Electromagnetic fields may act via calcineurin inhibition to suppress immunity, thereby increasing risk for opportunistic infection: Conceivable mechanisms of action

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Immunosuppression  
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A B S T R A C T

While a good number of studies have demonstrated that modern, man-made ambient electromagnetic fields can have both stimulatory and inhibitory effect on immune system function, the precise mechanisms have yet to be completely elucidated. It is hypothesized here that, depending on the parameters, one of the means by which long-term electromagnetic field exposure has the potential to eventually lead to immunosuppression is via downstream inhibition of the enzyme calcineurin — a protein phosphatase, which activates the T-cells of the immune system and can be blocked by pharmaceutical agents. Calcineurin is the target of a class of pharmaceuticals called calcineurin inhibitors (e.g., cyclosporine, pimecrolimus and tacrolimus). When organ transplant recipients take such pharmaceuticals to prevent or suppress organ transplant rejection, one of the major side effects is immunosuppression leading to increased risk of opportunistic infection: e.g., fungal, viral (Epstein-Barr virus, cytomegalovirus), atypical bacterial (Nocardia, Listeria, mycobacterial, mycoplasma), and parasitic (e.g., toxoplasmosis) infections. Frequent anecdotal reports, as well as a number of scientific studies, have shown that electromagnetic field exposures may indeed produce the same effect: a weakened immune system leading to an increase in the same or similar opportunistic infections: i.e., fungal, viral, atypical bacterial, and parasitic infections.

Furthermore, numerous research studies have shown that man-made electromagnetic fields have the potential to open voltage-gated calcium channels, which can in turn produce a pathological increase of intracellular calcium, leading downstream to the pathological production of a series of reactive oxygen species. Finally, there are a number of research studies demonstrating the inhibition of calcineurin by a pathological production of reactive oxygen species. Hence, it is hypothesized here that exposures to electromagnetic fields have the potential to inhibit immune system response by means of an eventual pathological increase in the influx of calcium into the cytoplasm of the cell, which induces a pathological production of reactive oxygen species, which in turn can have an inhibitory effect on calcineurin. Calcineurin inhibition leads to immunosuppression, which in turn leads to a weakened immune system and an increase in opportunistic infection.

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Introduction

In the last thirty years we have seen a substantial increase in a number of disease states and functional impairments, many new or previously rare (e.g., autism spectrum disorder [ASD], chronic fatigue syndrome [CFS], attention deficit hyperactivity disorder [ADHD], etc.) [1–4]. Many of these tend not only to be coupled with a weakened immune system, but also to involve immune system disorders (e.g., allergies, food and chemical sensitivities, autoimmune disorders, etc.) (e.g. [5,6]). These disease states have paralleled major increases in ambient levels of man-made electromagnetic fields (EMFs) in our immediate environments. Furthermore, a number of experimental and epidemiological studies are continuing to link electromagnetic radiation (EMR) exposure with many of these disease states (e.g., [7–10]), their immune system dysfunctions, and their symptomologies.

Since calcium (Ca2+) is necessary for numerous enzymatic functions, a number of researchers have postulated that an EMF effect
may transpire via Ca2+ signaling transcription due to the influence of EMFs on Ca2+ cellular flux. While one such study by Manikonda et al. [11], for example, found that a 90-day extremely-low-frequency (ELF) 50 Hz magnetic field exposure (at 50 mT and 100 mT) induced an increase in calcineurin activity concomitant with an increase in intracellular Ca2+ ([Ca2+]i) levels in the hippocampal brain regions. Erkut et al. [12] found that with increasing radiofrequency (RF) EMF (1800 MHz) exposure duration (6, 12, and 24 h, respectively) of pregnant rats exposed for 20 days, "there was... an increased reduction in calcineurin activities" in both bone and muscle tissues of newborn rats. Hence, it is assumed here that, depending on the changing complexity of parameters (e.g., cell type; quality, duration, and intensity of exposure, etc.), EMFs can have a stimulatory, inhibitory, or no effect on intracellular calcineurin activity. It is further postulated here that while the influx of Ca2+ into the cell can initially have a stimulatory effect on the enzyme calcineurin, a pathological increase in Ca2+ can also stimulate the enzyme nitric oxide synthase to produce more nitric oxide (NO), leading to the production of peroxynitrite and other reactive oxygen species (ROS), which may have the downstream potential to inhibit the enzyme calcineurin.

An abundant number of studies have shown a substantial increase in ROS with EMF exposure. And while ROS, depending on concentration, can have both beneficial and deleterious effects, a recent review by Pall [13] has drawn attention to a process by which EMFs can induce the opening of voltage-gated calcium channels (VGCCs) in the plasma membrane via a change in its electric potential, triggering a pathological increase in [Ca2+]i via Ca2+ influx into cells (Fig. 1a). This in turn can trigger an increase in a number of ROS by means of a chain reaction initiated by the production of nitric oxide (NO) via Ca2+ stimulation of enzyme nitric oxide synthase (Figs. 1c–1f). Under normal circumstances, in a physiological context, one of the feedback-control mechanisms mediating Ca2+ entry into the cell through VGCCs is via Ca2+ binding proteins (one of which is the protein phosphatase enzyme, calcineurin), which help to control homeostasis of Ca2+ within the cell [14] (Fig. 1b). Nitric oxide is involved in a reverse feedback loop by which the enzyme guanylate cyclase is activated, inducing an increase in intracellular cyclic guanosine monophosphate (cGMP), which, in turn, again under normal circumstances, causes an inhibition of Ca2+ entry into the cell, thus decreasing intracellular concentrations [15]. However, with the advent of VGCC-opening EMFs into the equation, there is the real possibility that this feedback mechanism becomes impeded or stops working altogether, allowing the uncontrolled flow of Ca2+ into the cell.

