10 IMMUNE SYSTEM AND HAEMATOLOGY

10.1 Epidemiological studies

No epidemiological studies on potential effects on the immune system and haematology were available at the time of the publication of the previous Environmental Health Criteria document in 1993. At the search performed for the current review five potentially relevant studies were identified. Three of these studies were excluded based on the exclusion criteria, and the remaining two are only briefly described below as they did not provide sufficient information to determine representativeness of participants.

Studies with insufficient information for assessment of inclusion criteria

Tuschl and colleagues investigated the effect of long-term occupational exposure to radiofrequency fields (RF) on immune function parameters in two cross-sectional studies including workers from different sectors (Tuschl et al., 1999; Tuschl et al., 2000). No information was provided on how the study participants were selected, and participation rates were not reported. Therefore, the results are not reported in a table.

The first study (Tuschl et al., 1999) included employees of three Austrian public health services: 18 physiotherapists exposed to RF (16 women and 2 men; aged 36 years on average ±10 yrs) and 13 sex- and age-matched unexposed controls (11 women and 2 men). The exposed and unexposed groups included similar proportions of smokers (44% and 39% respectively). The exposure sources consisted of short wave (27.12 MHz), decimeter (433.92 MHz), and microwave (2.45 GHz) diathermy devices. The exposure level could not be individually assessed, because of the high variability of time spent with the patient next to the diathermy devices, and of other exposure determinants (i.e. frequency and power of the diathermy device, cables and electrodes used, patient's dimension and position, and the way the electrodes are adjusted on the patient). However, based on measurements next to 18 diathermy devices (7 short wave, 10 decimeter, and 1 microwave), the whole-body exposure of the operator in the worst case condition was estimated and compared to the frequency specific exposure limits in force in Austria at the time of the survey. While no excess of the exposure limits were recorded for the microwave and decimeter wave devices, remarkable overexposure occurred at 6 out of 7 short wave devices, with maximum measured field strengths of 1000 V/m and 1.2 A/m. Blood samples were taken on Monday morning, in order to not interfere with the daily work of therapists and to avoid potentially confounding effects of stress during working hours. The end-points measured consisted of total leukocyte and lymphocyte counts, along with lymphocyte subsets [B cells (CD19+), total T cells (CD3+), T-helper (CD4+), T-suppressor (CD8+), natural killer cells (CD16+/CD56+), and activated T cells] determined by flow cytometry and monoclonal antibodies against surface antigens. The functional capacity of immunocompetent cells was also measured in both native blood (HLA-DR+ activated T cells), and after mitogenic stimulation ex-vivo with phytohemagglutinin, PHA (expression of interleukein-2 receptor determined by anti-CD25 antibody in PHA stimulated lymphocyte cultures). Total and differential leukocyte counts were in normal ranges for all study subjects and did not differ between exposed and unexposed groups. Similarly, there was no difference between groups in either percentage of activated T cell (HLA-DR+) in blood, or of CD25+ lymphocytes in PHA stimulated cultures. The CD4+/CD8+ ratio in lymphocyte cultures was positively correlated with the stimulation rate (number of S-phase cells).

In the second study (Tuschl et al., 2000), an extensive set of immunological indexes (total and differential leukocyte counts; T and B cell activation, along with production of interleukine-2, interferon-γ, and TNF-α in PHA and PWM stimulated cultures; serum level of immunoglobulins IgA, IgG, IgM; oxidative burst in monocytes and granulocytes after phagocytosis of E. coli) were compared between two groups of RF-exposed workers [10 industrial workers at induction heaters (IH), and 10 medical assistants operating magnetic resonance tomographs (MRI)] and 23 unexposed controls subjects employed at the same companies. Subjects in the MRI group were on average slightly younger (32 ± 6 years) than members of the IH (42 ± 12 years) and the control (40 ± 11 years) groups. Exposure at the individual level could not be assessed. However, magnetic flux density was measured at the center of the coil and at a distance of 0-20 cm from the MRI for each of the 5 tomographs operated by the study subjects; the Austrian exposure limit (8.75 mT) was exceeded in all 5 MRI rooms in percentages varying from 1006% to 8149%, but not in the surrounding rooms were the personnel was usually seated. The 21 induction heaters operated by the workers in IH groups differed in frequency (50 Hz to 21.3 kHz) and power (520-1200 KVA for the ELF devices; 6-75 kW for the devices operating at frequencies between 2.8 and 21.3 kHz); the estimated whole body or partial body exposures were often well above the Austrian limits, even though no exact value for overexposure could be given since the working process changed frequently and workers moved from one oven to another. No differences in total and differential leukocyte counts were observed between exposed and unexposed groups, apart from an increased frequency of natural killer (NK) cells

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in the IH group (average cell number per µl blood = 533 ± 388) compared to controls (276 ± 124). This finding was driven by particularly high numbers of NK cells (>700 / µl) detected in two workers often working in conditions where the exposure limit was exceeded by 2000%. Cultured monocytes from the IH group, compared to controls, showed a reduced oxidative burst, however, counteracted by an increased number of monocytes staining positively (thus the two groups did not differ in net capacity for oxidative burst).

Excluded studies:

(Boscol et al., 2001; Del Signore et al., 2000; Marino, 1995)

10.2 Volunteer studies

The previous WHO report on the effects of RF exposure issued in 1993 reported no study on the immune system and haematology. The current search identified only three relevant papers in this area of which one had uncertainties related to inclusion criteria (see Appendix X) and is therefore only presented in the text.

The table by the end of the section summarizes results of studies forming the basis for the final analysis and provide information about study details including study design. Similar and further details are included in the following text. Comments about particularly small samples sizes are made since the smallest samples are attached with highest uncertainties provided other study details are similar. Exposure was controlled in all studies that are included in the analysis as basis for the health risk assessment. If SAR was provided, it is specified in both the tables and text. Otherwise other exposure measures are provided.

