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Disturbance of the immune system by electromagnetic fields—A potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment

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Abstract

A number of papers dealing with the effects of modern, man-made electromagnetic fields (EMFs) on the immune system are summarized in the present review. EMFs disturb immune function through stimulation of various allergic and inflammatory responses, as well as effects on tissue repair processes. Such disturbances increase the risks for various diseases, including cancer. These and the EMF effects on other biological processes (e.g. DNA damage, neurological effects, etc.) are now widely reported to occur at exposure levels significantly below most current national and international safety limits. Obviously, biologically based exposure standards are needed to prevent disruption of normal body processes and potential adverse health effects of chronic exposure.

Based on this review, as well as the reviews in the recent Bioinitiative Report [http://www.bioinitiative.org/] [C.F. Blackman, M. Blank, M. Kundi, C. Sage, D.O. Carpenter, Z. Davanipour, D. Gee, L. Hardell, O. Johansson, H. Lai, K.H. Mild, A. Sage, E.L. Sobel, Z. Xu, G. Chen, The Bioinitiative Report—A Rationale for a Biologically-based Public Exposure Standard for Electromagnetic Fields (ELF and RF), 2007)], it must be concluded that the existing public safety limits are inadequate to protect public health, and that new public safety limits, as well as limits on further deployment of untested technologies, are warranted.

Keywords: Immunology; Radiofrequency fields; Magnetic fields; Power-frequency

1. Introduction

Around the world, for a number of years, there has been an active debate involving the general public, scientists, journalists, politicians, and people from the electric power and telecom companies, all trying to answer the basic question: Is biology compatible with the ever-increasing levels of electromagnetic fields (EMFs)? Or, to put it in more layman's terms: Can we, as human beings, survive all the radiation? Are we built for a 24-h, whole-body irradiation life? Are we immune to these signals, or are we actually playing with our planet's future, putting life at stake? The answers appear to be: *No, we are not designed for such EMF exposure loads. We are not immune. We are gambling with our future.*

Very often the biggest threat from EMF exposure is said to be cancer. However, this is not the most horrifying scenario. Just imagine if some basic *and general* molecular and/or cellular mechanism were altered. For instance, imagine if one morning the nitrogen-binding bacteria in the soil or the honey bees in the air had been destroyed beyond repair. Or, as this paper will indicate, imagine if our immune system, trying to cope with the ever-increasing electromagnetic signals, finally could not do so any longer!

Is the immune system designed to deal with "allergens" never present before, but now being invented, manufactured and used? Is it likely that our immune system, by some enormously intelligent 'glitch' in the evolutionary process has that capacity? Is that even remotely likely? *Of course, not.*

The recommended safe exposure levels have not taken this into account, since the existing standards are only based on the immediate heating of cells and tissues [most often evaluated in fluid-filled plastic dolls!]. They certainly do not take into consideration long-term effects or non-thermal effects that occur before heating can be detected. Furthermore, the recommendations do not take into account all available sci-

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entific reports. *The recommended exposure levels are not in any sense safe levels and are entirely inadequate.*

2. Basic concepts and components of the immune system

The human immune system is part of a general defense barrier towards our surrounding environment. We live in a biological system, the world, dominated by various microorganisms, including microbes and viruses, many of which can cause harm. The immune system serves as the primary line of defense against invasion by such microbes. As we are, practically speaking, built as a tube, the outer surface – the skin – and the innermost surface – the gastrointestinal tract – are the major borders between us and the outside world. These borders must be guarded, protected and constantly repaired since any damage to them could be fatal. In addition to these major borders there are number of other organ/tissue interfaces at which cellular conduct is monitored, evaluated and dealt with 24 h around the clock. Damage that is not detected and properly repaired in time can develop into cancer; something well known for ultraviolet light overexposure.

The skin and the mucous membranes are part of the innate or non-adaptive immune system. However, if these barriers are broken (e.g. after cutting a finger), then microbes, including potential pathogens (i.e. harmful microbes) can enter the body and begin to multiply rapidly in the warm, moist, nutrient-rich environment. The cut may not be as abrupt as a knife cut, it could also very well be an internal leakage, such as the one found after microwave exposure of the fragile blood–brain barrier [2]. Such a leakage could indeed be fatal, causing nerve cell damage and followed by cellular death [3].

One of the first cell types encountered by a foreign organism after a cut in the skin is the phagocytic white blood cell. These cells congregate within minutes and begin to attack the invading foreign microbes. The next cell type to be found in the area of such a local infection will be the so-called neutrophils. They are also phagocytic and use pattern-recognizing surface receptor molecules to detect structures commonly found on the surface of bacteria. As a result, these bacteria – as well as other forms of particulate materials – will be ingested and degraded by the neutrophils. Various other protein components of serum, including the complement components may bind to the invader organisms and facilitate their phagocytosis, thereby further limiting the source of infection/disease. Other small molecules, the interferons, mediate an early response to viral infection by the innate system.

The innate immune system is often sufficient to destroy invading microbes. If it fails to clear an infection, it will rapidly activate the adaptive or acquired immune response, which – as a consequence – takes over. The molecular messenger connection between the innate and the adaptive systems are molecules known as cytokines. (The interferons are part of this molecular family.)

The first cells in this cellular orchestra to be activated are the T- and B-lymphocytes. These cells are normally at rest and are only recruited when needed, i.e. when encountering a foreign (=non-self) entity referred to as an antigen. The T- and B-lymphocytes, together with a wide spectrum of other cell types, have antigen receptors or antigen-recognizing molecules on their surface. Among them you find the classical antibodies (=B-cell antigen receptors), T-cell antigen receptors as well as the specific protein products of special genetic regions (=the major histocompatibility complexes). The genes of humans are referred to as human leukocyte antigen (HLA) genes and their protein products as HLA molecules. The antibodies – apart from being B-cell surface receptors – are also found as soluble antigen-recognizing molecules in the blood (immunoglobulins). The adaptive immune response is very highly effective but rather slow; it can take 7–10 days to mobilize completely. It has a very effective pathogen (non-self) recognition mechanism, a molecular memory and can improve its production of pathogen-recognition molecules during the response.

A particularly interesting set of cells are the various dendritic cells of the skin as well as elsewhere. In the outermost cutaneous portion, the epidermis, you find both dendritic melanocytes, the cells responsible for the pigment-production, as well as the Langerhans cells with their antigen-presenting capacity. In the deeper layer, the dermis, you find corresponding cells, as well as the basophilic mast cells, often showing a distinct dendritic appearance using proper markers such as chymase, tryptase or histamine. All these cells are the classical reactors to external radiation, such as radioactivity, X-rays and UV light. For that reason, our demonstration [4] of a high-to-very high number of somatostatin-immunoreactive dendritic cells in the skin of persons with the functional impairment electrohypersensitivity is of the greatest importance. Also, the alterations found in the mast cell population of normal healthy volunteers exposed in front of ordinary household TVs and computer screens [5] are intriguing, as are the significantly increased number of serotonin-positive mast cells in the skin ($p < 0.05$) and neuropeptide tyrosine (NPY)-containing nerve fibers in the thyroid ($p < 0.01$) of rats exposed to extremely low-frequency electromagnetic fields (ELF-EMF) compared to controls. This indicates a direct EMF effect on skin and thyroid vasculature [[6–8]; for further details and refs., see below]. In the gastrointestinal tract, you will find corresponding types of cells guarding our interior lining against the outside world.

The immune system can react in an excessive manner and it can cause damage to the local tissue as well as generally to the entire body. Such events are called hypersensitivity reactions and they occur in response to three different types of antigens: (a) infectious agents, (b) environmental disturbances, and (c) self-antigens. The second one is, as you will

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see, of utmost importance when we discuss the impact of the new electromagnetic fields of today's world.

For environmental substances to trigger hypersensitivity reactions, they must be fairly small in order to gain access to the immune system. Dust triggers a range of responses because the particles are able to enter the lower extremities of the respiratory tract, an area that is rich in adaptive immune-response cells. These dust particles can mimic parasites and may stimulate an antibody response. If the dominant antibody is IgE, the particles may subsequently trigger immediate hypersensitivity, which is manifest as allergies, such as asthma or rhinitis. If the dust stimulates IgG antibodies, it may trigger a different kind of hypersensitivity, e.g. farmer's lung [9].

Smaller molecules sometimes diffuse into the skin and these may act as haptens, triggering a delayed hypersensitivity reaction. This is the basis of contact dermatitis caused by nickel [9].

Drugs administered orally, by injection or onto the surface of the body can elicit hypersensitivity reactions mediated by IgE or IgG antibodies or by T-cells. Immunologically mediated hypersensitivity reactions to drugs are very common and even very tiny doses of drugs can trigger life-threatening reactions. These are well classified as idiosyncratic adverse drug reactions.

In this respect, electromagnetic fields could be said to fulfil the most important demand: they penetrate the entire body.

The hypersensitivity classification system was first described by Coombs and Gell [cf. ref. 10]. The system classifies the different types of hypersensitivity reaction by the types of immune responses involved. Hypersensitivity reactions are reliant on the adaptive immune system. Prior exposure to antigen is required to prime the adaptive immune response to produce IgE (type I), IgG (type II and III) or T-cells (type IV). Because prior exposure is required, hypersensitivity reactions do not take place when an individual is first exposed to antigen. In each type of hypersensitivity reaction the damage is caused by different adaptive and innate systems, each of which has its respective role in clearing infections.

In essence, the immune system is a very complex one, built up of a large number of cell types (B- and T-lymphocytes, macrophages, natural killer cells, mast cells, Langerhans cells, etc.) with certain basic defense strategies. It has evolved during an enormously long time-span and is constructed to deal with its known enemies. *Among the known enemies one will not find modern electromagnetic fields, such as power-frequency electric and magnetic fields, radiowaves, TV signals, mobile phone or WiFi microwaves, radar signals, X-rays or artificial radioactivity.* They have been introduced during the last 100 years, in many cases during the very last decades. They are an entirely new form of exposure and could pose to be a biological "terrorist army" against which there are no working defences. They penetrate the body, and some have already proven to be fatal. Today no-one would consider having a radioactive wrist watch with glowing digits (as you could in the 1950s), having your children's shoes fitted in a strong X-ray machine (as you could in the 1940s), keeping radium in open trays on your desk (as scientists could in the 1930s), or X-raying each other at your garden party (as physicians did in the 1920s). In retrospect, that was just plain madness. However, the persons doing so and selling these gadgets were not misinformed or less intelligent. The knowledge at the time was deficient, as was a competent risk analysis coupled to a parallel analysis of public needs.

3. Electromagnetic fields – now and previously

The electromagnetic spectrum covers a broad range of frequencies (over 20 orders of magnitude), from low frequencies in electricity supplies, radiowaves and microwaves, infrared and visible light, to X-rays, radioactivity and cosmic rays. Electromagnetic fields are present everywhere in our environment, and except for the visible spectrum, they are invisible to the human eye.