Calcineurin, a serine-threonine phosphatase, found extensively in a variety of tissues with vital functions in neural, cardiac, skeletal, muscle, and immune cells, has not only been shown to play a central role in immunity, but it is also involved in a wide array of signaling pathways related to both cellular development and cell cycle progression.

Certain pharmaceutical agents known as calcineurin inhibitors, used mainly to prevent organ rejection of transplant recipients, also have an immunosuppressive side effect, known to lead to an increase in the following opportunistic infections: fungal/yeast (e.g., Cryptococcus neoformans); viral (esp. herpes-family viruses such as Epstein-Barr virus [EBV], cytomegalovirus [CMV]); atypical bacterial (e.g., mycoplasma, Nocardia, Listeria, mycobacteria); and parasitic (e.g., toxoplasmosis) infections [16–21].

Since ROS (e.g., superoxide [O2•–], nitric oxide [NO], hydrogen peroxide [H2O2], and ROS-like singlet oxygen) have also been shown to inhibit calcineurin activity [22–28], it is hypothesized here that one possible mechanism by which EMFs inhibit immune system function is via a downstream pathological increase in Ca2+...
Fig. 1b. Increased levels of intracellular calcium ions (Ca2+) activate calcineurin (CN).

Fig. 1c. Increased levels of intracellular calcium ions (Ca2+) stimulate nitric oxide synthase to produce nitric oxide (NO).
Fig. 1d. Electromagnetic fields (EMFs) can stimulate a pathological increase of calcium ions (Ca2+) within the cell.

Fig. 1e. A pathological increase in calcium ions (Ca2+) can lead to a pathological increase in nitric oxide (NO).
in the cytoplasm of the cell (Fig. 1d), inducing a pathological production of ROS [13] and also possibly singlet oxygen [29] (Fig. 1f), which in turn has an inhibitory effect on calcineurin [22–28] (Fig. 1g). One other possible mechanism could be an EMF-induced blockage of Ca2+ uptake by T-lymphocytes, which might also prevent the activation of calcineurin [30,31]. Either
way, calcineurin inhibition leads to immunosuppression, which in turn leads to a weakened immune system and an increase in opportunistic infections.

Calcineurin and T-lymphocytes

Calcineurin is a protein phosphatase enzyme, (named for its attraction to Ca2+ and affinity for neurons), which activates the T-lymphocytes involved in cell-mediated immunity (as opposed to humoral immunity). Calcineurin activated by Ca2+ thus generates nuclear factor of activated T-cells (NFAT), a transcription factor, via the process of dephosphorylation. The activated NFAT then moves into the nucleus binding to T-cell DNA stimulating production of interleukin 2 (IL-2), an important immune system cytokine. IL-2, in turn, enables the activation, proliferation, and differentiation of inactive or naive T-lymphocytes. (T-lymphocytes are one type of differentiated lymphocyte: the others being natural killer (NK) and B-cells. And it is important to note here that NK cells are also activated by IL-2 and thus would also be indirectly affected by calcineurin inhibition.) T-lymphocytes or T-cells are further differentiated into helper T-cell and cytotoxic T-cell subsets.

Calcineurin inhibitors

Calcineurin inhibitors are pharmaceutical agents (e.g., cyclosporine, tacrolimus, pimecrolimus) that act by inhibiting the function of calcineurin to produce NFAT, which in turn suppresses the ability of IL-2 to activate, differentiate, and proliferate T cells. They act pharmacologically as immunosuppressants in order to prevent or suppress organ rejection in transplant patients — and also to suppress inflammation in psoriasis and rheumatoid arthritis. Consequential side-effects of taking calcineurin inhibitors are (1) an increased risk of infection, especially opportunistic infections such as fungal, yeast, viral (Epstein-Barr virus [EBV], cytomegalovirus [CMV]), and atypical bacterial infection (Nocardia, Listeria, mycobacterial, mycoplasma), and (2) an increased risk of neoplasms.

There is evidence which also suggests that calcineurin inhibitors act via increases in oxidative stress. Research by Hong et al. [22] has shown that at least one calcineurin inhibitor, cyclosporine (CsA), acts via the generation of high levels of ROS. Further research by Moreno et al. [23] showing increased oxidative stress markers in transplant recipients taking calcineurin inhibitors (cyclosporine and tacrolimus) vs. healthy subjects lends further evidence to this hypothesis.

EMF-induced calcium flux/uptake blockage

Specific frequency windows, power densities, modulations, and cellular states

Numerous studies [32–57] demonstrate EMF-induced Ca2+ flux and/or its possible related effects (Table 1), with the more recent of