10.2.1 Mobile phone related studies

Radon et al. (Radon et al., 2001) evaluated the effects of GSM 900 MHz mobile phone signal (SAR = 0.025 W/kg) on two markers of immune system activation: immunoglobulin A (IgA) and neopterin, in eight male volunteers. The signals were emitted by circularly polarized antenna positioned 10 cm behind the head. The experimental protocol consisted of twenty 4-hour sessions per participant in the experimental chamber, with sessions 2 days apart after the experiment had been performed during the day and 3 days apart after nightly sessions. Half of the experiments (ten 4-hour sessions) were conducted with EMF exposure and the others with sham exposure in random order, and the sessions were evenly distributed between day and night. The study was performed double blind. The same time of day was used for all day and night sessions, respectively, and saliva was collected every 30 minutes during and after exposure. The results did not show any significant differences in salivary IgA and neopterin between the exposed and sham exposed conditions. [The weight of this study is limited due to its small sample size.]

Papers with uncertainties related to inclusion criteria

In the single blind and crossover study reported by Kimata et al. (Kimata, 2005) 30 patients with atopic eczema dermatitis syndrome who reacted to latex were exposed to mobile phone radiation for 30 minutes. After exposure, mononuclear cells from blood were cultured and specific-allergen immunoglobulin E (IgE) and cytokine production were determined. Three of the tested compounds increased significantly from before to after exposure and two other showed a significantly decrease. No significant changes were observed from before to after the sham exposure. [The paper did not present any statistical comparison between RF and sham exposure. Furthermore, no exposure information was provided, just that the phone was "transmitting but without sound". Since the session with real exposure was always 2 weeks before the similar session with sham exposure the results might have been biased by an order effect. Therefore interpretation of the reported results is difficult.]

10.2.2 Mobile phone base station related studies

Augner et al. (Augner et al., 2010) investigated whether GSM 900 MHz base station signals from a real base station mounted on the façade of the testing room may have an effect on bodily defence systems, by using saliva IgA concentration as an indicator for alteration of the immune system. By applying different types of shielding, three different exposure levels were obtained. The power density was measured during all exposure sessions and the average values were calculated for each condition: high (2126.8 µW/m²), medium (153.6 µW/m²) and low (5.2 µW/m²). Fifty-seven participants were randomly assigned to receive one of three exposure scenarios, each consisting of five 50-minute exposure sessions separated from each other by 5-minute intervals. The scenarios were "HM" (low exposure, high exposure, low, medium, low) with 22 volunteers, "MH" (low, medium, low, high, low) with 26 volunteers and "LL" (low, low, low, low, high), the control scenario with 9

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volunteers. All scenarios were conducted at the same time of day. Saliva samples were taken after 10, 25, and 45 minutes of each exposure, and analyses were performed by including age, gender, and degree of self-rated electromagnetic hypersensitivity as covariates. IgA concentrations were investigated without detecting any effect of exposure. [A limitation of this study is the low number of participants in the control condition (n = 9) that used the lowest exposure level in all sessions. In the same study (Augner et al., 2010) tested potential effects on cortisol and alpha amylase, see Section 7.2.2.]

Table 10.2.1. Studies assessing effects of RF EMF on immune system and haematology

Endpoint and participants	Exposure[a]	Response	Comment	Reference
Mobile phone handset related studies				
Salivary, IgA and neopterin samples taken during and after exposure 8 male volunteers (20-30 years)	GSM mobile phone signal emitted by circularly polarized antenna 10 cm behind head, 900 MHz SAR_{10g} 0.025 W/kg 4 h: 12:00 – 16:00 or 22:00 – 02:00, 10 times with RF and 10 with sham	No effect of exposure.	Double blind, randomized, counterbalanced. Small group. For neuroendocrine results see Section 7.1.	Radon et al. (2001)
Mobile phone base station related studies				
Salivary IgA samples taken before and after exposure 57 volunteers (18–67 years; 22 males, 35 females)	Real GSM 900 MHz base station on the building, shielding to reduce exposure.. L = 5.2 µW/m^2 M = 153.6 µW/m^2 H = 2126.8 µW/m^2 5 sessions of 50 min each between 09:00 and 13:30 HM scenario:L+H+L+M+L (n=22) MH ccenario:L+M+L+H+L (n=26) LL scenario:L+L+L+L+H (n=9)	No effect of exposure.	Double blind, randomized, between groups. Small group in control scenario (3). Not specified corrections for multiple comparisons. For neuroendocrine results see Section 7.1.2; for well-being see Augner et al. (2009) in Section 5.2.4.	Augner et al. (2010)

Abbreviations: GSM: Global System For Mobile Communication; IgA: immunoglobulin A

[a] SAR with relevant averaging volume (e.g. SAR_{10g}) is specified if included in the paper

10.3 Animal studies

In the WHO (1993) report, haematological effects were evidenced in animals exposed to RF, mainly associated with a significant rise in temperature. In the same way, exposure has been reported to affect various components of the immune system, with both inhibitory and stimulatory responses, mostly in a transient mode and usually consequent to thermal stress.

Through an extensive database search, 27 publications on effects on the immune system and hematologic parameters carried out in animal models were retrieved. Among them 23 articles are on immune system. Four articles specifically dealing with haematology are also reported. A distinction is made between studies on adult animals (19 papers) and studies employing prenatal exposure and exposure of newborn animals (four papers). The immune system in early life stages is not yet completely formed and activated, thus it can be more vulnerable to EMF exposure.

10.3.1 Immune system

10.3.1.1 Exposure of adult animals

Studies with exposure to RF alone

Veyret et al. (1991) exposed BALB/c mice to pulsed 9.4 GHz microwaves, both with and without concurrent amplitude modulation (AM) for 10 h a day during 5 days at a whole-body SAR of 0.015 W/kg. Fourteen experiments were conducted, one without AM, and the others with AM in the range 14–41 MHz. For

each of these experiments there were 12 RF EMF-exposed mice and 12 sham-exposed. Each mouse was immunized intraperitoneally on day 1 with sheep red blood cells (SRBC) just before exposure, and killed on day 6. Other groups of 20 mice for each different frequency (10 RF EMF exposed and 10 sham-exposed) received glutaric-anhydride-conjugated bovine serum albumin (GA-BSA) just before the first exposure. These mice were immunized a second time on the 8th day and were killed 3 weeks after the first immunization. Splenic cells and sera were collected to evaluate titers of antibodies (IgM and IgG). Exposure to the pulsed field alone resulted in a modest increase in antibody responsiveness, in particular in IgM ($p<0.01$-0.05). Addition of the Plaque-forming cell (PFC) counts (number of cells that produce antigen-specific antibodies) and antibody titers were significantly decreased by amplitude modulation at 14, 36, and 41 MHz and increased at 21 and 32 MHz.