An electromagnetic field consists of an electrical part and a magnetic part. The electrical part is produced by a voltage gradient and is measured in volts/metre. The magnetic part is generated by any flow of current and is measured in Tesla. Magnetic fields as low as around 0.2 μT (a millionth of a Tesla) can produce biological effects. For comparison, using a mobile (cell) phone or a PDA exposes you to magnetic pulses that peak at several tens of microTesla [11,12], which is well over the minimum needed to give harmful effects. Because mobile phones and other wireless gadgets are held close to the body and are used frequently, these devices are potentially the most dangerous sources of electromagnetic radiation that the average person possesses.

Even the extremely low frequencies (ELF) that are widely used in powerlines and domestic appliances should be viewed with caution. In June 2007, the World Health Organization (WHO) pointed out that they are believed to be one of the causes for children's leukemia. Pulsed or amplitude-modulated, at a biologically active lower frequency (i.e. when the radio signal strength rises and falls in time with the lower frequency), high frequencies are the hallmark of mobile phones, WiFi systems, PDAs, etc. At radiofrequencies, electric and magnetic fields are closely interrelated and we typically measure their levels as power densities in Watts per square metre (W/m^2).

4. Electromagnetic fields and health

Life on Earth, since its beginning more than 3.5 billion years ago, has developed under the influence of the practically static geomagnetic field and the radiation from the sun. All living organisms that have not been able to directly cope with these influences are either gone or have adapted in one of several ways. Living under-ground, only being active during night, living in the deeper waters (at least from 1 m and down

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below) of our oceans and lakes, under the foliage of the jungle trees, or having developed a skin (or, for plants, a cortex) containing a pigment (animals and plants have very similar ones) that will shield from some heat and some sunshine. Any fair-skinned Irish or Scandinavian person learns very early to avoid even the bleak sun up-north to avoid a nasty sunburn. That sunburn will develop into a postinflammatory hyperpigmentation, that may have cosmetic value, but will also cause a redness of the skin as well as heat and pain/itch sensations.

But, during the last 100 years we have found that the pigment in our skin does not furnish any protection against other frequencies. Cosmic rays, radioactivity, X-rays, UVC, UVB and now even UVA are considered, together with radar-type microwaves to be very dangerous to health. We are translucent to power–frequency magnetic fields as well as mobile phone and WiFi microwaves, but this does not mean that they are without possible effects, through thermal or non-thermal mechanisms.

For me, as a scientist, this poses the main relevant questions: Is it possible to adapt our biology to altered exposure conditions in less than 100 years, or do we have to have thousands of years – or longer – for such an adaptation? And, in the meantime, what kind of safety standards must we adopt? A 'prudent avoidance' strategy, ALARA, recommendation levels based only on thermal effects, or is the only actual safe safety level for such exposures 0 (zero) Watts/kg until we really know? Or is "human progress", profit and greed more important than possible damage to our health? How far can we push the Russian roulette? And who should decide about this? Who should be held responsible if something goes wrong?

Our limited understanding of the biological effects of the vast majority of frequencies gives reason for concern. Although there is still a debate in this regard, *tinnitus, brain tumours and acoustic neuroma clearly are associated with cell phones and mobile phones, as is childhood leukemia with powerlines* (for references, see Blackman et al. [1]).

Communications and radar antennae expose those who live or work near these installations to their emissions. The radiation travels through buildings, and can also be conducted along electrical wires or metal plumbing. Wireless communications create levels within buildings that are many orders of magnitude higher than natural background levels. The same is true for appliances using power frequencies.

There are four phenomena that emerge from the use of electricity: ground currents; "electromagnetic smog" from communications equipment; electric and magnetic fields from power supplies and specialized equipment; and high frequencies on powerlines or so-called "dirty electricity". They may all be potential environmental toxins and this is an area of research that must be further pursued.

It is worth noting that off-gassing of electrical equipment may also contribute to sensitivities. Different sorts of technology (e.g. various medical equipment, analogue or digital telephones; flat screen monitors and laptop computers or larger older monitors) may vary significantly in strength, frequency and pattern of electromagnetic fields. One challenging question for science is to find out if, for instance, 50- or 60-Hz ELF pure sine wave, square waves or sawtooth waveform, ELF-dirty (e.g. radiofrequencies on powerlines), ELF-modulated radiofrequency fields, continuous wave radiofrequency radiation and particularly pulsed radiofrequency signals are more or less bioactive, e.g. as neurotoxic, immune-disrupting and/or carcinogenic environmental exposure parameters. As will be discussed below, hazardous effects on the immune system of this potential environmental toxin must be seriously considered.

5. Effects of electromagnetic fields on the immune system

An ever-increasing number of studies has clearly shown various biological and medical effects at the cellular level due to electromagnetic fields, including power–frequency, radiofrequency and microwaves. Such fields are present in everyday life, at the workplace, in homes and places of leisure.

5.1. The functional impairment electrohypersensitivity (EHS)

One of the first observations of a direct effect on the immune system was the finding in the 1980s of persons with the functional impairment electrohypersensitivity (EHS), namely those who claim to suffer from subjective and objective skin- and mucosa-related symptoms, such as itch, smarting, pain, heat sensation, redness, papules, pustles, etc., after exposure to visual display terminals (VDTs), mobile phones, DECT telephones, WiFi equipments, as well as other electromagnetic devices. Frequently, symptoms from internal organ systems, such as the heart and the central nervous system, are also encountered [13].

Persons with EHS experience facial skin symptoms (sensory sensations of the facial skin including stinging, itching, burning, erythema, rosacea), eye irritation, runny or stuffy nose, impaired sense of smell, hoarse dry throat, coughing, sense of pressure in ear(s), tinnitus, fatigue, headache, "heaviness" in the head, sleeplessness, nausea/dizziness, cardiac symptoms and difficulties in concentrating. In the Cox [14] report on electrical hypersensitivity in the United Kingdom, mobile phone users' symptoms included headaches (85%), dizziness (27%), fatigue (24%), nausea (15%), itching (15%), redness (9%), burning (61%), and cognitive problems (42%). For those individuals reporting EHS symptoms in the UK population, the percentage of persons with symptoms from cell phone masts was 18%, DECT cordless phones (36%), landline phones (6%), VDTs (27%), television (12%) and fluorescent lights (18%). In addition, Fox [15] reported that a questionnaire survey of EHS individuals revealed symptoms of nausea, and of dizziness/disorientation.

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Levallois et al. [16] in 2002 reported on their study of prevalence of self-perceived hypersensitivity to EMF in California. They found that about 3% of the population reports to be electrohypersensitive. About 0.5% of the population reported the necessity to change jobs or remain unemployed due to the severity of their symptoms. Underestimation of these percentages is discussed, since the population surveyed was found through contact with either an occupational clinic or a support group, and electrohypersensitive people very frequently cannot engage in normal outings (go out, travel, meet in buildings with EMF exposures, etc.). The study concludes that while there was no clinical confirmation of the reported symptoms of electrohypersensitivity, the perception is of public health importance in California, and North America. The results were based on a telephone survey among a sample of 2072 Californians. Being 'allergic or very sensitive" to getting near electrical devices was reported by 68 subjects resulting in an adjusted prevalence of 3.2% (95% confidence interval: 2.8–3.7). Twenty-seven subjects (1.3%) reported sensitivity to electrical devices but no sensitivity to chemicals. Alleging that a doctor had diagnosed "environmental illness or multiple chemical sensitivity" was the strongest predictor of reporting being hypersensitive to EMF in this population (adjusted prevalence odds ratio = 5.8, 95% confidence interval: 2.6–12.8). This study confirms the presence of this self-reported disability in North America.

A recent German survey [17] suggests that the prevalence of subjects who attribute health complaints to EMF exposures is not negligible. In a sample of 2500 interviewees, 8% specifically attributed health complaints to exposures from mobile phone base station antennas or the use of mobile or cordless phones. In Sweden, 3.1% of the population claimed to be hypersensitive to EMF. Considerable variation across countries, regions within countries, and surveys in the same regions has been noted before. In 1997, the European Group of Experts reported that electrical hypersensitivity had a higher prevalence in Sweden, Germany, and Denmark than in the United Kingdom, Austria, and France. All these data suggest that the true number is still uncertain and requires further research (cf. Schüz et al. [18]).

Roosli et al. [19,20] estimate that the proportion of individuals in Switzerland with EHS symptoms is about 5%, where the exposures of concern are cited to be mobile phone base stations (74%), followed by mobile phones (36%), cordless phones (29%), and powerlines (27%). They reported that about half the Swiss population is concerned about health effects from EMF exposures in general.

The WHO has acknowledged the condition of electrohypersensitivity, and published in 2006 a research agenda for radiofrequency fields. The WHO recommends that people reporting sensitivities receive a comprehensive health evaluation. It states: "Some studies suggest that certain physiological responses of EHS individuals tend to be outside the normal range. In particular, hyperactivity in the central nervous system and imbalance in the autonomic nervous system need to be followed up in clinical investigations, and the results for the individuals taken as input for possible treatment". Studies of individuals with sensitivities ought to consider sufficient acclimatization of subjects as recommended for chemical sensitivities, as well as recognition of individuals' wavelength-specific sensitivities. Reduction of electromagnetic radiation may ameliorate symptoms in people with chronic fatigue.

Lyskov et al. [21] in 2004 reported that EHS individuals exhibited sensitivity to VDTs, fluorescent lights and television, all of which produce flickering light. EHS individuals who were given provocation tests with flickering light exhibited a higher critical flicker frequency (CFF) than normal, and their visual evoked potential (VEP) was significantly higher than in controls. In follow-up studies, individuals with EHS demonstrated increased CFF, increased VEP, increased heart rate, decreased heart rate variability (HRV) and increased electrodermal (EDA) reaction to sound stimuli. These results indicate an imbalance in the autonomic nervous system and a lack of normal circadian rhythms in these EHS individuals. [N.B. It may just show that they feel ill. It is very hard for me to understand how sensitivity to flickering light could account for EHS in conjunction with e.g. mobile phones and base stations.]

Mueller and Schierz [22], also in 2004, reported that soundness of sleep and well-being in the morning, but not sleep quality, were affected by overnight EMF exposure in EHS individuals. An effect was reported where EHS individuals shifted their position in the bed during sleep to the non-exposed (or probably less exposed) side of the bed, something which may have strong implications for disease development (cf. Hallberg and Johansson, submitted).

Markovà et al. [23] reported that non-thermal microwave exposure from global system for mobile communication (GSM) mobile telephones at lower levels than the International Commission for Non-Ionizing Radiation Protection (ICNIRP) safety standards affect 53BP1 and γ-H2AX foci and chromatin conformation in human lymphocytes. They investigated effects of microwave radiation of GSM at different carrier frequencies on human lymphocytes from healthy persons and from persons reporting hypersensitivity to EMFs. They measured the changes in chromatin conformation, which are indicative of stress response and genotoxic effects, by the method of anomalous viscosity time dependence, and analyzed tumour suppressor p53-binding protein 1 (53BP1) and phosphorylated histone H2AX (γ-H2AX), which have been shown to co-localize in distinct foci with DNA double-strand breaks (DSBs), using immunofluorescence confocal laser microscopy. The authors reported that microwave exposure from GSM mobile telephones affect chromatin conformation and 53BP1/γ-H2AX foci similar to heat shock. For the first time, they reported that effects of microwave radiation from mobile telephones on human lymphocytes are dependent on carrier frequency. On average, the same response was observed in lymphocytes from hypersensitive and healthy subjects. *N.B. These effects occurred at non-*

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thermal microwave exposure levels from mobile telephones that are permissible under safety standards of ICNIRP!