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<tr>
<td><strong>Selected research studies</strong></td>
<td><strong>EMF type</strong></td>
</tr>
<tr>
<td>Kaczmarek LK, Adey WR. Weak electric gradients change ionic and transmitter fluxes in cortex. (1974) [32]</td>
<td>Electric field</td>
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<tr>
<td>Bawin SM, Adey WR. Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. (1976) [33]</td>
<td>Weak sinusoidal electric fields</td>
</tr>
<tr>
<td>Blackman CF, et al. Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effects of modulation frequency and field strength. (1979) [34]</td>
<td>Modulated RF field</td>
</tr>
<tr>
<td>Joines WT, Blackman CF. Power density, field intensity, and carrier frequency determinants of RF-energy-induced calcium-ion efflux from brain tissue. (1980) [35]</td>
<td>Modulated RF field</td>
</tr>
<tr>
<td>Blackman CF, et al. Calcium-ion efflux from brain tissue: power-density versus internal field-intensity dependencies at 50-MHz RF radiation. (1980) [36]</td>
<td>Modulated RF field</td>
</tr>
<tr>
<td>Joines WT, Blackman CF. Equalizing the electric field intensity within chick brain immersed in buffer solution at different carrier frequencies. (1981) [37]</td>
<td>Modulated RF field</td>
</tr>
<tr>
<td>Joines WT, Blackman CF, Hollis MA. Broadening of the RF power-density window for calcium-ion efflux from brain tissue. (1981) [38]</td>
<td>Modulated RF field</td>
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<tr>
<td>Blackman et al. Effects of ELF fields on calcium-ion efflux from brain tissue in vitro. (1982) [39]</td>
<td>ELF field</td>
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<tr>
<td>Blackman et al. Effects of ELF (1–120 Hz) and modulated (50 Hz) RF fields on the efflux of calcium ions from brain tissue in vitro. (1985) [40]</td>
<td>ELF (1–120 Hz) field Modulated RF (50 Hz) field</td>
</tr>
<tr>
<td>Blackman et al. A role for the magnetic field in the radiation-induced efflux of calcium ions from brain tissue in vitro. (1985) [41]</td>
<td>Local Geomagnetic Field [LGF]</td>
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<th>Biological effect/Conclusion</th>
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<tr>
<td>Conti P, et al. A role for Ca2+ in the effect of very low frequency electromagnetic field on blastogenesis of human lymphocytes. (1985) [30]</td>
<td>Pulsed ELF field</td>
<td>3 Hz</td>
<td>6 mT</td>
<td>Effective frequencies could be rendered ineffective by altering the field strength whereas other ineffective frequencies could be made effective via the same means. Field exposure caused DNA synthesis and Ca2+ uptake inhibition in mitogen-stimulated thymocytes, the effect being seemingly synergistic with calcium blocker agent, verapamil.</td>
</tr>
<tr>
<td>Conti P, et al. Mitogen dose-dependent effect of weak pulsed electromagnetic field on lymphocyte blastogenesis. (1986) [31]</td>
<td>Pulsed ELF field</td>
<td>3 Hz</td>
<td>50 G</td>
<td>Field exposure induced intense inhibition of DNA synthesis with use of optimal doses of mitogens, while opposite effects were induced with suboptimal concentration of mitogens. “Hypothetical ordering of the frequency-response profile provides the basis for future experimental designs to test each possible interaction model and for their connection to the calcium-ion efflux endpoint.”</td>
</tr>
<tr>
<td>Blackman et al. Influence of electromagnetic fields on the efflux of calcium ions from brain tissue in vitro: a three-model analysis consistent with the frequency response up to 510 Hz. (1988) [42]</td>
<td>ELF Field</td>
<td>15 Hz</td>
<td>15 Vrms/m, 59 and 69 nTrms</td>
<td>The brains of chicks born of eggs exposed to either 50 or 60 Hz for a 21-day incubation period had differing Ca2+ efflux reactions, demonstrating that the “exposure of a developing organism to ambient power-line-frequency electric fields at levels typically found inside buildings can alter the response of brain tissue to field-induced calcium-ion efflux.” Exposure increased response of lymphocytes to suboptimal phytohemagglutinin (PHA) levels via the influx of Ca2+. Calcium blocker verapamil blocked PHA-stimulated Ca2+ influx while PEMF antagonized the verapamil block suggesting that the PEMF effect was on Ca2+ flux across the plasma membrane.</td>
</tr>
<tr>
<td>Blackman et al. Effect of ambient levels of power-line-frequency electric fields on a developing vertebrate. (1988) [43]</td>
<td>Sinusoidal electric fields</td>
<td>Either 50 or 60 Hz</td>
<td>Average intensity of 10 V rms/m</td>
<td>Calcium influx increased during mitogen-stimulated DNA synthesis with use of optimal doses of mitogens, while opposite effects were induced with suboptimal concentration of mitogens. “Hypothetical ordering of the frequency-response profile provides the basis for future experimental designs to test each possible interaction model and for their connection to the calcium-ion efflux endpoint.”</td>
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<tr>
<td>Cadoss R, et al. Lymphocytes and pulsing magnetic fields. (1988) [47]</td>
<td>Pulsed magnetic field</td>
<td>75 Hz</td>
<td>1 mV/m</td>
<td>Amplitude-modulated radiofrequency radiation can induce responses in cells of nervous tissue origin from widely different animal species, including humans. Amplitude-modulated radiofrequency radiation can induce responses in cells of nervous tissue origin from widely different animal species, including humans. Exposed neuroblastoma cells (NG108) (30 min) showed increased AChE activity.</td>
</tr>
<tr>
<td>Blackman et al. Multiple power-density windows and their possible origin. (1989) [44]</td>
<td>Modulated RF field</td>
<td>50 MHz</td>
<td>SAR of 0.05 and 0.005 W/kg</td>
<td>Calcium influx increased during mitogen ConA uptake in lymphocytes activated with the mitogen Concanavalin A (Con A) while having no effect on 45Ca2+ uptake in resting lymphocytes. Furthermore, thymocytes with a reduced capacity to mobilize Ca2+ in response to Con A were most affected by the magnetic field. Human polymorphonuclear leukocytes (PMNs) treated with the calcium channel antagonists diltiazem, nifedipine, and verapamil prior to being exposed to a magnetic field had no significant change in degranulation when compared to control and sham-exposed PMNs similarly treated. Calcium influx increased during mitogen-activated signal transduction in thymic lymphocytes exposed for 60 min.</td>
</tr>
<tr>