Chou et al. (1992) investigated the effects of long-term exposure to pulsed RF EMF in 100 Sprague Dawley rats. The animals were exposed or sham-exposed for 21.5 h per day for 13 or 25 months to pulsed 2450 MHz at SARs ranging from 0.4 W/kg for a 200 g rat to 0.15 W/kg for a 800 g rat. B and T-cells numbers, and complement-receptor-bearing (CRPC) lymphocytes, proliferative response of spleen lymphocytes to Phytohaemagglutinin (PHA), Concanavalin A (ConA), Pokeweed mitogen (PWM), lipopolysaccharide (LPS), Mycobacterium tuberculosis-extracted Purified Protein Derivative (PPD) and direct Plaque-Forming Cell (PFC) assay were assessed. The first set of analysis was performed at 13 months and the final examination at the end of exposure time (25 months). At 13 months, both B- and T-cells were significantly increased in exposed animals, while there was no difference in CPRC. At 25 months, no significant difference in both B- and T-cells and CPRC lymphocytes were detected. [This paper is also discussed in sections 7.3.2 (Other hormones) and 12.2.2 (Cancer).]

Elekes et al. (1996) exposed BALB/c mice to 2450 MHz fields, either continuous wave (CW) or 50 Hz square wave amplitude-modulated (AM), 3 h per day for 6 consecutive days at a whole-body SAR of 0.014 ± 0.02 W/kg. The mice were immunized on the second day of the exposure and bled 5 days later. The authors observed a moderate and statistically significant increase in antibody production in male mice only and after both types of exposure conditions, but no significant effects on spleen parameters..

Chagnaud and Veyret (1999) analysed spleen lymphocyte subpopulations and their mitogenic activity in Sprague Dawley rats exposed to GSM-modulated RF EMF 2 h per day for 10 days at a whole-body SAR of 0.075 or 0.27 W/kg. None of the investigated endpoints (expression of CD4, CD8, and MHC class II molecules and mitogenic response) were affected by the exposure. No effect was seen on the number of cells expressing the surface markers CD4 (helper T-cells), CD8 (cytotoxic T-cells) or immunoglobulin A (IgA-expressing B-cells). In addition, the mitogenic response of splenic lymphocytes to the mitogen Con-A was unchanged [Lymphocyte subpopulations were studied by flow-cytometry on their pattern of surface markers.]

Three studies by the same group evaluated the effects of RF EMF on the immune system in mice: two dealing with peripheral lymphocytes and B-cell peripheral differentiation and antibody response (Gatta et al., 2003; Nasta et al., 2006) and one with the immuno-hematopoietic potential of bone marrow cells (Prisco et al., 2008). In these studies, C57BL/6 female mice were exposed or sham-exposed to a 900 MHz GSM signal at whole-body SARs of 1 or 2 W/kg for 2 h per day, 5 days per week up to 4 consecutive weeks.

The first study (Gatta et al., 2003) examined the effects on numbers and proliferation of T- and B-lymphocytes, expression of activation markers (CD25 and CD69) and cytokine (IL2 and IFNγ) production. In the second study they investigated peripheral differentiation of B-lymphocytes in spleen, basal serum concentrations of antibodies (IgM and IgG), and production of IgM and IgG in response to mitogen (LPS)-induced in vitro stimulation or antigen-specific in vivo immunization (Nasta et al., 2006). No differences between exposed and sham-exposed animals were reported in both studies.

In the study by Prisco et al. (2008), X-irradiated mice were transplanted with bone marrow cells from donor mice that had been previously either sham-exposed or exposed to 900 MHz RF EMF at an SAR of 2 W/kg. All recipient animals survived for 6 weeks (when they were sacrificed) whereas not-transplanted X-irradiated mice died by ten days. Several immunological parameters were examined at different time points after bone marrow cell transplantation. No differences were observed in the ability of exposed or sham-exposed bone marrow precursor cells to home and colonize lymphoid organs and differentiate in phenotypically and functionally mature T- and B-lymphocytes.

Jin et al. (2012) assessed the effect of simultaneous exposure to two types of RF EMF signals, single code division multiple access (CDMA), 849 MHz and wideband code division multiple access (WCDMA), 1900 MHz, on the immune system of rats (SAR = 2 or 4 W/kg). Animals were exposed for 45 min per day, 5 days per

week up to 8 weeks. Body weight, histopathological changes in the spleen, spleen lymphocyte subpopulations, haematological analysis and apoptosis were evaluated. None of the haematological parameters showed significant alterations, no apoptosis was detected and no tumours were observed in the spleen upon autopsy (see Section 12.2.2). No alterations were found in the production of antibodies (IgM and IgG). The expression of genes for TGFβ, IFNγ, IL1β, IL6 and TNFα did not differ between real and sham-exposed rats. No differences in IL6 and TNFα production were also found at protein level.

A group of Soviet studies published in Russian during the 1970–'80s described several effects on the immune system of rats following chronic exposures to radiofrequencies. Replication of these studies was recommended in the WHO's International EMF Project research agenda (WHO, 2006). In particular, experiments with a protocol similar to that of Shandala and Vinogradov (1982) were conducted in Bordeaux (Poulletier de Gannes et al., 2009) and Moscow (Grigoriev et al., 2010) under the supervision of an international oversight committee. In both laboratories, Wistar rats were exposed or sham-exposed to continuous 2450 MHz fields for 7 h per day, 5 days per week for a total of 30 days at a whole-body SAR of 0.16 ± 0.04 W/kg. In the Russian laboratory the complement fixation assay (CFA) was used to assess the presence of antibodies to brain and liver tissues in sera from exposed vs. sham-exposed animals, according to the original protocol. In both laboratories, antibodies directed to a panel of self and altered self antigens were assessed by an ELISA (Enzyme Linked Immunosorbent Assay) test. The "pathogenic potential" of the antibodies present in sera from exposed (and sham-exposed) animals was assessed by injecting sera in pregnant rats, according to the original protocol.