The same group after having described frequency-dependent effects of mobile phone microwaves (MWs) of GSM on human lymphocytes from EHS persons and healthy persons (see above), went ahead asking themselves this: Contrary to GSM, universal global telecommunications system (UMTS) mobile phones emit wide-band MW signals. Hypothetically, UMTS MWs may result in higher biological effects compared to GSM signals because of eventual "effective" frequencies within the wideband. Based on this hypothesis they have very recently reported for the first time that UMTS MWs affect chromatin and inhibit formation of DNA double-strand breaks co-localizing 53BP1/γ-H2AX DNA repair foci in human lymphocytes from hypersensitive and healthy persons and confirm that effects of GSM MWs depend on carrier frequency [24]. Remarkably, the effects of MWs on 53BP1/γ-H2AX foci persisted up to 72 h following exposure of cells, even longer than the stress response following heat shock. The data are in line with the hypothesis that the type of signal, UMTS MWs, may have higher biological efficiency and possibly larger health risk effects compared to GSM emissions. No significant differences in effects between groups of healthy and hypersensitive subjects were observed, except for the effects of UMTS MWs and GSM – 915 MHz MWs on the formation of the DNA repair foci, which were different for hypersensitive ($p < 0.02$[53BP1]//0.01[γ-H2AX]) but not for control subjects ($p > 0.05$). The non-parametric statistics used here did not indicate specificity of the differences revealed between the effects of GSM and UMTS MWs on cells from hypersensitive subjects and more data are therefore needed to study the nature of these differences.

5.2. EHS as radiation damage/the mast cell hypothesis

Persons claiming adverse skin reactions after having been exposed to computer screens or mobile phones could be reacting in a highly specific way and with a completely correct avoidance reaction, especially if the provocative agent was radiation and/or chemical emissions – just as you would do if you had been exposed to e.g. sun rays, X-rays, radioactivity or chemicals. My working hypothesis, thus, became that they react in a cellularly correct way to the electromagnetic radiation, maybe in concert with chemical emissions such as plastic components, flame retardants, etc., something later focussed upon by professor Denis L. Henshaw and his collaborators at the Bristol University [25,26]. This is also covered in great depth by the author Gunni Nordström in her latest book [27].

Very early, immune cell alterations were observed when exposing two EHS individuals to a TV monitor [4]. In this article, we used an open-field provocation, in front of an ordinary TV set, of persons regarding themselves as suffering from skin problems due to work at video display terminals. Employing immunohistochemistry, in combination with a wide range of antisera directed towards cellular and neurochemical markers, we were able to show a high-to-very high number of somatostatin-immunoreactive dendritic cells as well as histamine-positive mast cells in skin biopsies from the anterior neck taken before the start of the provocation. At the end of the provocation the high number of mast cells was unchanged, however, all the somatostatin-positive cells had seemingly disappeared. This latter finding may be due to loss of immunoreactivity, increase of breakdown, etc. The high number of mast cells present may explain the clinical symptoms of itch, pain, edema and erythema.

In facial skin samples of electrohypersensitive persons, the most common finding is a profound increase of mast cells as monitored by various mast cell markers, such as histamine, chymase and tryptase [28]. From these studies, it is clear that the number of mast cells in the upper dermis is increased in the electrohypersensitivity group. A different pattern of mast cell distribution also occurred in the electrohypersensitivity group, namely, the normally empty zone between the dermo-epidermal junction and mid-to-upper dermis had disappeared in the electrohypersensitivity group and, instead, this zone had a high density of mast cell infiltration. These cells also seemed to have a tendency to migrate towards the epidermis (=epidermiotrophism) and many of them emptied their granular content (=degranulation) in the dermal papillary layer. Furthermore, more degranulated mast cells could be seen in the dermal reticular layer in the electrohypersensitivity group, especially in those cases showing mast cell epidermiotrophism. Finally, in the electrohypersensitivity group, the cytoplasmic granules were more densely distributed and more strongly stained than in the control group, and, generally, the size of the infiltrating mast cells was found to be larger in the electrohypersensitivity group as well. It should be noted, that increases of similar nature were demonstrated later on in an experimental situation employing normal healthy volunteers in front of visual display units, including ordinary television sets [5].

Mast cells, when activated, release a wide range of mediators, among them histamine, which is involved in a variety of biological effects with clinical relevance, e.g., allergic hypersensitivity, itch, edema, local erythema, and many types of dermatoses. From the results of the cited studies, it is clear that electromagnetic fields affect the mast cell and the dendritic cell population, and may degranulate these cells.

The release of inflammatory substances, such as histamine, from mast cells in the skin results in a local erythema, edema, and sensation of itch and pain, and the release of somatostatin from the dendritic cells may give rise to subjective sensations of ongoing inflammation and sensitivity to ordinary light. These are common symptoms reported from persons suffering from EHS/screen dermatitis. Mast cells occur in the brain [29] and their presence may, under the influence of EMF and/or radiofrequency radiation exposure lead to a chronic inflammatory response by the mast cell degranulation.

Mast cells are also present in the heart tissue and their localization is of particular relevance to their function. Data

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from studies made on interactions of EMF with cardiac function have demonstrated that changes are present in the heart after exposure. Some electrically sensitive people have symptoms similar to heart attacks or strong heart palpitations after exposure to EMF.

We have also, in more detail, compared facial skin from EHS persons with corresponding material from normal healthy volunteers [30]. The aim of the study was to evaluate possible markers to be used for future double-blind or blind provocation investigations. Differences were found for the biological markers calcitonin gene-related peptide (CGRP), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine amide (PHI), NPY, protein S-100 (S-100), neuron-specific enolase (NSE), protein gene product (PGP) 9.5 and phenylethanolamine *N*-methyltransferase (PNMT). The overall impression in the blind-coded material was such that it turned out easy to blindly separate the two groups from each other. However, no single marker was 100% able to pin-point the difference, although some were quite powerful in doing so (CGRP, SOM, S-100). In our ongoing investigations, we have also found alterations of the Merkel cell number in the facial skin of electrohypersensitive persons (Yoshimura et al., in preparation). However, it has to be pointed out that we cannot draw any definitive conclusions about the cause of the changes observed, based upon those results. Blind or double-blind provocations in a controlled environment [5] are necessary to elucidate the underlying causes for the changes reported in this particular investigation. So far, unfortunately, I and my co-workers have not been able to attract funding for such studies.

Gangi and Johansson [31,32] have proposed models for how mast cells and substances secreted from them (e.g., histamine, heparin, and serotonin) could explain sensitivity to EMF similar to those used to explain UV- and ionizing radiation-related damages. We discuss the increasing number of persons who report cutaneous problems as well as symptoms from certain internal organs, such as the central nervous system and the heart, when being close to electric equipment. Many of these respondents are users of video display terminals, and have both subjective and objective skin- and mucosa-related symptoms, such as pain, itch, heat sensation, erythema, papules, and pustules. The nervous system-derived symptoms are, e.g., dizziness, tiredness, and headache, erythema, itch, heat sensation, edema, and pain which are also common symptoms of sunburn (UV dermatitis). Alterations have been observed in cell populations of the skin of EHS persons similar to those observed in the skin damaged due to UV light or ionizing radiation.

Dr. Shabnam Gangi and I, in two theoretical papers [31,32], have put forward a model for how mast cells and substances secreted from them could explain sensitivity to EMF. The model starts from known facts in the fields of UV- and ionizing radiation-related damages, and uses all the new studies dealing with alterations seen after e.g. power frequency or microwave EMF to propose a simple summarizing model for the phenomenon of electrohypersensitivity.

Mast cells are able to secrete an array of potent mediators which may orchestrate neuroinflammation and affect the integrity of the blood–brain barrier. The "cross-talk" between mast cells, lymphocytes, neurons and glia constitutes a neuroimmune axis which is implicated in a range of neurodegenerative diseases with an inflammatory and/or autoimmune component.

Mast cells are involved in numerous activities ranging from control of the vasculature, to tissue injury and repair, allergic inflammation and host defences. They synthesize and secrete a variety of mediators, activating and modulating the functions of nearby cells and initiating complex physiological changes. Interestingly, nitric oxide (NO) produced by mast cells and/or other cells in the microenvironment appears to regulate these diverse roles. Some of the pathways central to the production of NO by mast cells and many of the tightly controlled regulatory mechanisms involved have been identified. Several cofactors and regulatory elements are involved in NO production, and these act at transcriptional and post-translational sites. Their involvement in NO production and the possibility that these pathways are critically important in mast cell functions in EHS persons should be investigated. The effects of NO on mast cell functions such as adhesion, activation and mediator secretion ought to be examined with a focus on molecular mechanisms by which NO modifies intracellular signalling pathways dependent or independent of cGMP and soluble guanylate cyclase. Metabolic products of NO including peroxynitrite and other reactive species may be the critical elements that affect the actions of NO on mast cell functions. Further understanding of the actions of NO on mast cell activities may uncover novel strategies to modulate inflammatory conditions.

It is important to remember that mastocytosis – an abnormal accumulation of mast cells in one or more organ system – can occur secondarily to other causes, such as inflammation and some kinds of leukemia. The increase in EHS being described here is more accurately thought of as "primary" mastocytosis, meaning that the increased number of mast cells occurs independently of any other cause. However, because of the increased number of mast cells in primary mastocytosis, conditions such as osteoporosis and inflammation may arise as a result of the activity of those mast cells. The manner in which primary mastocytosis can be distinguished from secondary mastocytosis and other conditions should also be addressed in controlled studies.

Patients with mastocytosis may or may not have constitutional symptoms, including weight loss, pain, nausea, headache, malaise, or fatigue. These symptoms may be due to uncontrolled proliferation of mast cells or involvement of distinct organs, such as the stomach and intestines, or bone or bone marrow. Constitutional symptoms also can result from high levels of mast cell mediators in the blood stream. The severity of symptoms varies from mild to life threatening.

Holmboe and Johansson [33] reported on testing EHS persons for increased levels of IgE or signs of a positive Phadiatop Combi (which is a screening test for allergies towards

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certain foods, pollens, insects, and other animals) both of which would be indicators of an immune system alert. Five men and 17 women participated in the study. Skin and nervous system effects were the primary symptoms reported. The most frequently reported symptoms were skin redness, eczema and sweating, loss of memory, concentration difficulties, sleep disturbances, dizziness, muscular and joint-related pain, and muscular and joint-related weakness. Headache, faintness, nasal stuffiness, and fatigue were also common. In addition, 19 of the people had disturbances of the gastrointestinal tract. All the EHS persons had tinnitus. However, no connection between IgE blood levels and symptoms was found. All EHS people had normal values (<122 kU/l). Only three people had a positive Phadiatop Combi.

In summary, it is evident from our preliminary experimental data that various biological alterations are present in EHS persons claiming to suffer from exposure to EMF. The alterations are themselves enough to fully explain the EHS symptoms, and the involvement of the immune system is evident.