<td>Dutta SK, Ghosh B, Blackman CF. Radiofrequency radiation-induced calcium ion efflux enhancement from human and other neuroblastoma cells in culture. (1989) [45]</td>
<td>Modulated RF field</td>
<td>13–16 Hz and 57.5–60 Hz</td>
<td>Modulation ranges</td>
<td>Amplitude-modulated radiofrequency radiation can induce responses in cells of nervous tissue origin from widely different animal species, including humans. Amplitude-modulated radiofrequency radiation can induce responses in cells of nervous tissue origin from widely different animal species, including humans. Exposed neuroblastoma cells (NG108) (30 min) showed increased AChE activity.</td>
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<td>Dutta SK, et al. Dose dependence of acetylcholinesterase activity in neuroblastoma cells exposed to modulated radio-frequency electromagnetic radiation. (1992) [46]</td>
<td>Modulated RF field</td>
<td>147 MHz</td>
<td>SINUSOIDALLY MODULATED AT 16 Hz 60 Hz</td>
<td>Beta2 receptor antagonist (90) activation. Sinusoidal exposure increased the plasma membrane. Calcium influx increased during mitogen-ConA uptake in lymphocytes activated with the mitogen Concanavalin A (Con A) while having no effect on 45Ca2+ uptake in resting lymphocytes. Furthermore, thymocytes with a reduced capacity to mobilize Ca2+ in response to Con A were most affected by the magnetic field. Human polymorphonuclear leukocytes (PMNs) treated with the calcium channel antagonists diltiazem, nifedipine, and verapamil prior to being exposed to a magnetic field had no significant change in degranulation when compared to control and sham-exposed PMNs similarly treated. Calcium influx increased during mitogen-activated signal transduction in thymic lymphocytes exposed for 60 min.</td>
</tr>
<tr>
<td>Walleczek J, Liburdy RF. Nonthermal 60 Hz sinusoidal magnetic-field exposure enhances 45Ca2+ uptake in rat thymocytes: dependence on mitogen activation. (1990) [63]</td>
<td>Sinusoidal magnetic field</td>
<td>1.0 mV/cm</td>
<td>SINUSOIDALLY MODULATED AT 16 Hz 60 Hz</td>
<td>Calcium influx increased during mitogen-ConA uptake in lymphocytes activated with the mitogen Concanavalin A (Con A) while having no effect on 45Ca2+ uptake in resting lymphocytes. Furthermore, thymocytes with a reduced capacity to mobilize Ca2+ in response to Con A were most affected by the magnetic field. Human polymorphonuclear leukocytes (PMNs) treated with the calcium channel antagonists diltiazem, nifedipine, and verapamil prior to being exposed to a magnetic field had no significant change in degranulation when compared to control and sham-exposed PMNs similarly treated. Calcium influx increased during mitogen-activated signal transduction in thymic lymphocytes exposed for 60 min.</td>
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<tr>
<td>Papatheofanis FJ. Use of calcium channel antagonists as magnetoprotective agents. (1990) [48]</td>
<td>Static magnetic field</td>
<td>0.1 T</td>
<td>SINUSOIDALLY MODULATED AT 16 Hz 60 Hz</td>
<td>Calcium influx increased during mitogen-ConA uptake in lymphocytes activated with the mitogen Concanavalin A (Con A) while having no effect on 45Ca2+ uptake in resting lymphocytes. Furthermore, thymocytes with a reduced capacity to mobilize Ca2+ in response to Con A were most affected by the magnetic field. Human polymorphonuclear leukocytes (PMNs) treated with the calcium channel antagonists diltiazem, nifedipine, and verapamil prior to being exposed to a magnetic field had no significant change in degranulation when compared to control and sham-exposed PMNs similarly treated. Calcium influx increased during mitogen-activated signal transduction in thymic lymphocytes exposed for 60 min.</td>
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<tr>
<td>Liburdy RP. Calcium signaling in lymphocytes and ELF fields. Evidence for an electric field metric and a site of interaction involving the calcium ion channel. (1992) [49]</td>
<td>ELF field</td>
<td>60 Hz</td>
<td>22 mT 1.7 mV/cm</td>
<td>Various field effects were observed in response to a pulsed magnetic ELF field in lymphocytes: (1) stimulation, (2) inhibition, and (3) no effect. These were dependent on Ca2+ signal transduction activation, which was in turn dependent on lymphocyte responsiveness to the mitogen ConA. Furthermore, the magnitude of field response increased in line with increasing field flux densities.</td>
</tr>
<tr>
<td>Walleczek J, Budinger TF. Pulsed magnetic field effects on calcium signaling in lymphocytes: dependence on cell status and field intensity. (1992) [62]</td>
<td>Pulsed Magnetic ELF field</td>
<td>3 Hz</td>
<td>Bpeak = 6.5 mT, Emin = 0.69 mV/cm, Jmax = 2.6 microA/cm²</td>
<td>Various field effects were observed in response to a pulsed magnetic ELF field in lymphocytes: (1) stimulation, (2) inhibition, and (3) no effect. These were dependent on Ca2+ signal transduction activation, which was in turn dependent on lymphocyte responsiveness to the mitogen ConA. Furthermore, the magnitude of field response increased in line with increasing field flux densities.</td>
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<tr>
<td>Fitzsimmons RJ, et al. Combined magnetic fields increased net calcium flux in bone cells. (1994) [56]</td>
<td>Combined magnetic field (CMF)</td>
<td>Between 15.3 and 16.3 Hz</td>
<td>10(-5) V/m</td>
<td>Calcium influx increased in human osteoblast-like cells</td>
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these studies [47–57] implicating the EMF effect of opening of VGCCs as being responsible for this flux. These have predominantly shown not only an influx of Ca2+ with EMF exposures, but also that both calcium channel blockers and a reduction in extracellular Ca2+ can impede this effect. Furthermore, EMF stimulation of Ca2+ influx occurs dependent on specific frequency windows, specific power densities, specific modulations, (but not in others lying between them), and the specific biological parameters of the cell. A limited number of studies [30,31] have also shown blockage of Ca2+ uptake by cells — similar to the effect of the voltage-gated calcium channel (VGCC) blocker verapamil — along with inhibition of lymphocyte blastogenesis when a 3-Hz (6 mT) EMF was used. (It should be noted that with Ca2+ influx and efflux both processes are induced via VGCC activation; Ca2+ efflux has been studied via the loading of cells with radioactively labeled 45Ca2+. When VGCCs are activated raising intracellular Ca2+ levels via non-radioactive Ca2+ influx, 45Ca2+ efflux ensues.)

