMF signals identical in intensity and pulse frequency to signals that inhibited PHA-induced DNA synthesis in HPBL (refs. 24, 26 in Table 1) also inhibited PHA-dependent Ca^{2+} uptake in these cells (24, 25). Furthermore for nonactivated, resting lymphocytes it was consistently found that Ca^{2+} uptake as well as DNA synthesis was unaffected by all the exposure protocols tested so far ($B \geq 0.1$ mT). In contrast, in mitogen-activated cells these same EMF signals proved to be effective modulators of both Ca^{2+} uptake and DNA synthesis (Table 1, refs. 24-27, 29, 31). These latter results strongly suggest that activation of transmembrane Ca^{2+} signalling is required in order to obtain field effects on both Ca^{2+} uptake and DNA synthesis (29).

Another set of data also points to a link between field-induced Ca^{2+} changes with cell proliferation: lymphocyte populations with a diminished ability to mobilize Ca^{2+} in response to mitogen were observed to be significantly more sensitive to EMF influences than cells displaying a normal mitogen response (29). Against this background, the results from studies investigating the effects of EMFs on mitogen- or

ionophore-induced DNA synthesis in HPBL are revealing: it was found that lymphocytes with an impaired responsiveness to mitogen-stimulation responded to the EMF influence more strongly than fully functioning cells (27). This observation is consistent with the above report on EMF-induced Ca^{2+} uptake in cells which are less responsive to Con A stimulation (29). Further, Ca^{2+}-ionophore (A23187)-induced ^{3}H-thymidine uptake in HPBL at suboptimal ionophore concentrations was stimulated several-fold ($p < 0.0001$) in the presence of a 3-Hz pulsed magnetic field (B_p = 5 mT) compared to nonexposed controls, whereas at optimal ionophore concentrations DNA synthesis was reduced by the magnetic field (38). One possible interpretation is that the suboptimal influx of Ca^{2+} was enhanced by the magnetic field to optimal levels; in contrast, a reduction in ionophore-induced DNA synthesis was caused by field perturbation of already optimal Ca^{2+} influx rates.

There are preliminary data from three laboratories using the classical Ca^{2+} channel blocker verapamil in lymphocyte field-exposure studies. If confirmed, these studies would suggest that verapamil can interfere with the effects of EMFs on Ca^{2+} uptake (24, 25), DNA synthesis (47) and the viability of HPBL (48). For example, a 12%-drop in the viability of HPBL after a 60-min exposure to a 100-mT, steady state magnetic field was found to be reversed in the presence of appropiate concentrations of verapamil in a dose-dependent manner, suggesting the involvement of altered Ca^{2+} membrane permeability in the mediation of EMF effects in lymphocytes (48). This must remain a tentative

conclusion, however, since it is unclear if the verapamil effects are indeed caused by directly affecting Ca^{2+}-membrane permeability, by an interaction with voltage-activated K^+ channels or possibly by nonspecific effects of the drug (e.g. 49).

Time Dependence of Lymphocyte DNA Synthesis and the Role of Calcium

If early magnetic field-induced changes in cellular Ca^{2+} homeostasis are indeed participating in the mediation of later stage biological effects such as lymphocyte replication, exposure times much shorter than the reported 66 to 72 hours for ELF-pulsed fields should be sufficient to alter DNA synthesis (Table 1). This would be expected from the known Ca^{2+} requirement of stimulated lymphocytes during early and late stages (> 12 h) of mitogenesis. Although the exact phases in the cell cycle which are Ca^{2+}-dependent have not been clearly defined yet, based on present knowledge it is safe to assume that lymphocyte proliferation is dependent on Ca^{2+} for the first 24 hours of mitogen-activated proliferation only (e.g. 50, 51). Thus, it is of interest to review the exposure time dependence of field-altered DNA synthesis in relationship to this Ca^{2+} requirement: Cadossi et al. (52) found that after a 24-h exposure of PHA-activated HPBL to a 75-Hz, pulsed magnetic field (B_p = 2 mT) the mitotic index of these cells was increased by 50% compared to nonexposed controls. Interestingly, no additional field effect on

the cell cycle was seen when the exposure period was extended up to 40 or even 72 h. In agreement with these findings is the observation that exposures of PHA-activated HPBL restricted to the first 24 h of the 72-h incubation period appeared to be as effective as subjecting the cells to the field influence for as long as 66 h (27). There is also information that an exposure time of only 6 h, but not 1 h, might be sufficient for modifying lymphocyte DNA synthesis (38). However, this result is in contrast to another study reporting that a 12-h exposure period was not effective under similar conditions (26). Finally, work studying the effects of 50-Hz, pulsed magnetic fields (B_p = 2.5 mT) on surface receptor expression revealed that an 18-h exposure period was long enough to enhance the number of Interleukin 2-receptors expressed on the cell surface of HPBL by 22% ($p < 0.001$) compared to controls (53).