Thus, it is of paramount importance to continue investigating persons with EHS. We would favour studies of EMF interaction with mast cell release of histamine and other biologically active substances, studies of lymphocyte viability, as well as studies of the newly described serotonin-containing melanocytes. Also, continued analysis of the intraepidermal nerve fibers and their relations to these mast cells and serotonin-containing melanocytes is very important. Finally, not to be forgotten, a general investigation of persons with EHS versus normal healthy volunteers regarding the above markers as well as other markers for cell traffic, proliferation and inflammation is very much needed. Such research may lay a firm foundation for necessary adjustment of accessibility, thus helping all persons with EHS.

5.3. Rat skin and thyroid: animal model studies

In addition to the studies in humans, we have also done a series of animal experiments [6–8]. These have been a collaborative effort between the Department of Biology, Faculty of Sciences, Novi Sad, Serbia, and my own research group at the Karolinska Institute, Stockholm, Sweden.

These papers go back to early observations in persons with EHS where large increases in the cutaneous mast cell count could be demonstrated as compared to normal healthy volunteers. A corresponding effect on cutaneous mast cells from normal healthy volunteers placed in front of ordinary TVs/PCs could also be shown. My working hypothesis since then is that EHS is a kind of radiation damage, since the observed cellular changes are very much the same as the ones you would find in tissue subjected to UV-light or ionizing radiation (for refs., see above).

One very fierce criticism has been that such mast cell alterations in persons with electrohypersensitivity (or in normal healthy volunteers!) can not be due to EMFs and/or airborn chemicals, but must be due to psychological or psychiatric personality disturbances, cognitive malfunction, or likewise.

The aim of these studies has therefore been to investigate the influence of extremely low-frequency electromagnetic fields (ELF-EMFs) on mast cells, parafollicular cells, and nerve fibers in rat skin and thyroid gland, as seen using light and transmission electron microscopy. The experiments were performed on 2-month-old Wistar male rats exposed for 4 h a day, 5 or 7 days a week for 1 month to power–frequency (50 Hz) EMFs (100–300 μT, 54–160 V/m). After sacrifice, samples of skin and thyroid were processed for indirect immunohistochemistry or toluidine blue staining and were then analyzed using the methods of stereology. Antibody markers to serotonin, substance P, CGRP, and PGP 9.5 were applied to skin sections and PGP 9.5, CGRP, and neuropeptide Y (NPY) markers to the thyroid. A significantly increased number of serotonin-positive mast cells in the skin ($p < 0.05$) and NPY-containing nerve fibers in the thyroid ($p < 0.01$) of rats exposed to ELF-EMF was found compared to controls, indicating a direct EMF effect on skin and thyroid vasculature.

After ultrastructural examination, a predominance of microfollicles with less colloid content and dilated blood capillaries was found in the EMF group. Stereological counting showed a statistically significant increase of the volume density of follicular epithelium, interfollicular tissue and blood capillaries as well as the thyroid activation index, as compared to the controls. The volume density of colloid significantly decreased. Ultrastructural analysis of thyroid follicular cells in the EMF group revealed the frequent finding of several colloid droplets within the same thyrocyte with the occasional presence of large-diameter droplets. Alterations in lysosomes, granular endoplasmic reticulum and cell nuclei compared to the control group were also observed. Taken together, both the light microscope and the ultrastructural results show the stimulating effect of power–frequency EMFs.

The results obtained in animal studies cannot be understood by psychological or psychiatric theories, but are clearly related to the EMF exposure. In view of recent epidemiological studies, pointing to a correlation between long-term exposure from power-frequency magnetic fields or radio-/microwaves and cancer, our data have to be taken seriously.

5.4. Cutaneous heat shock protein/stress response pathway

Recent evidence by Leszczynski et al. has indicated activation of stress-induced pathways in cultivated cells in response to microwaves [34]. Their article indicated that mobile telephone microwaves activate a variety of cellular signal transduction pathways, among them the hsp27/p38MAPK stress response pathway [34]. Whether activation of stress response pathways relates to apoptosis, blood–brain barrier permeability, or increased cancer in humans remains to be investigated. Further work reported gene and protein expres-

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sion changes in human endothelial cell lines with microwave 900 MHz mobile phone exposure [35].

5.5. Childhood leukemia and power-frequency magnetic fields; CNS tumours and cell phone use

Childhood leukemia was connected to power-frequency magnetic fields already in the pioneering work by Wertheimer and Leeper [36]. More recently, Scandinavian scientists have identified an increased risk for acoustic neuroma (i.e., a benign tumour of the eighth cranial nerve) in cell phone users, as well as a slightly increased risk of malignant brain tumours such as astrocytoma and meningioma on the same side of the brain as the cell phone was held [37–40]. In addition, a clear association between adult cancers and FM radio broadcasting radiation has been noticed, both in time and location [41–43]. Initial studies on facial nevi indicate that nowadays young children can have a substantial number of these (Hallberg and Johansson, unpublished data). If, in addition to the low-frequency EMF, there is a radiofrequency and/or microwave correlation, then this must be considered in future research and safety programs.

5.6. Effects by microwaves on acute experimental allergic encephalomyelitis

Turning back to the immune system, Anane et al. [44] have studied the effects of acute exposure to GSM-900 microwaves (900 MHz, 217 Hz pulse modulation) on the clinical parameters of the acute experimental allergic encephalomyelitis (EAE) model in rats in two independent experiments: rats were either habituated or non-habituated to the exposure restrainers. EAE was induced with a mixture of myelin basic protein and Mycobacterium tuberculosis. Female Lewis rats were divided into cage control, sham-exposed, and two groups exposed either at 1.5 or 6.0 W/kg local specific absorption rate (SAR averaged over the brain) using a loop antenna placed over their heads. No effect of a 21-day exposure (2 h/day) on the onset, duration, and termination of the EAE crisis was seen.

5.7. Effects of electromagnetic fields on immune system parameters, including cellular markers

5.7.1. Residential exposure effects/occupational studies

The object of the study by Boscol et al. [45] in 2001 was to investigate the immune system of 19 women with a mean age of 35 years, for at least 2 years (mean = 13 years) exposed to electromagnetic fields induced by radio–television broadcasting stations in their residential area. In September 1999, the EMFs (with range 500 kHz–3 GHz) in the balconies of the homes of the women were (mean ± S.D.) 4.3 ± 1.4 V/m. Forty-seven women of similar age, smoking habits and atopy composed the control group, with a nearby resident EMF exposure of <1.8 V/m. Blood lead and urinary trans–trans muconic acid (a metabolite of benzene), markers of exposure to urban traffic, were higher in the control women. The EMF exposed group showed a statistically significant reduction of blood NK CD16+–CD56+, cytotoxic CD3(−)–CD8+, B and NK activated CD3(−)–HLA–DR+ and CD3(−)–CD25+ lymphocytes. In vitro production of IL-2 and interferon-gamma (INF-gamma) by peripheral blood mononuclear cells (PBMC) of the EMF exposed group, incubated either with or without phytohaemoagglutinin (PHA), was significantly lower; the in vitro production of IL-2 was significantly correlated with blood CD16+–CD56+ lymphocytes. The stimulation index (S.I.) of blastogenesis (ratio between cell proliferation with and without PHA) of PBMC of EMF exposed women was lower than that of the control subjects. The S.I. of blastogenesis of the EMF exposed group (but not blood NK lymphocytes and the in vitro production of IL-2 and INF-gamma by PBMC) was significantly correlated with the EMF levels. Blood lead and urinary trans–trans muconic acid were barely correlated with immune parameters: the urinary metabolite of benzene of the control group was only correlated with CD16+–CD56+ cells indicating a slight effect of traffic on the immune system. In conclusion, this study demonstrates that high-frequency EMFs reduce cytotoxic activity in the peripheral blood of women without a dose-response effect. [Such an effect could only be considered as very serious, since this could hamper the immune system in its daily struggle against various organisms/agents.]

A more general reaction pattern was found by Dmoch and Moszczynski [46] who assessed immunoglobulin concentrations and T-lymphocyte subsets in workers of TV re-transmission and satellite communication centres. An increase in IgG and IgA concentrations, an increased count of lymphocytes and T8 lymphocytes, a decreased count of NK cells and a lower value of T-helper/T-suppressor ratio were found.

The immunoglobulins' concentrations and T-lymphocyte subsets during occupational exposures to microwave radiation were also assessed in 1999 by Moszczynski et al. [47]. In the workers of re-transmission TV center and center of satellite communications on increased IgG and IgA concentration and decreased count of lymphocytes and T8 cells was found. However, in the radar operators IgM concentration was elevated and a decrease in the total T8 cell count was observed. The different behaviors of examined immunological parameters indicate that the effect of microwave radiation on the immune system depends on the exposure. However, disorders in the immunoglobulins' concentrations and in the T8 cell count had not, so far, caused any reported clinical consequences in their workers.

Tuschl et al. [48] recorded a considerable excess of recommended exposure limits in the vicinity of shortwave diathermy devices used for medical treatment of patients. Different kinds of field probes were used to measure electric and magnetic field strength and the whole body exposure of medical personnel operating shortwave, decimetre wave and microwave units was calculated. To investigate the influence of chronic exposure on the immune system of operators,

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blood was sampled from physiotherapists working with the above mentioned devices. Eighteen exposed and 13 control persons, matched by sex and age, were examined. Total leucocyte and lymphocyte counts were performed and leucocytic subpopulations determined by flow cytometry and monoclonal antibodies against surface antigens. In addition, to quantifying subpopulations of immunocompetent cells, the activity of lymphocytes was measured. Lymphocytes were stimulated by mitogen phytohemagglutinin and their proliferation measured by flow cytometry. No statistically significant differences between the control and exposed persons were found. In both study groups all immune parameters were within normal ranges.

5.7.2. Electromagnetic fields and atopy

In an attempt to understand how non-atopic and atopic fertile women with uniform exposure to toxic compounds produced by traffic immunologically react to high or low frequency electromagnetic fields (ELMF), Del Signore et al. [49] performed a preliminary study. Women were divided in group A (non-atopic, non-exposed to ELMF); B (atopic, non-exposed to ELMF); C (non-atopic, exposed to ELMF); D (atopic, exposed to ELMF). In vitro cell proliferation of PBMC of atopic women (groups B and D) stimulated by PHA was reduced. The ELMF exposed women (groups C and D) showed lower levels of blood NK CD16(+)–CD56+ lymphocyte subpopulations and of in vitro production of interferon-gamma (both spontaneously and in presence of PHA) by PBMC, suggesting that ELMF reduces blood cytotoxic activity. Serum IgE of the atopic women exposed to ELMF (group D) was higher than that of the other groups. Linear discriminant analysis including serum zinc and copper (essential enzymes for immune functions), blood lead and urinary trans–trans muconic acid, a metabolite of benzene (markers of exposure to traffic) and key parameters of immune functions (CD16(+)–CD56+ lymphocyte subset, serum IgE, interferon-gamma produced by PBMC in presence of PHA, stimulation index of blastogenesis) showed absence of significant difference between groups A and C and a marked separation of groups B and D. This datum suggests that ELMF have a greater influence on atopic women exposed to traffic than on non-atopic ones, again pointing out differing reaction capacities in the human population – possibly dependent on varying immune functions based on variations in genetic make-up. [This is of particular interest since EHS persons have certain atopic features (Liden, personal communication) that may make them more susceptible to EMFs.]