In the French laboratory (Poulletier de Gannes et al., 2009), ELISA was used to assess auto-antibodies and injection of sera from RF-exposed rats in pregnant rats was used to evaluate pathologic effects. No effects were detected on circulating antibody levels in blood serum using a panel of 16 antigens. No teratological effects or postnatal changes in development were observed in offspring of rats injected with sera from exposed or sham-exposed rats. This study therefore did not confirm the original results (Shandala & Vinogradov, 1982). In contrast, results from the Russian laboratory (Grigoriev et al., 2010) partially confirmed the observations from the early studies indicating possible effects of radiofrequencies on autoimmune processes. An increase in titers of antibodies (as assessed by CFA) to brain and liver tissue antigens was reported, but the authors conclude that the changes did not appear to be pathogenic. Adverse effects (embryo mortality) due to the injection of sera from exposed rats into pregnant rats were also described. The International Oversight Committee (IOC) published a paper with comments and conclusions on the differing results between the two studies on both replication studies (Repacholi et al., 2011). According to the IOC, when viewed as a whole, the results of Poulletier de Gannes et al. (2009) and Grigoriev et al. (2010) did not provide support for the original Soviet study results. Although Grigoriev et al. (2010) reported that they did confirm some of the immunological and teratological findings of the Soviet studies, following a very detailed analysis of both studies, the IOC concluded that this was not convincing (Repacholi et al., 2011). More recently, a review with an appendix containing the translation of the Soviet-era studies that constituted the scientific basis for the Soviet and Russian radiofrequency standards for the general public has been published (Repacholi et al., 2012).

Studies with exposure to RF and effect modifiers

Some studies have shown that millimeter EMF (range of tens of GHz) can beneficially modulate immune responses. In cancer treatment, millimeter EMF therapy has been used in combination with chemo- and radiotherapy to increase immunity, and to reduce the toxic effects of chemotherapy. The group of Ziskin published a number of systematic studies on the modulation of the immune system by millimeter EMF and the anti-cancer drug cyclophosphamide (CPA) (Logani et al., 2012; Makar et al., 2003; 2005; 2006). BALB/c mice were exposed locally on the nose to 60 Hz-modulated 42.2 GHz or 61.2 GHz EMF, for 30 min per day on three days, at thermographically-estimated local (in the nose area) peak SAR levels ranging from 600 to 800 W/kg, resulting in a temperature increase in the nose area of 1–1.5 °C. On day 2, CPA was administered intraperitoneally just before the EMF exposure. The 2003 study indicated that 42.2 GHz EMF can protect T-cell functions from the toxicity of CPA (Makar et al., 2003). After the EMF exposure, they also observed upregulation of cytolytic activity of NK cells, as well as T-lymphocyte recovery through upregulation of activation and effector functions of CD4 (Makar et al., 2005). T- lymphocytes, B- lymphocytes and macrophage functions were investigated in a later study (Makar et al., 2006). Here they showed upregulation of TNFα production in peritoneal macrophages that were immunosuppressed by CPA administration (a single i.p. injection), as well as a significant increase in IFNγ production by splenocytes and enhanced proliferative activity of T-lymphocytes. Conversely, no changes were observed in IL10 level and proliferation of B-lymphocytes. [The data strongly suggest that the EMF exposures accelerated recovery through the activation of cell-mediated immunity, where both CD4+ and CD8+ T-lymphocytes play a significant role. Furthermore, the results indicated that TNFα and IFNγ are important cytokines mediating the process.] Finally in Logani et al. (2012) they

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observed that CPA treatment caused suppression of cytokines, shifting the balance toward the production of Th2 cytokines. Production of Th1 cytokines that had been suppressed by CPA treatment was upregulated (exposed vs. sham $p<0.005$ in CPA+β-funaltrexamine treated mice; exposed vs. sham $p<0.005–0.05$ in CPA+naltrindole methansulfonate treated mice) after EMF exposure.

10.2.1.2 Exposure of foetal and juvenile animals

The effects of exposure to RF EMF during pregnancy were reported by Nakamura and co-workers in a series of papers (Nakamura et al., 1997; 1998). Pregnant and non-pregnant Wistar rats were exposed to 2450 MHz RF EMF for 90 min at different whole-body SAR levels. Pregnant rats exposed to a SAR of 1.8–2.2 W/kg showed a significant reduction of splenic activity of NK cells ($p<0.05$), while the splenic activity in exposed virgin rats was similar to that in unexposed pregnant rats (Nakamura et al., 1997) It cannot be excluded that this is a thermal effect, since a significant increase of tail skin temperature ($p<0.05$ for 15 min exposure, $p<0.01$ for 30 min, $p<0.001$ for 45–90 min) was reported in exposed versus sham rats. In a subsequent paper (Nakamura et al., 1998) they demonstrated again that there are differences in the endocrine and immune responses to RF EMF between non-pregnant and pregnant rats. An i.p. injection of the opioid receptor antagonist naloxone was given prior to EMF exposure. This resulted in an increase in natural killer cell activity, blood parameters and placental β-endorphin in pregnant rats. Both pregnancy and EMF exposure were found to induce immunosuppression, which could be of benefit to maintain the pregnant status, mediated by activation of placental progesterone and placental or pituitary β-endorphin..

Sambucci et al. (2010) examined the early and late effects of daily exposure to a WiFi signal during pregnancy, with particular emphasis on the immune system, in C57BL/6 mice. Pregnant mice were exposed to a WiFi-like 2450 MHz field (SAR = 4 W/kg) for 2 h per day on days 5–19 after conception. No significant effects on pregnancy outcome and on immune parameters in offspring, including B-lymphocyte numbers, proliferation and antibody production were found in male and female offspring at 5 and 26 weeks of age. Conversely, differences due to confinement stress during exposure, or to gender- and/or age-related changes were observed. In a following study Sambucci et al. (2011) investigated the effects of the exposure to WiFi signals during the first 5 weeks after birth. Newborn mice were exposed daily to an SAR of 0.08 or 4 W/kg, 2 h per day, starting the day after birth. No effects on body weight and development were found in mice of both sexes. From the immunological analyses no effects on T-lymphocytes maturation in thymus were observed. In the spleen CD4, CD8 and B-lymphocyte frequencies as well as cell proliferation and IL2 production were not affected by the exposure. Nevertheless, a reduced IFNγ production in spleen cells from males exposed to an SAR of 4 W/kg) compared with sham-exposed animals was described ($p<0.05$). This effect was not observed in females. No effects on antibody levels in serum were found. A further study (Laudisi et al., 2012) on the effects of WiFi exposure (SAR = 4 W/kg) during pregnancy reported no effects on T- lymphocytes differentiation in thymus and in peripheral organs (spleen) in 5 and 26 weeks old offspring, showing also no alterations in cell phenotype, proliferation and cytokine production, comparing sham-exposed and exposed mice. [Effects of restraint and gender/age-associated differences were observed: there was a reduction in thymus cell number and in thymocyte proliferation in males; IL2 production in spleen cells was reduced in males and females; IFNγ production in spleen cells was reduced due to constraint in old male (not in young males and not in females.]