In summary there is evidence that exposures of only 24 h, or in some cases even less, are capable of inducing field effects on DNA synthesis which are similar in magnitude compared to the effects from much longer exposure times. Thus, the hypothesis that EMFs interact with Ca^{2+} regulated processes during early and late phases in mitogenesis, thereby causing later-stage proliferative effects, appears to be in line with the observed kinetics of field influences on DNA synthesis. At the same time, however, it seems unlikely that an EMF effect on early (< 1 h) ligand-induced Ca^{2+} signals alone could be sufficient to modify lymphocyte replication to a similar extent.

The Possible Role of Calcium in the Mediation of Field Effects on RNA Transcription and Lymphocyte-mediated Cytotoxicity

With regards to the effects of magnetic fields on RNA transcrip. levels, field-induced alterations in cellular Ca^{2+} homeostasis might again be important (see refs. 28 and 32 in Table 1). For example, it is known that c-myc gene transcription in lymphoid cells can be enhanced 10 to 20-fold upon addition of an Ca^{2+} ionophore within one hour (54, 55). Thus, the rather modest 1.5 to 2-fold increase in c-myc transcript levels, measured after field exposures of HL-60 cells, a human promyelocytic leukemic cell line, for 20 min could be explained by a slight field-induced increase in $[Ca^{2+}]_i$ alone (ref. 28 in Table 1). This interpretation is substantiated by the results of Carson et al. (30) who found that a single 23-min exposure of HL-60 cells to weak magnetic fields resulted in a small, but significant ($p < 0.01$), rise in $[Ca^{2+}]_i$ by 34 ± 10 nM from a basal level of 121 ± 8 nM in the nonexposed control cells as measured by spectrofluorimetry (ref. 30 in Table 1).

EMF-triggered changes in $[Ca^{2+}]_i$ in target or cytotoxic effector cells after 24 or 48-h field exposures, respectively, could also be responsible for the described influence of 60-Hz, electric fields on lymphocyte-mediated cytotoxicity (refs. 36 and 37 in Table 2). The increased susceptibility of field-exposed target cells to natural killer (NK) cell-induced cytolysis could be caused by an elevated $[Ca^{2+}]_i$ in the target cells after the electric field exposure (36); sustained increases in $[Ca^{2+}]_i$ are

known to be an important contributing factor to achieving target cell lysis by NK cells (e.g. 56). Since lethal hit delivery by the effector cell is also a Ca^{2+}-dependent process and can be negatively affected by Ca^{2+} channel blockers or in the presence of Ca^{2+} chelators (e.g. 57), it is speculated that reduced lymphocyte cytotoxicity after 48-h exposure to 60-Hz electric fields (37), could again be caused by an EMF influence on Ca^{2+} regulated activity. However, direct evidence in support of a role for Ca^{2+} in triggering EMF effects on transcription or cytotoxicity is not available yet.

Summary of the Evidence and a Cautionary Note

The combined results suggest that the interference of an EMF with the regulation of cellular Ca^{2+} signals represents a plausible candidate for the mediation of EMF effects on cells of the immune system. The presented evidence includes correlations between EMF-induced Ca^{2+} changes and the cellular activation status, modifications in DNA synthesis, RNA transcription, cell-mediated cytotoxicity and cell viability. Furthermore, the kinetics of the EMF influence on DNA synthesis also seems to be consistent with a role for Ca^{2+} in elucidating field effects in lymphocytes. Currently, only very little can be said, however, about how an EMF acts to modify Ca^{2+} regulation at the molecular level: There are numerous possible pathways starting with an interference of the field with ligand-receptor binding as proposed by Chiabrera et al. (58). Theoretically many different steps in the signal