5.7.3. Animal and human cellular in vivo and in vitro studies

One very interesting set of experiments was performed by Cleary et al. [50] in 1990. Whole human blood was exposed or sham-exposed in vitro for 2 h to 27 or 2450 MHz radiofrequency (RF) radiation under isothermal conditions (i.e., $37 \pm 0.2\,^{\circ}C$). Immediately after exposure, mononuclear cells were separated from blood by Ficoll density-gradient centrifugation and cultured for 3 days at 37 °C with or without mitogenic stimulation by PHA. Lymphocyte proliferation was assayed at the end of the culture period by 6 h of pulse-labeling with 3H-thymidine (3H-TdR). Exposure to radiation at either frequency at specific absorption rates (SARs) below 50 W/kg resulted in a dose-dependent, statistically significant increase of 3H-TdR uptake in PHA-activated or unstimulated lymphocytes. Exposure at 50 W/kg or higher suppressed 3H-TdR uptake relative to that of sham-exposed cells. There were no detectable effects of RF radiation on lymphocyte morphology or viability. Notwithstanding the characteristic temperature dependence of lymphocyte activation in vitro, the isothermal exposure conditions of this study indicate that the biphasic, dose-dependent effects of the radiation on lymphocyte proliferation are not dependent on heating.

Half a decade later (1996), Cleary et al. [51] published yet another paper with the title "Effect of isothermal radiofrequency radiation on cytolytic T lymphocytes". Previous in vitro studies had provided evidence that RF radiation modulates proliferation of human glioma, lymphocytes, and other cell types. The mechanism of such cell proliferation modulation, as well as mechanisms for effects on other cell physiologic endpoints, however, was not well understood. To obtain insight regarding interaction mechanisms, they investigated effects of RF radiation exposure on interleukin 2 (IL-2)-dependent proliferation of cytolytic T-lymphocytes (CTLL-2). After exposure to RF radiation – in the presence or absence of IL-2 – cells were cultured at various physiological concentrations of IL-2. Treatment effects on CTLL-2 proliferation were determined by tritiated thymidine incorporation immediately or 24 h after exposure. Exposure to 2450 MHz RF radiation at specific absorption rates (SARs) of greater than 25 W/kg (induced E-field strength 98.4 V/m) induced a consistent, statistically significant reduction in CTLL-2 proliferation, especially at low IL-2 concentrations. At lower SARs, 2450 MHz exposure increased CTLL-2 proliferation immediately after exposure but reduced 24 h postexposure proliferation. RF radiation effects depended on the mitotic state of the cells at the time of exposure.

In 1992, Czerska et al. [52] studied the effects of continuous and pulsed 2450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. Normal human lymphocytes were isolated from the peripheral blood of healthy donors. One-milliliter samples containing one million cells in chromosome medium 1A were exposed for 5 days to conventional heating or to continuous wave (CW) or pulsed wave (PW) 2450-MHz radiation at non-heating (37 °C) and various heating levels (temperature increases of 0.5, 1.0, 1.5, and 2 °C). The pulsed exposures involved 1-μs pulses at pulse repetition frequencies from 100 to 1000 pulses/s at the same average SAR levels as the CW exposures. Actual average SARs ranged to 12.3 W/kg. Following termination of the incubation period, spontaneous lymphoblastoid transformation was determined with an image analysis system. The results were compared

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among each of the experimental conditions and with sham-exposed cultures. At non-heating levels, CW exposure did not affect transformation. At heating levels both conventional and CW heating enhanced transformation to the same extent and correlate with the increases in incubation temperature. PW exposure enhanced transformation at non-heating levels. This finding is significant ($p < 0.002$). At heating levels PW exposure enhanced transformation to a greater extent than did conventional or CW heating. This finding is significant at the 0.02 level. It was concluded that PW 2450-MHz radiation acts differently on the process of lymphoblastoid transformation in vitro compared with CW 2450-MHz radiation at the same average SARs.

In 2003, Dabrowski et al. [53] exposed samples of mononuclear cells isolated from peripheral blood of healthy donors ($n = 16$) to 1300 MHz pulse-modulated microwaves at 330 pps with 5 μs pulse width. The samples were exposed in an anechoic chamber at the average value of power density of $S = 10\ W/m^2$ ($1\ mW/cm^2$). The average specific absorption rate (SAR) was measured in rectangular waveguide and the value of SAR = 0.18 W/kg was recorded. Subsequently, the exposed and control cells were assessed in the microculture system for several parameters characterizing their proliferative and immunoregulatory properties. Although the irradiation decreased the spontaneous incorporation of 3H-thymidine, the proliferative response of lymphocytes to PHA and to Con A as well as the T-cell suppressive activity (SAT index) and the saturation of IL-2 receptors did not change. Nevertheless, the lymphocyte production of interleukin (IL)-10 increased ($p < 0.001$) and the concentration of IFNg remained unchanged or slightly decreased in the culture supernatants. Concomitantly, the microwave irradiation modulated the monokine production by monocytes. The production of IL-1b increased significantly ($p < 0.01$), the concentration of its antagonist (IL-1ra) dropped by half ($p < 0.01$) and the tumour necrosis factor (TNF-α) concentration remained unchanged. These changes of monokine proportion (IL-1b versus IL-1ra) resulted in significant increase of the value of the LM (=the monokine influence on lymphocyte mitogenic response; cf. Dabrowski et al. [54]) index ($p < 0.01$), which reflects the activation of monocyte immunogenic function. The results indicate that pulse-modulated microwaves represent the potential of immunotropic influence, stimulating preferentially the immunogenic and proinflammatory activity of monocytes at relatively low levels of exposure!

Following these findings of G_o phase PBMC exposed to low-level (SAR = 0.18 W/kg) pulse-modulated 1300 MHz microwaves and subsequently cultured, demonstrating changed immune activity (as of above), in 2006 Stankiewicz et al. [55] investigated whether cultured immune cells induced into the active phases of cell cycle (G_1, S) and then exposed to microwaves will also be sensitive to EMF. An anechoic chamber containing a microplate with cultured cells and an antenna emitting microwaves (900 MHz simulated GSM signal, 27 V/m, SAR 0.024 W/kg) was placed inside an ASSAB incubator. The microcultures of PBMC exposed to microwaves demonstrated significantly higher response to mitogens and higher immunogenic activity of monocytes (LM index) than control cultures. The results suggest that immune activity of responding lymphocytes and monocytes can be additionally intensified by 900 MHz microwaves. [The above described effects of an immune system activity-intensifying effect of 900 MHz microwaves are a very important warning signal as well as a very important piece of the explanatory jigsaw puzzle regarding EHS. In the latter, affected persons very often describe "influenza-like" sensations in their body. Maybe the mobile phones, as well as other high-frequency devices, have aroused the immune system to too high an activation level?]

Two papers of paramount importance are Donnellan et al. [56] and Harvey and French [57]. In the first, a mast cell line, RBL-2H3, was exposed to 835 MHz for 20 min, three times per day for 7 days at a power density of $8.1 \pm 3\ mW/cm^2$. From day 4 onwards, it was observed that the rate of DNA synthesis and cell replication increased, that actin distribution and cell morphology became altered, and that the amount of beta-hexosaminidase (a marker of granule secretion) released in response to a calcium ionophore was significantly enhanced, in comparison to unexposed cultures. No effects were seen on levels of cytoskeletal protein synthesis or of beta-actin mRNA. Morphological changes persisted following subculture for at least 7 days in the absence of further exposure. It is hypothesized that effects of exposure to an EMF at 835 MHz may be mediated via a signal transduction pathway. In the second publication, Harvey and French used a resonant cavity which delivered a continuous wave exposure at 864.3 MHz at an average SAR of 7 W/kg to determine non-thermal biological effects of microwave exposure. A human mast cell line, HMC-1, was used as the biological target. Cells were exposed three times for 20-min duration daily, for 7 days. The temperature of the cell culture medium during the exposure fell to 26.5 °C. Effects were seen on localization of protein kinase C, and expression of three of the 588 genes screened. The affected genes included the proto-oncogene c-kit, the transcription factor nucleoside diphosphate kinase B and the apoptosis-associated gene DAD-1. Stress response genes were variably upregulated. No significant effect on morphology or on F-actin distribution was detected. They concluded that low-power microwave exposure may act on HMC-1 cells by altering gene expression via a mechanism involving activation of protein kinase C, and at temperatures well below those known to induce a heat shock response.

Elekes et al. [58] in 1996 found a very interesting sex-difference. The effect of continuous (CW; 2.45 GHz carrier frequency) or amplitude-modulated (AM; 50 Hz square wave) microwave radiation on the immune response was tested. CW exposures (6 days, 3 h/day) induced elevations of the number of antibody-producing cells in the spleen of male Balb/c mice (+37%). AM microwave exposure induced elevation of the spleen index (+15%) and antibody-producing cell number (+55%) in the spleen of male mice. No changes were

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observed in female mice. It is concluded that both types of exposure conditions induced moderate elevation of antibody production only in male mice.

Irradiation with electromagnetic waves (8.15–18 GHz, 1 Hz within, 1 $\mu W/cm^2$) in vivo increases the cytotoxic activity of natural killer cells of rat spleen [59]. In mice exposed for 24–72 h, the activity of natural killer cells increased by 130–150%, the increased level of activity persisting within 24 h after the cessation of treatment. Microwave irradiation of animals in vivo for 3.5 and 5 h, and a short exposure of splenic cells in vitro did not affect the activity of natural killer cells.

Whole body microwave sinusoidal irradiation of male NMRI mice with 8.15–18 GHz (1 Hz within) at a power density of 1 $\mu W/cm^2$ caused a significant enhancement of TNF production in peritoneal macrophages and splenic T-lymphocytes [60]. Microwave radiation affected T-cells, facilitating their capacity to proliferate in response to mitogenic stimulation. The exposure duration necessary for the stimulation of cellular immunity ranged from 5 h to 3 days. Chronic irradiation of mice for 7 days produced the decreasing of TNF production in peritoneal macrophages. The exposure of mice for 24 h increased the TNF production and immune proliferative response, and these stimulatory effects persisted over 3 days after the termination of exposure. Microwave treatment increased the endogenously produced TNF more effectively than did lipopolysaccharide, one of the most potential stimuli of synthesis of this cytokine. Microwaves, thus, indeed can be a factor interfering with the process of cellular immunity!

A very intriguing investigation was carried out by Gapeev et al. [61], who compared horn, dielectric and channel antennae and their matching with various types of loads, including a biological object. The channel antenna in contrast to dielectric and horn provides the uniform spatial distribution of specific absorbed rating in the frequency range used and wide-band matching with the object both in near field and far field zones of the radiator. It is shown, that low-intensity electromagnetic radiation of extremely high frequency in near field zone of the channel radiator modifies the activity of mouse peritoneal neutrophils on a quasi-resonance manner. The interaction of electromagnetic radiation with the biological object has been revealed in the narrow-band frequencies of 41.8–42.05 GHz and consists in inhibition of luminol-dependent chemiluminescence of neutrophils activated by opsonized zymosan. No frequency dependence has been found of the electromagnetic radiation effects in the far field zone of the radiator. The results obtained suggest, that the quasi-resonance dependence of the biological effect on the frequency of the electromagnetic radiation in the near field zone is conditioned by structure and nature of the electromagnetic radiation in this zone.