In summary, ELF EMFs can induce calcium influx via the opening of VGCCs at specific frequencies coupled with specific field strengths and not at others. A change in frequency can stimulate VGCC openings at different field strengths and vice versa: i.e., specific frequencies can be made ineffective with a change in field strength, while, conversely, also rendering previously ineffective signals effective with, for example, the same change in the field strength. Radiofrequency (RF) EMFs at specific frequencies can also cause Ca2+ influx via the opening of VGCCs, but at only a specific range of intensities and only when they are amplitude-modulated with low frequencies. While continuous wave ELFs have been shown to induce Ca2+ influx, unmodulated RF EMFs have so far shown no effect on Ca2+ influx. Furthermore, biological effects induced by Ca2+ influx are also dependent on the qualitative state of the cell (e.g., whether or not blastogenesis is taking place).

**EMF effect on immune system cells**

**T-lymphocytes and VGCCs**

While by 1985, calcium channels had yet to be uncovered in T-lymphocytes [58], it is now well established that T-lymphocyte activation is associated with both antigen receptor — or mitogen — stimulation and a continuous increase in free [Ca2+]i levels via heightened transmembrane Ca2+ influx via both VGCCs [59] and

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<th>Biological effect/Conclusion</th>
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<tr>
<td>Kenny JS, et al. Quantitative study of calcium uptake by tumorigenic bone (TE-85) and neuroblastoma x glioma (NG108-15) cells exposed to extremely-low-frequency (ELF) electric fields. (1997) [57]</td>
<td>Capacitively coupled electric fields (CCEF)</td>
<td>16 Hz</td>
<td>18.3 mV/cm</td>
<td>45Ca2+ uptake enhanced in tumorigenic bone (TE-85) and neuroblastoma x glioma (NG108-15) cells at frequency-specific 16 Hz also increasing TE-85 [Ca2+]i from 140 to 189–210 nM and NG108-15 [Ca2+]i from 67 to 189–210 nM.</td>
</tr>
<tr>
<td>Fanelli C, et al. Magnetic fields increase cell survival by inhibiting apoptosis via modulation of Ca2+ influx. (1999) [50]</td>
<td>Magnetic field</td>
<td>6 G</td>
<td>2 G</td>
<td>Magnetic fields were show to increase cell survival via the inhibition of apoptosis via their modulation of Ca2+ influx. A continuous 30 min. electric field applied to human hepatoma (Hep3B) cells prompted a fourfold increase in [Ca2+]i. This [Ca2+]i increase was inhibited by a depletion in extracellular Ca2+, and also partially by cation channel inhibitor GdCl3 or the nonspecific calcium channel blocker CoCl3, with simultaneous treatment of both GdCl3 and CoCl3 completely inhibiting the increase in [Ca2+]i.</td>
</tr>
<tr>
<td>Cho MR, et al. Transmembrane calcium influx induced by ac electric fields. (1999) [51]</td>
<td>Electric field</td>
<td>1 or 10 Hz</td>
<td>10 V/cm</td>
<td>Proliferation of human neuroblastoma IMR32 (+40%) and rat pituitary GH3 cells (+38%) was significantly enhanced by exposure to ELF EMFs. Furthermore, pumonicin- and H2(3)O(2)-induced apoptosis in IMR32 cells was also inhibited by field exposure. Ca2+ channel blockade counteracted the field exposure effects on both proliferation and apoptosis.</td>
</tr>
<tr>
<td>Grassi C, et al. Effects of 50 Hz electromagnetic fields on voltage-gated Ca2+ channels and their role in modulation of neuroendocrine cell proliferation and death. (2004) [52]</td>
<td>ELF</td>
<td>50 Hz</td>
<td>100 mV</td>
<td>Proliferation of human neuroblastoma IMR32 (+40%) and rat pituitary GH3 cells (+38%) was significantly enhanced by exposure to ELF EMFs. Furthermore, pumonicin- and H2(3)O(2)-induced apoptosis in IMR32 cells was also inhibited by field exposure. Ca2+ channel blockade counteracted the field exposure effects on both proliferation and apoptosis.</td>
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<td>Craviso GL, et al. Nanosecond electric pulses: a novel stimulus for triggering Ca2+ influx into chromaffin cells via voltage-gated Ca2+ channels (2011) [54]</td>
<td>Pulsed electric field</td>
<td>5 MV/m</td>
<td>1 Hz, 10 Hz, and 1 kHz.</td>
<td>A single 5 ns, high-voltage electric pulse exposure stimulated Ca2+ entry into bovine chromaffin cells via the opening of L-type voltage-gated Ca2+ channels (VGCC) while VGCC blockers inhibited this response. A single 5 ns pulse exposure induced Ca2+ influx and a rapid, transient increase in intracellular calcium concentration ([Ca2+]i) in chromaffin cells. A temperature sensitivity was also noted with a number of cellular responses.</td>
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<td>Morotomi-Yano K, Akiyama H, Yano K. Different involvement of extracellular calcium in two modes of cell death induced by nanosecond pulsed electric fields. (2014) [55]</td>
<td>Pulsed electric field</td>
<td>5 MV/m</td>
<td>1 Hz, 10 Hz, and 1 kHz.</td>
<td>Various cellular responses (e.g., Ca2+ influx and cell death) were induced by nanosecond pulsed electric fields (nsPEFs). Necrosis was enhanced with the presence of extracellular Ca2+ in HeLa S3 cells. The presence of a Ca2+ ionophore boosted necrosis while the absence of extracellular Ca2+ made HeLa S3 cells less susceptible to nsPEFs. It was concluded that cell death induced by nsPEFs is cell-type dependent and that extracellular Ca2+ is essential for nsPEF-induced necrosis.</td>
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also a non-voltage-gated calcium channel (nVGCC) influx [60,61], the latter of which is most likely opening in response to T-cell activating mitogens, and facilitated by inositol trisphosphate (InsP3) — a secondary messenger molecule used in signal transduction and lipid signaling.