transduction cascade subsequent to ligand-receptor coupling, e.g. phosphorylation-dephosporylation events which act as effectors of selected intracellular responses, could be targets of the field action. There exist no data yet to exclude any of of these possible interactions. The work using the Ca^{2+} channel antagonist verapamil would indicate that EMF effects on Ca^{2+} channels could be important in eliciting cellular field responses (24, 25, 47, 48). Additionally, an EMF could alter the activity of the membrane-incorporated Ca^{2+}-ATPase which is responsible for pumping Ca^{2+} out of the cell. While this possibility has not been directly tested yet, there are data from two laboratories demonstrating that the activity of another membrane ion pump, Na^{+}/K^{+}-ATPase, can be influenced by low-frequency electric fields (59, 60). The lowest effective induced current density was 50 $\mu A/cm^2$ at an optimum field frequency of 100 Hz; by extrapolation of their measurements the authors estimated that a current density as low as 1 $\mu A/cm^2$ (E = 0.02 mV/cm under these conditions) would still be able to alter Na^{+}/K^{+}-ATPase activity in their <u>in-vitro</u> system (60).

It should be kept in mind that this effort only represents a first attempt to interpret field effects on the immune system within the framework of field-altered Ca^{2+} signalling. Obviously there are many open questions and only future research can clarify whether EMF-induced alterations in Ca^{2+} regulation are indeed causal in the chain of events leading to the observed field effects, or if Ca^{2+} changes represent effects which are only secondary to more fundamental field-induced molecular

changes. If this were the case, EMF-modified Ca^{2+} signals should only be considered a suitable marker for the early detection of EMF effects in cells of the immune system.

THE SEARCH FOR MECHANISMS: PHYSICAL CONSTRAINTS

Reports of biological effects of nonionizing EMFs have been neglected by biologists for many years (i) because it was widely assumed that nonthermal levels of EMFs are much too weak to be able to affect cellular activity in any significant manner, and (ii) because testable hypotheses which possibly could explain such field effects were missing. However, the careful documentation of selected biological EMF effects in independent laboratories has initiated a search for the underlying physical mechanisms. So far, however, no biophysical model can provide a satisfactory explanation of the reported field effects, although some theoretical progress has been made (i) in attempts to explain field effects on the basis of long-range, high-cooperativity phenomena in cells (for reviews see 2, 6, 8) or (ii) in modelling locally-restricted magnetic and electric field influences on ion interactions with cell membrane structures (e.g. 9, 61-64). Due to lack of space this discussion will only consider one approach which exclusively deals with local field effects at the cell membrane level for very weak electric fields: Weaver and Astumian (9) have recently examined the thermodynamic constraints that would be imposed on EMF-cell membrane interactions by the thermal noise associated with random membrane

fluctuations in a single cell. They calculated that an electric field intensity of about 1 mV/cm would be able to influence cells even in the absence of biological amplification mechanisms. Provided that field effects are frequency-specific and taking into account signal averaging, their computations predict that fields as weak as 0.01 to 0.001 mV/cm could still significantly affect membrane-associated proteins like receptors, enzymes or channels without violating the thermal noise limit. Although these calculations are based on a number of as yet unconfirmed assumptions, they still can give a rough estimate of field magnitudes that could be detectable by cells. Upon comparison of the computed values (E = 0.001 to 1 mV/cm) with the estimated electric field magnitudes present in the reviewed exposure experiments (E = 0.02 to 10 mV/cm; see Tables 1 and 2), it becomes clear that new theoretical developments are already beginning to bridge the gap between the experimentally observed EMF sensitivity of biological systems and physical constraints. It certainly will take many more years of intensive experimental and theoretical research, however, before any of the proposed physical interaction mechanisms can be verified.

CONCLUSIONS

This review has argued that there is now considerable evidence in support of the existence of low-frequency EMF effects in cells of the immune system (for examples see Tables 1 and 2) and that mechanisms involving Ca^{2+}-dependent signal transduction

mechanisms are probably participating in the mediation of weak field effects on immune cells. The fact that such field effects apparently do exist represents a remarkable discovery for immunology because it suggests that the immune system can be influenced by or respond to electrical currents and magnetic fields too weak in intensity to act through any thermal mechanism. A similar responsiveness to weak EMFs has also been documented for cells and tissues of the neuroendocrine and the musculoskeletal system indicating that EMF sensitivity might be a general property of biological systems (see refs. 1-7). The elucidation of the underlying cellular and molecular mechanisms which trigger such field responses represents a fascinating challenge to biologists and physicists alike. From the biologists' point of view the study of the role of Ca^{2+} signalling processes in the mediation of EMF effects in biological systems, including the immune system, clearly is a promising place to start. Furthermore, the observation that EMF signals can rather rapidly influence cellular Ca^{2+} regulation, in combination with recent advances in understanding the molecular details of Ca^{2+} signalling processes, establishes lymphoid cells as an excellent model system for the study of the fundamental field interaction mechanisms at the cellular and molecular level.