In 2003, Gatta et al. [62] studied the effects of in vivo exposure to GSM-modulated 900 MHz radiation on mouse peripheral lymphocytes. The aim of this study was to evaluate whether daily whole-body exposure to 900 MHz GSM-modulated radiation could affect spleen lymphocytes. C57BL/6 mice were exposed 2 h/day for 1, 2 or 4 weeks in a TEM cell to an SAR of 1 or 2 W/kg. Untreated and sham-exposed groups were also examined. At the end of the exposure, mice were killed and spleen cells were collected. The number of spleen cells, the percentages of B- and T-cells, and the distribution of T-cell subpopulations (CD4 and CD8) were not altered by the exposure. T- and B-cells were also stimulated *ex vivo* using specific monoclonal antibodies or LPS to induce cell proliferation, cytokine production and expression of activation markers. The results did not show relevant differences in either T- or B-lymphocytes from mice exposed to an SAR of 1 or 2 W/kg and sham-exposed mice with few exceptions. After 1 week of exposure to 1 or 2 W/kg, an increase in IFN-gamma (Ifng) production was observed that was not evident when the exposure was prolonged to 2 or 4 weeks. This suggests that the immune system might have adapted to RF radiation as it does with other stressing agents. All together, from their in vivo data, they concluded that the T- and B-cell compartments were not substantially affected by exposure to RF radiation and that a clinically relevant effect of RF radiation on the immune system is unlikely to occur. [Another explanation could be that the cells were unable to deal with the exposure and the obvious follow-up question then will be: What happened with the immune cells after months and years of exposure?]

On the other hand, Kolomytseva et al. [63], in their whole-body exposure experiment designed to study the dynamics of leukocyte number and functional activity of peripheral blood neutrophils under whole-body exposure of healthy mice to low-intensity extremely high-frequency electromagnetic radiation (EHF EMR, 42.0 GHz, 0.15 mW/cm^2, 20 min daily), showed that such a whole-body exposure of healthy mice to low-intensity EHF EMR has a profound effect on the indices of non-specific immunity. It was shown that the phagocytic activity of peripheral blood neutrophils was suppressed by about 50% ($p < 0.01$ as compared with the sham-exposed control) in 2–3 h after the single exposure to EHF EMR. The effect persisted for 1 day after the exposure, and then the phagocytic activity of neutrophils returned to the normal within 3 days. A significant modification of the leukocyte blood profile in mice exposed to EHF EMR for 5 days was observed after the cessation of exposures: the number of leukocytes increased by 44% ($p < 0.05$ as compared with sham-exposed animals), mostly due to an increase in the lymphocyte content. The supposition was made that EHF EMR effects can be mediated via the metabolic systems of arachidonic acid and the stimulation of adenylate cyclase activity, with subsequent increase in the intracellular cAMP level.

The modification of indices of the humoral immune response to thymus-dependent antigen (sheep erythrocytes) after a whole-body exposure of healthy mice to low-intensity extremely high-frequency electromagnetic radiation was reported by Lushnikov et al. in 2001 [64]. Male NMRI mice were exposed in the far-field zone of horn antenna at a frequency of 42.0 GHz and energy flux density of 0.15 mW/cm^2

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under different regimes: once for 20 min, for 20 min daily during 5 and 20 successive days before immunization, and for 20 min daily during 5 successive days after immunization throughout the development of the humoral immune response. The intensity of the humoral immune response was estimated on day 5 after immunization by the number of antibody-forming cells of the spleen and antibody titers. Changes in cellularity of the spleen, thymus and red bone marrow were also assessed. The indices of humoral immunity and cellularity of lymphoid organs changed insignificantly after acute exposure and a series of five exposures before and after immunization of the animals. However, after repeated exposures for 20 days before immunization, a statistically significant reduction of thymic cellularity by 17.5% ($p < 0.05$) and a decrease in cellularity of the spleen by 14.5% ($p < 0.05$) were revealed. The results show that single low-intensity extremely high-frequency electromagnetic radiation, at the frequency and energy flux density used, does not influence the humoral immune response intensity in healthy mice but influences immunogenesis under multiple repeated exposures.

Experiments have also been conducted to elucidate the effects of chronic low power-level microwave radiation on the immunological systems of rabbits [65]. Fourteen male Belgian white rabbits were exposed to microwave radiation at 5 mW/cm^2, 2.1 GHz, 3 h daily, 6 days/week for 3 months in two batches of seven each in specially designed miniature anechoic chambers. Seven rabbits were subjected to sham exposure for identical duration. The microwave energy was provided through S band standard gain horns connected to a 4K3SJ2 Klystron power amplifier. The first batch of animals was assessed for T-lymphocyte-mediated cellular immune response mechanisms and the second batch of animals for B-lymphocyte-mediated humoral immune response mechanisms. The peripheral blood samples collected monthly during microwave/sham exposure and during follow-up (5/14 days after termination of exposures, in the second batch animals only) were analysed for T-lymphocyte numbers and their mitogen responsiveness to ConA and PHA. Significant suppression of T-lymphocyte numbers was noted in the microwave group at 2 months ($p < 0.01$) and during follow-up ($p < 0.01$). The first batch of animals was initially sensitised with BCG and challenged with tuberculin (0.03 ml) at the termination of microwave irradiation/sham exposure and the increase in foot pad thickness (delta mm), which is a measure of T-cell-mediated immunity (delayed type hypersensitivity response, DTH) was noted in both the groups. The microwave group revealed a "better" response than the control group (delta % +12.4 versus +7.54).

Nasta et al. [66], very recently examined the effects of in vivo exposure to a GSM-modulated 900 MHz RF field on B-cell peripheral differentiation and antibody production in mice. Their results show that exposure to a whole-body average SAR of 2 W/kg, 2 h/day for 4 consecutive weeks does not affect the frequencies of differentiating transitional 1 (T1) and T2 B-cells or those of mature follicular B and marginal zone B-cells in the spleen. IgM and IgG serum levels are also not significantly different among exposed, sham-exposed and control mice. B-cells from these mice, challenged in vitro with LPS, produce comparable amounts of IgM and IgG. Moreover, exposure of immunized mice to RF fields does not change the antigen-specific antibody serum level. Interestingly, not only the production of antigen-specific IgM but also that of IgG (which requires T–B-cell interaction) is not affected by RF-field exposure. This indicates that the exposure does not alter an ongoing in vivo antigen-specific immune response. In conclusion, the results of Nasta et al. [66] do not indicate any effects of GSM-modulated RF radiation on the B-cell peripheral compartment and antibody production.

Whole-body microwave sinusoidal irradiation of male NMRI mice, exposure of macrophages in vitro, and preliminary irradiation of culture medium with 8.15–18 GHz (1 Hz within) at a power density of 1 μW/cm^2 caused a significant enhancement of tumour necrosis factor production in peritoneal macrophages [67]. The role of microwaves as a factor interfering with the process of cell immunity must, thus, be seriously considered. Furthermore, the effect of 8.15–18 GHz (1 Hz within) microwave radiation at a power density of 1 μW/cm^2 on the tumour necrosis factor (TNF) production and immune response was tested by Novoselova et al. [68]. A single 5 h whole-body exposure induced a significant increase in TNF production in peritoneal macrophages and splenic T-cells. The mitogenic response in T-lymphocytes increased after microwave exposure. The activation of cellular immunity was observed within 3 days after exposure. A diet containing lipid-soluble nutrients (beta-carotene, alpha-tocopherol and ubiquinone Q9) increased the activity of macrophages and T-cells from irradiated mice. These results demonstrate that irradiation with low-power density microwaves stimulates the immune potential of macrophages and T-cells, and the antioxidant treatment enhances the effect of microwaves, in particular when the effect of irradiation is reduced.

In the experimental study by Çetin et al. [69] in 2006, the hematological effects of pulsed EMFs chronic exposure were investigated on mice. Sixty, 6-week-old male Swiss mice, weighing 40–45 g were used, and were divided into two groups: in one group, animals ($n = 30$) were exposed to pulsed EMFs (60 Hz, intensity 3 μT, 12 h by day) for a 120-day period, whereas the second group ($n = 30$) was used as control. On days 15, 30, 90 and 120, samples were taken by cardiac puncture for the hematological analysis (red blood cell and white blood cell counts, leukocyte distribution). Whereas no significant difference was noted between control and exposed animals at the 15th and the 30th days, a macrocytic anemia characterized by decreases in of hemoglobin concentration, hematocrit values and erythrocyte counts and by increases in mean corpuscular volume, occurred in the exposed animals on day 90. Furthermore, they have shown significant reductions of leukocyte, lymphocyte and neutrophil counts, while monocyte counts were increased. On day 120, these leukocyte alterations were still

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observed, whereas erythrocyte parameters approached control values. These results suggest that pulsed electromagnetic fields (60 Hz and 3 μT) affect the hematological parameters of mice, probably by reducing proliferation and differentiation of marrow stem cells.

Obukhan [70] has performed cytologic investigations designed to study bone marrow, peripheral blood, spleen, and thymus of albino rats irradiated by an EMF of 2375, 2450, and 3000 MHz. Structural and functional changes in populations of megakaryocytes, immunocompetent cells as well as of undifferentiated cells, and of other types of cells that are dependent on the intensity of irradiation were revealed. The results permitted establishing the probability-threshold levels of exposure taking account of reactions of perception and physiologic adaptation together with compensatory and regenerative processes and the injury sustained. It was shown that changes in bone marrow cells differentiation and reproduction, rather than integral shifts in the peripheral blood, acquired the utmost significance. The blast cell population increased in low-intensity exposure, along with disturbances in mitosis.

The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with X-rays was recently investigated in vitro using several assays on human lymphocytes by Stronati et al. [71]. The chosen SAR values are near the upper limit of energy absorbed by localized tissue during the use of some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen. Blood specimens from 14 donors were exposed continuously for 24 h to a GSM basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp X-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling. By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition, RFR did not modify any measured effects of the X-radiation. In conclusion, this study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of X-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic *per se* or whether, it can influence the genotoxicity of the well-established clastogenic X-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed. [Of course, DNA damage is a well characterized effect of electromagnetic irradiation of other cell types, including lymphoblastoid cells [72], fibroblasts [73], and brain cells [74].]