No effects on levels of antibodies directed to antigens related to damage and/or pathological markers, were observed also by Aït-Aïssa et al. (2012) in Wistar rats exposed to WiFi signals 2 h per day 5 days per week both during pregnancy (days 6 to 21 after conception) and the first 5 weeks after birth. Whole body SARs in the embryos of 0 (sham), 0.08, 0.4 and 4 W/kg were considered.

Studies not included in the analysis

Fesenko et al. (1999) examined the effect of weak RF EMF on the secretion of tumour necrosis factor (TNF) and the proliferative response of T-lymphocytes in NMRI mice. The animals were exposed to RF EMF with a single frequency of 10 GHz or with a multiple frequency signal, known as swept frequency (range 8.15–18 GHz), over periods ranging from 2 h to 7 days. For the 10 GHz exposure, TNF production in macrophages was enhanced with exposure durations between 3 h and 3 days, while after 7 days it was decreased (no p-values were provided). Following a 24-h swept frequency exposure, TNF production in macrophages was significantly increased immediately after exposure and 24 and 72 h later, while in T-cells the increase was only measured at 0 and 24 h after exposure ($p<0.05$). They also observed stimulation of proliferation of T-cells (no p-values provided). [The presentation of the results and the assessment of the significance of differences is unclear. This prevents a meaningful analysis of this study.]

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In Novoselova et al. (1999) the same researchers assessed the effect of 5 h exposure to the swept frequency signal. In macrophages and T-cells they observed an increased TNF production at 0 and 24 h following exposure ($p<0.05$) [at 48 h after exposure in both cells types an increase larger than that at 0 h is reported, but without significance]. Mice fed antioxidants before and during exposure showed a similar increase in both TNF production in both cell lines immediately after exposure, a reduced increase at 24 and 48 h after exposure and a higher increase at 72 h after exposure [differences were only assessed between exposed and control groups, not between group with and without antioxidants]. Proliferation of T-cells was observed in some cases, but significance was indicated only for the control groups. [The presentation of the results and the assessment of the significance of differences is unclear. This prevents a meaningful analysis of this study.]

Table 10.3.1. Animal studies on the immune system

Endpoint, animals, number per group, age at start	Exposure: source, schedule, level, freely moving or restrained, co-exposure	Response	Comments	Reference
Exposure of adult animals				
Antibody response Mouse: BALB/c (n =10 or 12) 6 weeks	Coaxial antenna, pulsed 9.4 GHz (1 µs width, 5 ns rise/fall time, 1000 pulses per s) and AM (14–41 MHz), 10 h/d, 5 d WBA SAR 0.015 W/kg Free	Increase of IgM titers but not of IgG and PFC with pulse modulation alone. Increase, decrease or no effect on PFC and antibody titers dependent on frequency of signal modulation.	Immunization just before exposure with SRBC or GA-BSA.	Veyret et al. (1991)
Lymphocyte number and function Rat: Sprague Dawley (n=100) 8 weeks	Loop antenna, pulsed 2450 MHz, 10 µs width, 800 pulses per s 21.5 h/day, 13 or 25 months WBA SAR 0.15–0.4 W/kg Free	Increase of B- and T-cells at 13 months. No effect at 25 months. No effect on complement-receptor-bearing (CRPC) lymphocytes.	First set of analysis at 13 months and the final examination at the end of exposure time (25 months).	Chou et al. (1992)
Spleen index (spleen weight in mg/body weight in g), number and function of spleen cells; antibody production Mouse: BALB/c (n=8 or 16) Age not provided	Horn antenna, 2450 MHz CW or AM modulated 3 h/day, 6 days, 1 weeks WBA SAR 0.14 W/kg Free	After both types of exposure elevation of antibody production in males, but not in females. No effect on spleen parameters.	Immunization on the second day of exposure by SRBC.	Elekes et al. (1996)
Spleen lymphocytes subpopulation and mitogenic activity Rat: Sprague Dawley (n=10 or 15) 10 weeks	Horn antenna, 900 MHz GSM 2 h/day, 10 days WBA SAR 0.075 or 0.270 W/kg Restrained	No effects.	Lymphocyte subpopulation studied by flow-cytometry on their pattern of surface markers.	Chagnaud & Veyret (1999)
Spleen lymphocytes number and functions Mouse: C57BL/6 female (n=4) 2 months	Long TEM cell, 900 MHz GSM 2 h/day, 5 days/week, 1, 2, 4 weeks WBA SAR 1–2 W/kg Restrained	No effects.		Gatta et al. (2003)
B- lymphocytes peripheral differentiation and antibody production. Mouse: C57BL/6 female (n=8) 3 months	Long TEM cell, 900 MHz GSM 2 h/day , 5 days/week, 4 weeks WBA SAR 2 W/kg Restrained	No effects.		Nasta et al. (2006)