**Qualitatively different field responses: inhibition, stimulation or no field effect**

Walleczek and Budinger [62], in investigating the effects of pulsed magnetic fields on Ca2+ signaling in T-lymphocytes, found that magnetic fields at 3 Hz inhibited Ca2+ uptake (i.e., influx) in mitogen-stimulated thymocytes (immature T-lymphocytes) and that this inhibition increased with increasing magnetic flux densities, noting that variations in both physical field exposure characteristics as well as biological parameters (e.g., the state of the cell) can determine the outcome of cellular EMF exposure experiments. Inhibition, stimulation, or no field effect can be observed in direct dependence on physical and biological boundary conditions [62].

This is in line with the Conti et al. [28] experiment also applying a 3-Hz (6-mT) pulsed magnetic field on mitogen-dependent human lymphocytes and finding a reduction in uptake of Ca2+.

In an earlier study, however, Walleczek and Liburdy [63], this time looking at 60-Hz sinusoidal magnetic field (22-mT) effect on thymocytes, found that Ca2+ uptake (i.e., influx) under the same experimental conditions was enhanced, and not reduced as with the case of the 3-Hz model, further highlighting the fact that varying EMF frequency differentials can induce field responses that are qualitatively different. This is in line with the conclusion of Blackman et al. [41] that there are specific frequency windows that can change in accordance with changes in power density.

Over the past 35 years, a large number of papers [64–108] have addressed effects — often contradictory — on immune system function with exposures to EMF of both extremely low frequency (ELF) and modulated radiofrequency (RF). Outcomes have been both stimulatory and inhibitory, with both pathological and — some — potentially therapeutic effects. Contradictory EMF effect can be attributed to the fact that the immune system will show varied responses based on the interactions of a number of parameters: (1) EMF exposure quality, (2) EMF exposure duration, (3) EMF exposure frequency, (4) EMF exposure power density, and (5) the specific state of the cell. And this is seemingly closely related to changes in Ca2+ ion flux.

Based on accumulated evidence showing that nonthermal exposure of ELF-EMF can elicit changes in immune cells, in 1992, Walleczek [64] had hypothesized that EMFs indirectly affect the immune system via cell membrane-mediated Ca2+ signaling processes. Eichwald and Walleczek [65], in 1996, further noting that in studies of immune system cellular Ca2+ -uptake regulation, depending on the degree of cellular activation, various field effects — stimulatory, inhibitory, or no field effect — have been observed under identical field parameters. They hypothesized that cellular biochemical stimulation results in specific signaling pathway activation, which regulates cellular Ca2+ dynamics (the release of Ca2+ from [Ca2+]i stores and capacitative Ca2+ entry), hypothesizing that a specific EMF-sensitive enzyme may be activating a feedback control loop on signaling processes, which in turn would modulate entry of Ca2+ into the cell, and affect other Ca2+ -dependent cellular processes (e.g., DNA synthesis). More recent studies, detailed in a review by Nejabakhsh and Feng [14], have established that this is indeed the case. Nejabakhsh and Feng state that calcium ion entry through voltage-gated calcium channels is essential for cellular signaling in a wide variety of cells and multiple physiological processes. Perturbations of voltage-gated calcium channel function can lead to pathophysiological consequences. Calcium binding proteins serve as calcium sensors and regulate the calcium channel properties via feedback mechanisms [14].

Pall [13], in a review of the literature, has described how EMFs may produce — what is usually — a pathological increase in [Ca2+]i via the opening of VGCCs that trigger an influx of Ca2+ from extracellular medium, leading downstream to the production of nitric oxide and peroxynitrite and consequent oxidative stress, and further that a wide range of these EMF effects have been shown to be blocked by calcium channel blockers, concluding that “voltage-gated calcium channels are essential to the responses produced by extremely low frequency (including 50/60 Hz) EMFs and also to microwave frequency range EMFs, nanosecond EMF pulses, and static electrical and magnetic fields [13].”