Despite the important role of the immune system in defending the body against infections and cancer, only rather few investigations on possible effects of RF radiation on function of human immune cells have been undertaken. One of these is the investigation by Tuschl et al. [75] in 2006 where they assessed whether GSM modulated RF fields have adverse effects on the functional competence of human immune cells. Within the frame of a multidisciplinary project "Biological effects of high frequency electromagnetic fields (EMFs)" sponsored by the National Occupation Hazard Insurance Association (AUVA), in vitro investigations were carried out on human blood cells. Exposure was performed at GSM basic 1950 MHz, an SAR of 1 mW/g in an intermittent mode (5 min "ON", 10 min "OFF") and a maximum ΔT of 0.06 °C for the duration of 8 h. The following immune parameters were evaluated: (1) the intracellular production of interleukin-2 (IL-2) and interferon (INF) gamma in lymphocytes, and IL-1 and TNF-alpha in monocytes were evaluated with monoclonal antibodies. (2) The activity of immune-relevant genes (IL 1-alpha and beta, IL-2, IL-2-receptor, IL-4, macrophage colony stimulating factor (MCSF)-receptor, TNF-alpha, TNF-alpha-receptor) and housekeeping genes was analyzed with real time PCR. (3) The cytotoxicity of lymphokine activated killer cells (LAK cells) against a tumour cell line was determined in a flow cytometric test. For each parameter, blood samples of at least 15 donors were evaluated. No statistically significant effects of exposure were found and there was no indication that emissions from mobile phones are associated with adverse effects on the human immune system.

Chagnaud and Veyret [76] in 1999 could also not demonstrate an effect of low-level pulsed microwaves on the integrity of the immune system. They investigated the effects of GSM-modulated microwaves on lymphocyte sub-populations of Sprague-Dawley rats and their normal mitogenic responses using flow cytometry analysis and a colorimetric method. No alterations were found in the surface phenotype of splenic lymphocytes or in their mitogenic activity.

[N.B. One must always be very cautious when it comes to negative findings. For example: of the 100 or so papers on genotoxic effects of RF fields, a majority has been done with peripheral blood lymphocytes. Except for special cases, these cells are highly protected from their upregulated repair enzymes. These cells are often used to investigate chemical genotoxicity, because in these cases the toxicity often occurs due to the action of the repair enzymes. Repair-deficient cells remain intact! The mechanisms of action of EMFs may not be clearly understood, but it is unlikely they mimick such chemical enzyme-induced genotoxicity. — Yet another example is the use of mice and rats for the study increases in brain tumour incidence due to mobile telephony exposure. Since the incidence data from human studies point to a needed exposure time of at least 5 years, and mice and rats do not live longer

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than 2 years, the rats will die long before they have had a chance to develop the tumours!]

Irradiation by pulsed microwaves (9.4 GHz, 1 μs pulses at 1000/s), both with and without concurrent amplitude modulation (AM) by a sinusoid at discrete frequencies between 14 and 41 MHz, was assessed for effects on the immune system of Balb/c mice [77]. The mice were immunized either by sheep red blood cells (SRBC) or by glutaric–anhydride conjugated bovine serum albumin (GA-BSA), then exposed to the microwaves at a low rms power density (30 $\mu W/cm^2$; whole-body averaged SAR approximately 0.015 W/kg). Sham exposure or microwave irradiation took place during each of 5 contiguous days, 10 h/day. The antibody response was evaluated by the plaque-forming cell assay (SRBC experiment) or by the titration of IgM and IgG antibodies (GA-BSA experiment). In the absence of AM, the pulsed field did not greatly alter immune responsiveness. In contrast, exposure to the field under the combined-modulation condition resulted in significant, AM-frequency-dependent augmentation or weakening of immune responses.

To study the possible RF effects on human lymphocyte activation, Capri et al. [78] analyzed CD25, CD95, CD28 molecules in unstimulated and stimulated CD4+ or CD8+ T-cells in vitro. Peripheral blood mononuclear cells (PBMCs) from young and elderly donors were exposed or sham-exposed to RF (1800 MHz, SAR 2 W/kg) with or without mitogenic stimulation. No significant changes in the percentage of these cell subsets were found between exposed and sham-exposed lymphocytes in both young and elderly donors. Nevertheless, after RF exposure they observed a slight, but significant, downregulation of CD95 expression in stimulated CD4+ T-lymphocytes from elderly, but not from young donors. This age-related result is noteworthy given the importance of such a molecule in regulation of the immune response.

In the paper by Yurekli et al. [79], a GHz transverse electromagnetic (GTEM) cell was used as an exposure environment for plane wave conditions of far-field free space EMF propagation at the GSM base transceiver station (BTS) frequency of 945 MHz, and effects on oxidative stress in rats were investigated. When EMFs at a power density of 3.67 W/m^2 (SAR = 11.3 mW/kg), which is well below current exposure limits, were applied, MDA (malondialdehyde) level was found to increase and GSH (reduced glutathione) concentration was found to decrease significantly ($p < 0.0001$). Additionally, there was a less significant ($p = 0.019$) increase in SOD (superoxide dismutase) activity under EMF exposure.

Since genotoxic effects of the second generation standard GSM have been reported after exposure of human cells in vitro, Schwarz et al. [80] decided to use human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures and expose them to 1950 MHz UMTS below the SAR safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at an SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN ($p = 0.02$). At an SAR of 0.1 W/kg the CTF was significantly increased after 8 h of incubation ($p = 0.02$), the number of MN after 12 h ($p = 0.02$). However, under these conditions, no UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with phytohemagglutinin. The authors conclusion was that UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

Simkó and Mattsson [81] have presented a hypothesis of a possible initial cellular event affected by exposure to ELF-EMF, an event that is compatible with the multitude of effects observed after exposure. Based on an extensive literature review, they suggested that ELF-EMF exposure is able to perform such activation by means of increasing levels of free radicals. Such a general activation is compatible with the diverse nature of observed effects. Free radicals are intermediates in natural processes, like mitochondrial metabolism, and are also a key feature of phagocytosis. Free radical release is inducible by ionizing radiation or phorbol ester treatment, both leading to genomic instability. EMFs might be a stimulus to induce an "activated state" of the cell such as phagocytosis, which then enhances the release of free radicals, in turn leading to genotoxic events. Simkó and Mattsson envisaged that EMF exposure can cause both acute and chronic effects that are mediated by increased free radical levels: (1) Direct activation of, for example macrophages (or other cells) by short-term exposure to EMF leads to phagocytosis (or other cell-specific responses) and consequently, free radical production. This pathway may be utilized to positively influence certain aspects of the immune response, and could be useful for specific therapeutic applications. (2) EMF-induced macrophage (cell) activation includes direct stimulation of free radical production. (3) An increase in the lifetime of free radicals by EMF leads to persistently elevated free radical concentrations. In general, reactions in which radicals are involved become more frequent, increasing the possibility of DNA damage. (4) Long-term EMF exposure leads to a chronically increased level of free radicals, subsequently causing an inhibition of the pineal gland hormone melatonin. Taken together, these EMF-induced reactions could lead to a higher incidence of DNA damage and therefore, to an increased risk of tumour development. While the effects on melatonin and the extension of the lifetime of radicals can explain the link between EMF

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exposure and the incidence of for example leukaemia, the two additional mechanisms described by them specifically for mouse macrophages, can explain the possible correlation between immune cell system stimulation and EMF exposure.

5.7.4. Effects of EMFs on the immune system at pregnancy

Nakamura et al. [82] have investigated a very important issue, namely what happens to pregnant rats exposed to microwaves. Earlier data had indicated that these microwaves produce various detrimental changes based on actions of heat or non-specific stress, although the effects of microwaves on pregnant organisms were not uniform. This study was therefore designed to clarify the effect of exposure to microwaves during pregnancy on endocrine and immune functions. Natural killer cell activity and natural killer cell subsets in the spleen were measured, as well as some endocrine indicators in blood: corticosterone and adrenocorticotrophic hormone (ACTH) as indices of the hypothalamic–pituitary–adrenal axis, beta-endorphin, oestradiol, and progesterone in six female virgin rats and six pregnant rats (9–11 days gestation) exposed to microwaves at 10 mW/cm^2 incident power density at 2450 MHz for 90 min. The same measurements were performed in control rats (six virgin and six pregnant rats). Skin temperature in virgin and pregnant rats increased immediately after exposure to microwaves. Although splenic activity of natural killer cells and any of the subset populations identified by the monoclonal antibodies CD16 and CD57 did not differ in virgin rats with or without exposure to microwaves, pregnant rats exposed to microwaves showed a significant reduction of splenic activity of natural killer cells and CD16+ CD57- ones. Although corticosterone and ACTH increased, and oestradiol decreased in exposed virgin and pregnant rats, microwaves produced significant increases in beta-endorphin and progesterone only in pregnant rats. So, in summary, Nakamura et al. [82] showed that microwaves at the power of 10 mW/cm^2 produced activation of the hypothalamic–pituitary–adrenal axis and increased oestradiol in both virgin and pregnant rats, indicating that microwaves are a great stress in pregnancy.

In the following year, 1998, the same groups of scientists published a new study [83] in which they examined the involvement of opioid systems in reduced natural killer cell activity (NKCA) in pregnant rats exposed to microwaves at a relatively low level (2 mW/cm^2 incident power density at 2450 MHz for 90 min). They assayed beta-endorphin (betaEP) in blood, pituitary lobes, and placenta as well as splenic NKCA in virgin and/or pregnant rats. Although microwaves elevated colonic temperatures by 0.8 °C for virgin and 0.9 °C for pregnant rats, and betaEP in blood and anterior pituitary lobes (AP) significantly, it did not change blood corticosterone as an index of hypothalamic-pituitary adrenal axis. There were significant interactions between pregnancy and microwave exposure on splenic NKCA, betaEP in both blood and AP, and blood progesterone. Intra-peritoneal administration of opioid receptor antagonist naloxone prior to microwave exposure increased NKCA, blood, and placental betaEP in pregnant rats. Alterations in splenic NKCA, betaEP and progesterone in pregnant rats exposed to microwaves may be due to both thermal and non-thermal actions. These results suggest that NKCA reduced by microwaves during pregnancy is mediated by the pituitary opioid system.

To further clarify the effects of microwaves on pregnancy, uterine or uteroplacental blood flow and endocrine and biochemical mediators, including corticosterone, estradiol, prostaglandin E(2) (PGE(2)), and prostaglandin F(2)alpha (PGF(2)alpha), Nakamura et al. published yet another study in 2000 [84]. In this article they measured these parameters and factors in rats exposed to continuous-wave (CW) microwave at 2 mW/cm^2 incident power density at 2450 MHz for 90 min. Colonic temperature in virgin and pregnant rats was not significantly altered by microwave treatment. Microwaves decreased uteroplacental blood flow and increased progesterone and PGF(2)alpha in pregnant, but not in virgin rats. Intraperitoneal (i.p.) administration of angiotensin II, a uteroplacental vasodilator, before microwave exposure prevented the reduction in uteroplacental blood flow and the increased progesterone and PGF(2)alpha in pregnant rats. Increased corticosterone and decreased estradiol during microwave exposure were observed independent of pregnancy and pretreatment with angiotensin II. These results suggest that microwaves (CW, 2 mW/cm^2, 2450 MHz) produce uteroplacental circulatory disturbances and ovarian and placental dysfunction during pregnancy, probably through non-thermal actions. The uteroplacental disturbances appear to be due to actions of PGF(2)alpha and may pose some risk for pregnancy! [Could the above findings be part of the explanation behind the sensational findings of Magras and Xenos [85] from 1997?]