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Immuno-hematopoietic functions of bone marrow cells Mouse: C57BL/6 female (n=8 or 16) 3 months	Long TEM cell, 900 MHz GSM 2 h/day , 5 days/week, 4 weeks WBA SAR 2 W/kg Restrained	No effects.	X-irradiated mice transplanted with bone marrow cells from mice previously exposed to 900 MHz GSM signal.	Prisco et al. (2008)
Body weight, histopathology, haematology, apoptosis, cytokines and antibody production. Rat: Sprague Dawley (n=80) 9 weeks	Patch antenna in reverberating chamber, CDMA-849 MHz and WCDMA-1900 MHz modulations 45 min/day, 5 days/week, up to 8 weeks SAR 2 or 4 W/kg for combined CDMA and WCDMA exposure Free	No effects.		Jin et al. (2012)
Immunological parameters Rat: Wistar (n=16) 4–5 weeks	Helical antenna, 2450 MHz 7 h/day, 5 days/week, 6 weeks SAR 0.16 W/kg (whole body and brain averaged) Free	No effects.	Replication of Soviet-era studies (Shandala & Vinogradov, 1982). ELISA used to assess auto-antibodies. Injection of sera from RF-exposed rats in pregnant rats used to evaluate pathologic effects.	Poulletier de Gannes et al. (2009) see also Grigoriev et al. (2010) and Repacholi et al. (2012)
Immunological parameters Rat: Wistar (n=16) 4–5 weeks	Helical antenna, 2450 MHz 7 h/day, 5 days/week, 6 weeks SAR 0.16 W/kg (whole body and brain averaged) Free	Minor increase in auto-antibody to brain tissue antigens (when assessed by CFA). Adverse effects of injected sera on pregnancy and foetal development.	Replication of Soviet-era studies (Shandala & Vinogradov, 1982). ELISA and CFA used to assess auto-antibodies. Injection of sera from RF-exposed rats in pregnant rats used to evaluate pathologic effects.	Grigoriev et al. (2010) see also Poulletier de Gannes et al. (2009) and Repacholi et al. (2012)
Studies including co-exposures				
Cyclophosphamide (CPA)-induced suppression of T-lymphocyte function Mouse: BALB/c male (n=24) Age not provided	Horn antenna 42.2 GHz, 60 Hz modulated 0.5 h/day, 3 days Power density 31 mW/cm^2 (310 W/m^2), local SAR 622 W/kg at nose tip Restrained	T-lymphocyte function protected from CPA toxicity.	Single i.p. CPA injection.	Makar et al. (2003)
NK cell activation after CPA treatment. Mouse: BALB/c male (n=6) Age not provided	Horn antenna 42.2 GHz, 60 Hz modulated 0.5 h/day, 3 days Power density 31 mW/cm^2 (310 W/m^2) , local SAR 622 W/kg at nose tip Restrained	Upregulation of NK activity and T-lymphocyte recovery.	Single i.p. CPA injection.	Makar et al. (2005)
T-cell, B-cell and macrophage function after CPA treatment Mouse: BALB/c male (n=9) Age not provided	Horn antenna 61.22 GHz 0.5 h/day, 3 days Power density 31 mW/cm^2 (310 W/m^2), local SAR 855 W/kg at nose tip Restrained	Accelerated recovery of immune cell activation.	Single i.p. CPA injection.	Makar et al. (2006)

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CPA-induced suppression of cytokine release by T-cells in the presence of selective opioid receptor antagonists (ORA) Mouse: Balb/C (n=8) 7 weeks	Horn antenna 42.2 GHz 0.5 h/day, 3 days Power density 38 mW/cm^2 (380 W/m^2), local peak SAR 681 W/kg in the nasal area Restrained	Upregulation of Th1 cytokine production that was suppressed by CPA. Selective kappa (κ) ORA further potentiated effect on Th1 cytokine production, whereas treatment with μ or δ ORA increased the imbalance of cytokine production in the Th2 direction.		Logani et al. (2012)
Exposure of foetal and juvenile animals				
Cellular immunity and placental steroids in pregnant rats Rat: Wistar female virgin or pregnant (n=6) Age not provided	Rectangular aperture antenna 2450 MHz, CW 90 min WBA SAR 1.8–2.2 W/kg Restrained	Significant reduction of splenic activity of NK and CD16+ and CD57-cells in pregnant rats.	Probable thermal mediated effects.	Nakamura et al. (1997)
Assessment of β-endorphin (βEP) in blood, pituitary lobes, and placenta as well as splenic NK activity in virgin and/or pregnant rats Rat: Wistar (n=6 or 18) Age not provided	Rectangular aperture antenna 2450 MHz 90 min WBA SAR 0.36–0.44 W/kg Restrained	Increase of body temperature and βEP in blood and pituitary, but not placenta. No effect on NK activity.	I.p. injection of opioid receptor antagonist naloxone prior to the exposure increases NK activity, blood parameters and placental βEP in pregnant rats.	Nakamura et al. (1998)
Pregnancy outcome; peripheral B-lymphocyte s and antibody production in offspring Mouse: C57BL/6 female pregnant (n=16); offspring (n=6–12) 3 months	Long TEM cell, 2450 MHz WiFi 2h/day, on days 5–19 after conception WBA SAR 4 W/kg Restrained	No effects.	Effects due to restraint and gender/age-associated differences. Evaluation of early and late effects in both offspring genders.	Sambucci et al. (2010)
T- and B-lymphocyte maturation, cytokines, cell proliferation, antibodies, in newborn mice Mouse: C57BL/6 male and female (n=16) 5 weeks	Long TEM cell, 2450 MHz WiFi 2h/day , 5 days/week, 5 weeks, starting 1 day after birth (newborn mice) WBA SAR 0.08, 4 W/kg Restrained	Reduction of IFNγ production in males (not in females) exposed to 4 W/kg. No effects on all the other parameters investigated.		Sambucci et al. (2011)
Thymocyte development and peripheral T-lymphocyte compartment (proliferation, cytokines) in mice exposed in utero Mouse: C57BL/6 male and female (n=11–12) Embryonal day 4.5	Long TEM cell, 2450 MHz WiFi 2h/day , on gestational days 5–-19 WBA SAR 4 W/kg Restrained	No effects.	Effects due to restraint and gender/age-associated differences. Evaluation of early and late effects in both offspring genders.	Laudisi et al. (2012)

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Screening of immune markers in sera and gestational outcome Rat: Wistar male (n=9 or 12) Embryonal day 6	Reverberation chamber,2450 MHz, WiFi 2 h/day, 5 days/week, from gestational day 6 up to 5 weeks after birth WBA SAR 0.08, 0.4, 4 W/kg Free	No effects.	SAR values refer to embryo exposure.	Aït-Aïssa et al. (2012)

Abbreviations: AM: amplitude modulation; βEP: β-endorphin; CDMA: code division multiple access; CFA: complement fixation assay; CPA: cyclophosphamide; CRPC: complement receptor bearing; CW: continuous wave; GA-BSA: glutaric-anhydride-conjugated bovine serum albumin; GSM: global system for mobile communication; ELISA: immune-linked sorbent assay; IgG: immunoglobulin G; IFNγ: interferon gamma; NK: natural killer; ORA: opioid receptor antagonist; PFC: plaque-forming cell; SRBC: sheep red blood cells; TEM: transverse electromagnetic mode; WBA SAR: whole body SAR; WCDMA: wideband code division multiple access.