**EMFs and ROS**

Over the past 10 years there has been an exponential increase in studies [109–211] showing increased ROS activity with EMF exposure at power density levels more in line with what people are now receiving in their daily lives. Increased intracellular ROS activity has been found to increase lipid peroxidation and protein oxidation, impair antioxidant enzyme function, and is correlated with DNA damage, reduced cell membrane integrity, and reduced mitochondrial function. The majority of these studies show increased levels in markers for oxidative stress, e.g., malondialdehyde (MDA) and nitric oxide (NO), and decreases in levels of the antioxidant enzymes, e.g., superoxide dismutase (SOD), glutathione peroxidase (GSH-px), and catalase (CAT) — with a number of studies showing a protective and ameliorative effect of these induced by certain antioxidant nutritional substances like Ginkgo biloba, caffeic acid phenethyl ester (CAPE), garlic, ginseng, lotus seedpod procyanidins (LSPCs), β-glucan, L-carnitine, melatonin, vitamin E, C, zinc, and selenium. In a recent review by Yakymenko et al. [211] (and the only one of its kind at present), 100 peer-reviewed studies were surveyed examining the oxidative effects of low-intensity RF EMF with 93 confirming RF-EMF-induced oxidative stress in biological systems.

**ROS and calcineurin inhibition**

Sommer et al. [24] found that the ROS — superoxide (O2—), nitric oxide (NO), and hydrogen peroxide (H2O2) — all had a potent inhibitory effect on calcineurin, concluding that ROS and reactive nitrogen species (RNS) have an inhibitory effect on calcineurin via the oxidation of both catalytic metals and critical thiol.

Lee et al. [25], in noting a correlation in increased ROS with calcineurin inactivity, describe the inactivation of calcineurin by hydrogen peroxide triggering the proteolytic cleavage and decreased enzymatic activity of calcineurin, with the cleaved form of calcineurin having no enzymatic ability to dephosphorylate NFATc.

Smit et al. [212] found — in noting similarities in the immunosuppressive effect of UV radiation with calcineurin inhibitors, when they investigated the UV effect on calcineurin in skin — a significant reduction in calcineurin activity in skin with exposure to UV radiation, along with a reduction in production of cytokines IL-2, gamma-interferon, IL-4, and IL-10, all controlled by the Ca2+ —calcineurin pathway — indicating that UV radiation can result in calcineurin inactivation in skin, suppressing skin immunity in a manner similar to calcineurin inhibitors. Musson et al. [26], in noting that calcineurin is a target of modulation by ROS, were able to show, both in vivo and in vitro, that UVA1 radiation suppresses calcineurin activity via the production of singlet oxygen and superoxide. Musson et al. [27], also further emphasizing calcineurin’s documented sensitivity to oxidative stress, assessed and contrasted the influences of UVA1 and arsenite on calcineurin, showing that these two agents had a strong inhibitory effect on calcineurin activity in Jurkat and skin cells with a reduction in
NFAT nuclear translocation in Jurkat cells, and that these effects could be partially ameliorated with an increase in the dismutation of superoxide in Jurkat and skin cells.

**EMFs and immune system dysfunction/ suppression**

While a diverse number of studies [66–108] show nonthermal EMF effect on the immune system function, many of these studies find effect with short-term exposure to EMFs at power densities many times more powerful than we would at present expect to encounter in our everyday lives. Notwithstanding, it has been proposed that long-term low-level non-ionizing radiation exposures may be equivalent to short-term high-intensity ionizing radiation exposures [101], and similarly that long-term low-level non-ionizing radiation effects to what are considered “low” power densities can also be equivalent to effects produced by short-term high-intensity non-ionizing radiation exposures at what are considered “high” power densities [102]. In other words, many of the biological effects produced in many of these short-term exposure conditions using power densities at levels higher than we would normally experience in everyday life may very well be mimicked by present-day long-term exposures to lower power densities many of us are currently encountering.

Experimental immunological research studies have demonstrated that nonthermal EMF exposures can affect immune-specific organs [66–70], innate and adaptive immune cell activity [30,31,68,69,71–73,77–79], antibodies [69,82,83], cytokines [87–89,95], and enzymes [65,131], with other research studies showing effects on the autoimmune system [86,91], cellular CA2+ uptake [30,31], augmentation of chemical toxicity [84], food sensitivities [92], and increased free-radical production [109–211].

The same opportunistic infections induced as side effects in transplant recipients taking immunosuppressant calcineurin inhibitors to prevent organ rejection — reactivated viral (EVB, CMV, Coxsackie, etc.), candida, mold, bacterial (mycoplasma), and parasitic (toxoplasmosis) infections — have also been uncovered in a number of what have been described as neuro-immune dysfunction syndromes (NIDS) [213]: e.g., CFS, ASD, ADHD, and Alzheimer's disease [AD]. [214–264] etc.

For example, Nicholson and Haier [214,215] have uncovered various systemic and central nervous system bacterial and viral infections (e.g., Mycoplasma species, Chlamydia pneumonia, Borrelia burgdorferi, human herpes virus-1, -6, and -7, and other bacterial and viral infections) in patients with atypical mycotic lateral sclerosis (ALS), multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), autism spectrum disorders (ASD), a number of psychiatric disorders (paranoia, dementia, schizophrenia, bipolar disorder, panic attacks, major depression, anorexia nervosa, and obsessive-compulsive disorder), neuropsychiatric movement disorders (e.g., Giles de la Tourette's syndrome, autoimmune diseases (Guillain-Barré syndrome, paediatric autoimmune neuropsychiatric disorders associated with Streptococci (“PANDAS”)), fatiguing illnesses (CFS/myalgic encephalomyelitis [ME]), Gulf War Syndrome, and Lyme disease — with many of these showing cytokine changes, increases in ROS with its resulting oxidative stress, changes in neurotransmitter levels, and decreased NK cell activity.