5.7.5. Synchronization of cerebral rhythms. Important for the brain-immune system axis?

Vecchio et al. [86] have reported that EMF from mobile phones affects the synchronization of cerebral rhythms. Their findings suggest that prolonged exposure to mobile phone emissions affect cortical activity and the speed of neural synchronization by interhemispherical functional coupling of EEG rhythms. This may be evidence that such exposure can affect the way in which the brain is able to process information, by interfering with the synchronization rhythms between the halves of the brain, and by deregulating the normal alpha wave 2 (about 8–10 Hz) and alpha 3 (10–12 Hz) bands. [Could such deregulation affect the brain-immune system axis? If so, what implications would it have in the short- as well as in the long-term?]

5.7.6. Classical contact allergy reactions

Finally, in addition, classical contact allergy reactions, such as chromate allergy, have been studied by Seishima et al. [87]. The background for the study was an earlier case

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report about a patient with allergic contact dermatitis caused by hexavalent chromium plating on a cellular phone. The new study described the clinical characteristics and results of patch tests (closed patch tests and photopatch tests were performed using metal standard antigens) in eight patients with contact dermatitis possibly caused by handling a cellular phone. The eight patients were four males and four females aged from 14 to 54 years. They each noticed skin eruptions after 9–25 days of using a cellular phone. All patients had erythema, and seven had papules on the hemilateral auricle or in the preauricular region. Three of eight patients had a history of metal allergy. Chromate, aluminium and acrylnitrile–butadiene–styrene copolymer were used as plating on the cellular phones used by these patients. The patch test was positive for 0.5%, 0.1% and 0.05% potassium dichromate in all eight patients. The photopatch test showed the same results. One patient was positive for 2% cobalt chloride and one for 5% nickel sulfate. Based on these data, it is important to consider the possibility of contact dermatitis due to a cellular phone, possibly caused by chromate, when the patients have erythema and papules on the hemilateral auricle or in the preauricular region.

6. Effects of electromagnetic fields on other biological systems

Some parallel investigations, pointing to severe biological effects that need to be mentioned are, for instance, the results of Roux et al. [88] in 2008. Using an especially designed facility, the Mode Stirred Reverberation Chamber, they exposed tomato plants (Lycopersicon esculentum Mill. VFN8) to low level (900 MHz, 5 V/m) EMF for a short period (10 min) and measured changes in abundance of three specific mRNA soon after exposure. Within minutes of stimulation, stress-related mRNA (calmodulin, calcium-dependent protein kinase and proteinase inhibitor) accumulated in a rapid, large and 3-phase manner typical of an environmental stress response. Accumulation of these transcripts into the polysomal RNA also took place (indicating that the encoded proteins were translated) but was delayed (indicating that newly-synthesized mRNA was not immediately recruited into polysomes). Transcript accumulation was maximal at normal Ca(2+) levels and was depressed at higher Ca(2+), especially for those encoding calcium-binding proteins. Removal of Ca(2+) (by addition of chelating agents or Ca(2+) channel blocker) led to total suppression of mRNA accumulation. Finally, 30 min after the electromagnetic treatment, ATP concentration and adenylate energy charge were transiently decreased, while transcript accumulation was totally prevented by application of the uncoupling reagent, CCCP. These responses occur very soon after exposure, strongly suggesting that they are the direct consequence of application of radiofrequency fields, and their similarities to wound responses strongly suggests that this radiation is perceived by plants as an injurious stimulus! [Furthermore, it is impossible to interpret these reactions as "psychological or psychiatric personality disturbances, cognitive malfunction, or likewise".]

Also, the data from Divan et al. [89] deserve to be mentioned. They examined the association between prenatal and postnatal exposure to cell phones and behavioral problems in young children. Mothers were recruited to the Danish National Birth Cohort early in pregnancy. When the children of those pregnancies reached 7 years of age in 2005 and 2006, mothers were asked to complete a questionnaire regarding the current health and behavioral status of children, as well as past exposure to cell phone use. Mothers evaluated the child's behavior problems using the Strength and Difficulties Questionnaire. Mothers of 13,159 children completed the follow-up questionnaire reporting their use of cell phones during pregnancy as well as current cell phone use by the child. Greater odds ratios for behavioral problems were observed for children who had possible prenatal or postnatal exposure to cell phone use. After adjustment for potential confounders, the odds ratio for a higher overall behavioral problems score was 1.80 (95% confidence interval = 1.45–2.23) in children with both prenatal and postnatal exposure to cell phones. Exposure to cell phones prenatally – and, to a lesser degree, postnatally – was associated with behavioral difficulties such as emotional and hyperactivity problems around the age of school entry. [An obvious follow-up question would be "What about immune function alterations?".] Naturally, and hopefully, these associations may be non-causal and may be due to unmeasured confounding. But if real, they would be of public health concern given the widespread use of this technology.

The exposure to non-thermal microwave EMF generated by mobile phones affects the expression of many proteins. This effect on transcription and protein stability can be mediated by the MAPK (mitogen-activated protein kinase) cascades, which serve as central signaling pathways and govern essentially all stimulated cellular processes. Indeed, long-term exposure of cells to mobile phone irradiation results in the activation of p38 as well as the ERK (extracellular signal-regulated kinase) MAPKs. Friedman et al. [90] recently have studied the immediate effect of irradiation on the MAPK cascades, and found that ERKs, but not stress-related MAPKs, are rapidly activated in response to various frequencies and intensities. Using signaling inhibitors, they delineated the mechanism that is involved in this activation. They found that the first step is mediated in the plasma membrane by NADH oxidase, which rapidly generates ROS (reactive oxygen species). These ROS then directly stimulate MMPs (matrix metalloproteinases) and allow them to cleave and release Hb-EGF (heparin-binding EGF (epidermal growth factor)). This secreted factor activates the EGF receptor, which in turn further activates the ERK cascade. Thus, their study demonstrates for the first time a detailed molecular mechanism by which electromagnetic irradiation from mobile phones induces the activation of the ERK cascade and thereby induces transcription and other cellular processes.

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The terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) assay, a well known technique widely used for detecting fragmented DNA in various types of cells, was used by Panagopoulos et al. [91] to detect cell death (DNA fragmentation) in a biological model, the early and mid stages of oogenesis of the insect *Drosophila melanogaster*. The flies were exposed in vivo to either GSM 900 MHz or DCS 1800 MHz radiation from a common digital mobile phone, for few minutes per day during the first 6 days of their adult life. The exposure conditions were similar to those to which a mobile phone user is exposed. Previous results from the same group [92–94] had shown a large decrease in the oviposition of the same insect caused by GSM radiation. The recent results suggest that this decrease in oviposition, is due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells, induced by both types of mobile telephony radiation. Induced cell death is recorded for the first time, in all types of cells constituting an egg chamber (follicle cells, nurse cells and the oocyte) and in all stages of the early and mid-oogenesis, from germarium to stage 10, during which programmed cell death does not physiologically occur. Germarium and stages 7–8 were found to be the most sensitive developmental stages also in response to electromagnetic stress induced by the GSM and DCS fields and, moreover, germarium was found to be even more sensitive than stages 7–8.

7. Conclusions

- Both human and animal studies report large immunological changes upon exposure to environmental levels of modern, human-made EMFs. Some of these exposure levels are equivalent to those of wireless technologies in daily life, and often at low or very low (i.e., non-thermal) levels.
- Measurable physiological changes (mast cells increases, for example) that are bedrock indicators of allergic response and inflammatory conditions are stimulated by EMF exposures.
- Chronic exposure to such factors that increase allergic and inflammatory responses on a continuing basis may be harmful to health. The data presented here, as well as the very rapid international increase in incidence of allergies, asthma and other oversensitivities, together form a clear warning signal.
- It is, thus, possible that chronic provocation by exposure to EMF can lead to immune dysfunction, chronic allergic responses, inflammatory responses and ill health if they occur on a continuing basis over time. This is an area that should be investigated immediately.
- Specific findings from studies on exposures to various types of modern equipment and/or EMFs report overreaction of the immune system; morphological alterations of immune cells; profound increases in mast cells in the upper skin layers, increased degranulation of mast cells and larger size of mast cells in electrohypersensitive individuals; presence of biological markers for inflammation which are sensitive to EMF exposure at non-thermal levels; changes in lymphocyte viability; decreased count of NK cells; decreased count of T-lymphocytes; negative effects on pregnancy (uteroplacental circulatory disturbances and placental disfunction); suppressed or impaired immune function; and inflammatory responses that can ultimately result in cellular, tissue and organ damage.
- The functional impairment electrohypersensitivity is reported by individuals in the United States, Sweden, Switzerland, Germany, Belgium, Italy, The Netherlands, Norway, Denmark and many other countries of the world. Estimates range from 3% to perhaps 10% of populations, and appear to be a growing condition of ill-health leading to lost work and productivity.
- The WHO and IEEE literature surveys do not include all of the relevant papers cited here, leading to the conclusion that evidence has been ignored in the current WHO ELF Health Criteria Monograph; and the proposed new IEEE C95.1 RF public exposure limits.
- The current international public safety limits for EMFs do not appear to be sufficiently protective of public health at all, based on the studies of immune function. New, biologically based public standards are warranted that take into account low-intensity effects on immune function and health that are reported in the scientific literature. Also the accessability needs of persons with the functional impairment electrohypersensitivity must be fully addressed and resolved as dictated by the UN 22 “Standard rules on the equalization of opportunities for people with disabilities” (about the UN 22 Standard Rules, see website: http://www.un.org; since 2007 they have been upgraded into the UN “Convention on Human Rights for Persons with Functional Impairments”).

The conclusion of the above must be that there are a number of very strong indications of EMFs being capable of disturbing the immune system and thus increasing disease, including cancer, risk. It is somewhat odd that professional epidemiologists for the last 50 years have not addressed the issue of reduced repair but only looked at increased cell damages from different agents and environments when trying to understand trend changes.

Based on this review as well as on the recent Bioinitiative Report [http://www.bioinitiative.org/] [1], it must be concluded that the existing public safety limits are inadequate to protect public health. From a public health policy standpoint, new public safety limits, and limits on further deployment of untested technologies, are warranted.

New biologically based public and occupational exposure are recommended to address bioeffects and potential adverse health effects of chronic exposure. These effects are now widely reported to occur at exposure levels significantly below most current national and international limits. Therefore, biologically based exposure standards are needed to prevent disruption of normal body processes. Effects are

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reported for DNA damage (genotoxicity that is directly linked to integrity of the human genome), cellular communication, cellular metabolism and repair, cancer surveillance within the body; and for protection against cancer and neurological diseases. Also reported are neurological effects including changes in brainwave activity during cell phone calls, impairment of memory, attention and cognitive function; sleep disorders, cardiac effects; and – as reported here – serious impact on the immune function (allergic and inflammatory responses).

The current recommendation must be a biologically based exposure limit that is completely protective against e.g. extremely low frequency and radiofrequency fields which, with chronic exposure, can reasonably be presumed to result in no adverse impacts on health and well-being. Today, such a completely protective safety limit would, for many exposure situations, be zero.

Finally, attention to the above need would also mean a great gain in future public health costs for the entire electrified world. To do the opposite could turn out to be very expensive.

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