10.2.2 Haematology

Braithwaite et al. (1991) investigated the effect of exposure (average of 9 minutes per hour, 24 h/day, for 20 days) of 1-week old chicken (n=22) to a continous-wave 2450 MHz field at a power density of 13 mW/cm^2 (130 W/m^2). No effects were reported on blood chemistry, leucocyte count and morphology and histology in spleen, bursa of Fabricius, adrenal and thyroid.

Trošić and co-workers investigated the influence of 2450 MHz RF EMF exposure at an SAR of 1–2 W/kg on blood-forming cells in Wistar rats (Trošić, Busljeta & Pavičić, 2004; Trošić & Busljeta, 2006). The animals were exposed 2 h/day for 7 days and blood samples were taken at 2, 8, 15 and 30 days. Trošić, Busljeta & Pavičić (Trošić, Busljeta & Pavičić, 2004) reported a decrease in lymphoblast count at days 15 and 30, but no effect on leukocyte and lymphocyte counts. They speculated that this could be due to an adaptation to stress related to the exposure conditions. Trošić & Busljeta (Trošić & Busljeta, 2006) observed an increase in erythrocyte count, haemoglobin, and haematocrit in peripheral blood on exposure days 8 and 15. Concurrently anuclear cells and precursor cells of erythrocytes were significantly decreased in the bone marrow on day 15.

The presence of an adaptive response in relation to ionising radiation-induced hematopoietic damage by pre-exposure of mice to 900 MHz fields was studied by Cao et al. (2011). Adult male Kunming mice were exposed to 900 MHz RF at various intensities (12, 120 and 1200 mW/cm^2 [120, 1200, 12000 W/m^2]) for 1 h/day for 14 days and then subjected to whole body gamma-irradiation. The results indicated a significant increase in survival time ($p<0.05$), spleen and thymus weight ($p<0.001$), and reduction in the hematopoietic tissue damage ($p<0.01$) in RF pre-exposed γ-irradiated mice (as compared with those exposed to ionizing radiation alone). They observed an increased number of colony-forming units in bone marrow (CFU-BM) and spleen (CFU-S) of lethally irradiated 'recipient' mice, and an increased expression of cyclin-D1, cyclin-E and cyclin-DK4 in mice pre-exposed to 900 MHz at 120 mW/cm^2 and subsequently subjected to γ-irradiation (as compared to those exposed to γ-irradiation alone).

Table 10.3.2. Animal studies on the hematological system

Endpoint, animals, number per group, age at start	Exposure: source, schedule, level, freely moving or restrained co-exposure)	Response	Comments	Reference
Blood chemistry, leucocyte count, morphology and histology of spleen, bursa of Fabricius, adrenal and thyroid Chicken (n=11) 1 week	2450 MHz CW 9 min/h, 24 h/day, 20 days 13 mW/cm^2 (130 W/m^2) Free	No effects.		Braithwaite et al. (1991)

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Blood-forming system Rat: Wistar (n=10 or 6) 13 weeks	2450 MHz, CW 2h/day, 7 days WBA SAR 1.25 W/kg Free	Decrease in lymphoblast count at day 15 and 30, no effect on leukocytes and lymphocyte counts.		Trošić, Busljeta & Pavičić (2004)
Kinetics of polychromatic erythrocytes (PCEs)in bone marrow (BM) and peripheral blood (PB) Rat: Wistar (n=10 or 6) 13 weeks	2450 MHz, CW 2h/day, 7 days WBA SAR 1.25 W/kg Free	BM PCEs increased on day 8 and 15, PB PCEs elevated on day 2 and 8.	Also discussed in Section 12.2.1.	Trošić & Busljeta (2006)
Haemapoietic damage Mouse: Kumming (n=12) Adult	900 MHz 1 h/day, 14 days 12, 120, 1200 mW/cm^2 (120, 1200, 12000 W/m^2) Restrained	Reduction in hematopoietic tissue damage after exposure with gamma rays. Increase of CFU-BM, CFU-S; cyclin-D1, cyclin-E and cyclin-DK4 expression. Increase of survival time, spleen and thymus weight.		Cao et al. (2011)

Abbreviations: BM: bone marrow; CFU; colony forming unit; CW: continuous wave; PB: peripheral blood; PCE: polychromatic erythrocyte; WBA-SAR:whole-body SAR.

Excluded studies

(Busljeta, Trošić & Milkovic-Kraus, 2004) (Nakamura et al., 2003)

10.4 In vitro studies

In the previous WHO monograph (1993) it was reported that in most cases no effects of RF EMF exposure were induced in haematopoietic tissues, although in some studies suppression of some immune responses was detected. The present bibliographic search recognized a large number of studies dealing with immune-competent cells, but only a small number is directly linked to cellular immunological functions and has been included. They have been mainly carried out on peripheral blood mononuclear cells of different origin, but also microglial cells have been investigated. The latter are immune-competent cells acting as the macrophages of the brain.

The present literature search resulted in 16 studies. Three papers were excluded because they did not meet the inclusion criteria for in vitro studies and three papers were in languages that could not be understood. Two papers were not included in the analysis due to methodological issues and have been only presented in the text. The remaining eight papers have been described in the text and summarized in table 10.3.1. Unless specifically mentioned, papers did not report on blinding of the investigators to the exposure condition.