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Table 1: Low-frequency Magnetic Field Exposure Effects on Cells of the Immune System: Representative Reports From Ten Different Laboratories.

Description of Field Effect	Exposure Parameters				ref.
	f[a] [Hz]	B[b] [mT]	E[c] [mV/cm]	t[d] [h]	
Field Effects on Calcium Metabolism					
70% reduction ($p<0.001$) in PHA-dependent $^{45}Ca^{2+}$-uptake in HPBL. No effect in resting cells.	3.0 square[e]	6.0[f]	n.r.[g]	1.0	24, 25
170% increase ($p<0.01$) in Con A-dependent $^{45}Ca^{2+}$-uptake in rat thymic lymphocytes. No effect in resting cells.	60.0 sine	22.0	1.0 (16.0)[h]	1.0	29
28% increase ($p<0.01$) in cytosolic free Ca^{2+} concentration in HL-60 cells exposed to magnetic fields generated by an NMR unit.	0-100[i]	0.1	n.r.	0.38	30

Table 1 continues on next page

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Field Effects on RNA Transcription					
50-100% increase in c-myc, histone H2B, β-actin RNA-transcript levels in HL-60 cells.	60.0 sine	1.5	n.r.	0.3	28
30-50% increase in ^{3}H-uridine uptake in HL-60 cells.	60.0 sine	1.0	0.034	0.5	32
Field Effects on DNA Synthesis					
55% reduction ($p<0.01$) in ^{3}H-thymidine uptake in PHA-activated HPBL. No effects in resting cells.	3.0 square	6.0[f]	n.r.	72	24, 25
Up to 60% reduction ($p<0.001$) in ^{3}H-thymidine uptake PHA-activated HPBL. No effects in resting cells.	3.0 saw tooth	4.5[f]	0.23	72	26
No effect on the cell cycle progression of PHA-activated HPBL.	60.0 sine	0.2	n.r.	69	19

Table 1 continues on next page

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30-60% increase ($p<0.0001$) in ^{3}H-thymidine uptake in mitogen-activated HPBL depending on mitogen concentration and donor age. No effects in resting cells.	50.0 saw tooth	2.5[f]	0.02	66	27
8% increase ($p<0.0001$) in cell cycle progression of PHA-activated HPBL.	50.0 sine	5.0	n.r.	48	20
30% reduction ($p<0.01$) in ^{3}H-thymidine uptake in Con A-activated HPBL. No effects in resting cells.	50.0 saw tooth	10.0[f]	n.r.	72	31

see next page for footnotes to Table 1.

Footnotes to Table 1

[a] f, signal frequency

[b] B, rms magnetic flux density

[c] E, induced electric field intensity

[d] t, exposure duration

[e] description of wave form

[f] peak magnetic flux density

[g] n.r., not reported

[h] electric current density, J, [$\mu A/cm^2$]

[i] approximate range of the frequency components ($B \geq 0.1$ mT) generated by the NMR unit.

Table 2: Electric Field Effects From the Application of 60-Hz Sinusoidal Electric Currents via Electrodes (over agar-bridges) to Cultured Cells.

Description of Field Effect	Exposure Parameters			ref.
	J^a [$\mu A/cm^2$]	E^b [mV/cm]	T^c [h]	
No effect on cell cycle progression of PHA-activated HPBL.	30.0	2.4	69	19
Three-fold increase in ornithine decarboxylase activity in human lymphoma CEM cells.	160.0	10.0	1	35
53-73% increases in human natural killer cell-mediated cytolysis of field-exposed target cells.	30.0	n.r.[d]	24	36
19-25% reduction ($p<0.005$) of murine T-lymphocyte cytotoxicity (CTLL-1 cells). No effect on T-lymphocyte proliferatiom or viability.	n.r.	1-10.0	48	37

see next page for footnotes to Table 2

Footnotes to Table 2:

[a] J, rms electric current density

[b] E, rms electric field intensity

[c] t, exposure duration

[d] n.r., not reported

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