Many of these mysterious disease states of unknown etiology have increased exponentially in the last thirty years in proportion with increases in ambient man-made electromagnetic fields. And with this correlation, the question “Is there causation?” begs to be asked. There is more research that suggests there is. For example, Grimaldi et al. [104] found that a 50-Hz EMF exposure could activate the Epstein-Barr virus genome in latently infected human lymphoid cells, and Canseven et al. [105] further found that a 50-Hz magnetic field could suppress NK cell activity on candida stel-
can occur with everyday wireless (e.g., cell phone and WiFi) radiation exposures.

The ROS nitric oxide, superoxide, hydrogen peroxide, and singlet oxygen have been shown in a number of studies to inhibit calcineurin. EMFs have been shown to induce an intracellular increase in nitric oxide [112,118,121,132,144,181] and changes in superoxide dismutase [109,121,126,132,133,144,158,163,194,198,208], the latter strongly suggesting an increase in superoxide. Both nitric oxide and superoxide are known calcineurin inhibitors. Furthermore, superoxide can dismutate into hydrogen peroxide, another known calcineurin inhibitor. Finally, there is evidence to suggest that nitric oxide and hydrogen peroxide react to produce singlet oxygen [29]. Hence, it is hypothesized that EMFs have the potential to indirectly inhibit calcineurin via the downstream production of any one or more of the following ROS or ROS-like molecules — nitric oxide, superoxide, hydrogen peroxide, and singlet oxygen — as a result of a pathological influx of Ca2+ into the cell.

Electromagnetic fields have been shown to induce either stimulatory, inhibitory, or no effect on the immune system in relationship to how specific EMF frequencies, specific EMF power densities, and specific EMF durations interact with specific field parameters of the cell — especially with regard to either Ca2+ flux into or blockage of Ca2+ uptake by the cell. While the research seems to suggest that at specific frequencies and certain power densities, nonthermal EMF exposures can have an initially stimulating effect on the immune system, at higher power densities and/or with longer durations, this effect can be or become inhibitory — most likely due to increases in intracellular ROS and singlet oxygen, with the research also suggesting certain antioxidant substances ameliorating these effects.

Johansson [282], in a recent review of the literature, concluded that EMFs disturb immune function through stimulation of various allergic and inflammatory responses, as well as having effects on tissue repair processes, which may potentially lead to an underlying cause for cellular damage and tissue repair reduction. Such disturbances increase the risks for various diseases and functional impairments, including cancer and electrohypersensitivity (EHS).

Besides leaving the organism open to opportunistic infection, calcineurin inhibition has been correlated with an increase in neoplasms and skin cancer — and it has also been shown that UV radiation can inhibit calcineurin via the production of singlet oxygen and superoxide [26]. The fact that a number of ROS and singlet oxygen can be produced via means other than UV radiation — i.e., downstream effects of pathological increases in intracellular Ca2+ levels [e.g. 29] — supports the hypothesis that these can also inhibit calcineurin. The hypothesis that EMFs also inhibit calcineurin via the production of ROS furthermore adds support to research by Hallberg and Johansson [283–286], finding increases in skin cancers in correlation with increasing exposures to ambient man-made EMFs.

In summary, there has been a significant rise in a number of disease states (autism spectrum disorder, chronic fatigue syndrome, etc.) over the last thirty years, and these have coupled increases in levels of artificial electromagnetic pollution. Many of these disease states are paired with a weakened immune system, not to mention immune system disorders. Moreover, an increasing number of experimental and epidemiological studies are connecting many of these disease states, and their symptomologies, with electromagnetic radiation exposures. Calcium (Ca2+) is necessary for a number of enzymatic functions, and numerous researchers have proposed an EMF effect due to Ca2+ influx and Ca2+ signaling transcription. Two studies to date have found an EMF effect on calcineurin: one finding ELF to have a stimulatory effect and the other finding RF to have an inhibitory effect. While this paper proposes that depending on the complexity of parameters, EMF exposure can have stimulatory, inhibitory, or no effect on calcineurin activity, it also proposes that a pathophysiological increase in intracellular Ca2+ levels due to EMF activation of VGCCs can stimulate the production of ROS, which may lead downstream to calcineurin inhibition and a weakened immunity — as ROS have been shown in a number of studies to inhibit calcineurin. The infections induced by patients taking calcineurin inhibitors have interestingly also been found in a number of disease states termed neuro-immune system disorders (NIDS), (e.g., CFS, ASD, ADHD, and AD).

In conclusion, this hypothesis proposes that one means by which man-made electromagnetic fields have the potential to disturb immune system homeostasis is by opening VGCCs (Fig. 1a) and pathologically increasing intracellular Ca2+ levels (Fig. 1d), leading to pathological intracellular levels of ROS (Figs. 1e and 1f), which in turn have the potential to inhibit calcineurin (Fig. 1g), leaving the body increasingly susceptible to opportunistic infection. Given this possibility, while it can be said that candida, mold, mycoplasma, EBV, CMV, and other opportunistic pathogens are certainly part of the etiological conundrum of many unexplained disease states, perhaps the root of the problem may have more to do with an overlooked invisible environmental factor potentially weakening immune systems and making many more susceptible to these pathogens. While there is much research showing that EMFs alter immune system function, original experimental research demonstrating whether or not long-term low-level EMF exposure may indeed lead to immunosuppression and increase in opportunistic infection is required. Furthermore, original experimental research needs to be undertaken to determine for certain whether or not long-term exposure to EMFs (especially RF) can effect a decrease in calcineurin levels within immune cells, as was found with calcineurin in muscle and bone cells exposed to RF EMFs. This would determine a clear mechanism by which EMF exposure can in fact lead to immunosuppression.

Conflict of interest statement

The authors confirm that there are no conflicts of interest.

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