Cleary et al. (1996) exposed murine cytolytic T lymphocytes (CTLL-2) to 2450 MHz RF EMF, either CW (SAR = 5–50 W/kg) or pulse modulated (average SAR = 5 W/kg) for 2 h to study the effects on interleukin-2 (IL-2)-dependent proliferation. Pulse modulation parameters (pulse repetition rate of 50 Hz, 6.67 ms pulse duration) simulated those of the modulation of the Personal Communication System (PCS). Exposures were carried out in a waveguide exposure chamber. The temperature in exposed and sham-exposed cultures was measured continuously during exposure and was 37 ± 0.1 °C. Cell cultures were RF exposed or sham exposed in the absence of IL-2 or in presence of 20 U/ml IL-2. After exposure, cells were cultured at various physiological concentrations of IL-2 up to 40 U/ml, and cell proliferation was measured by tritiated thymidine incorporation immediately, 2 h or 24 h post exposure. In cells exposed to CW, at SAR = 50 W/kg in presence of IL-2, a significant reduction in proliferation was detected in cells assayed immediately after RF exposure (9.2% decrease; $p<0.001$) and the effect was not dependent on the IL-2 concentration added post exposure. Such a significant reduction was also detected in cells assayed 24 h post exposure at all the IL-2 concentrations (19% decrease; p value not reported), except for 0 and 40 U/ml IL-2. No data have been reported for cultures exposed

in absence of 20 U/ml IL-2. In contrast, in cells exposed at a SAR of 25 W/kg in presence of IL-2, a slight increase in proliferation, not dependent on IL-2 concentration added post exposure, was recorded immediately after exposure ($p < 0.05$). When cells were assayed 24 h after exposure, a decrease in proliferation was detected at IL-2 concentrations in the range 0.6-2.5 U/ml ($p<0.004$). No significant effects were found at higher IL-2 concentrations and in cells exposed in absence of IL-2. Results on pulse-modulated RF exposure (SAR=5 W/kg) indicated an increase in cell proliferation, as measured 2h after exposure in absence of IL-2 ($p<0.0002$) across all the IL-2 concentrations, and no effect for exposure in the presence of 20 U/ml IL-2. Two to four independent experiments per experimental condition were performed. Cell cultures incubated at temperatures in the 38-41°C range served as positive controls and compared to RF exposed samples showed qualitative and quantitative different effects. On the whole, the results indicated that RF exposure is capable to affect the IL-2-dependent proliferation of murine T-lymphocytes. Such effects were not thermal. [The results presented in this paper seem to be consistent with the hypothesis that RF EMF can affect cell membrane receptors and ion channels. However, due to not reported results for some conditions and missing numerical data for others, the study cannot be fully evaluated and interpreted.]

Capri et al. (2006) studied the possible effects of RF EMF on activation of human lymphocytes by analysing several cluster differentiation antigens (CD), such as CD25, CD95 and CD28, in non-stimulated and stimulated CD4+ or CD8+ T-helper cells. Peripheral blood mononuclear cells (PBMCs) from 20 healthy donors (10 young and 10 elderly subjects) were exposed to an 1800 MHz talk-modulated GSM signal (SAR = 2 W/kg) for 44 h (10 min on, 20 min off cycles), in the presence or absence of a mitogen. For exposure, two waveguides were employed and EMF and sham-conditions were blindly assigned by a computer-controlled signal unit. The waveguides were placed in a cell culture incubator and temperature was monitored throughout the exposure. No significant changes in the percentage of these subsets of cells were found between exposed and sham-exposed non-stimulated lymphocytes in young or elderly donors, while a small, but statistically significant down-regulation of CD95 expression ($p<0.05$) was found in stimulated CD4+ T cells from elderly, but not from younger donors. Positive controls were not included in the study design.

Tuschl et al. (2006) exposed peripheral blood mononuclear cells from healthy donors to an 1950 MHz GSM-basic signal, at an SAR of 1 W/kg, in an intermittent mode (5 min on/10 min off cycles) for 8 h. As in Capri et al. (2006), two waveguides were placed in a cell culture incubator and EMF and sham conditions were blindly assigned by a computer-controlled signal unit. Temperature was monitored throughout the exposure and was never more than 0.06 °C higher than in the sham controls. After exposure, blood samples were tested for intracellular production of IL2 and interferon γ (INFγ) in lymphocytes and IL1 and tumour necrosis factor-α (TNFα) in monocytes, activity of immune-relevant genes (IL1α and β, IL2, IL2-receptor, IL4, macrophage colony-stimulating factor (MCSF) receptor, TNFα, TNFα-receptor), and cytotoxicity of lymphokine-activated killer cells (LAK cells) against a tumour cell line. Experiments on blood cells from 15–22 donors (depending on the parameter investigated) resulted in no statistically significant effects of exposure. Positive controls were not included in the study design. [This study is also presented in Section 12.3.2 – Intracellular and intercellular signalling.]

Chauhan et al. (2007) examined the ability of non-thermal RF EMF exposure to affect cytokine production and a variety of biological processes in several human-derived cell lines (TK6, HL60 and Mono-Mac-6). For each cell line five independent experiments were carried out. Exponentially growing cells were exposed to intermittent (5 min on/10 min off cycles) 1900 MHz pulse-modulated EMF for 6 h at SAR values of 0, 1 and 10 W/kg. Circularly polarized cylindrical fields were used and the temperature inside the cell cultures was maintained within 37 ± 0.5 °C, as monitored during the exposure. Concurrent negative (incubator) and positive (heat shock for 1 h at 43 °C) controls were included in each experiment. Cell pellets were analysed for cell viability, apoptosis and cell cycle kinetics immediately after the 6-h exposure period and 18 h after exposure, while the cell culture supernatants were assayed for the presence of pro- and anti-inflammatory cytokines (TNFα, IL1β, IL6, IL8, IL10, IL12). No detectable changes in the analysed endpoints were observed in any of EMF-exposed groups relative to the sham controls in any of the cell lines tested. The positive control samples displayed a significant decrease in cell viability, an increase in apoptosis, and an alteration in cell cycle kinetics (G2/M block). [This study has been also discussed in Section 12.3.2 – Intracellular and intercellular signalling.]

Huang et al. (2008) exposed human lymphoblastoid Jurkat T-cells to a 1763 MHz CDMA signal at an SAR of 2 or 10 W/kg (1 h/day for three days); three to six independent experiments were carried out. A rectangular cavity-type applicator was employed, specifically designed to assure proper environmental conditions (ventilation, humidity and temperature). During exposure, the temperature in the chamber was maintained at 37 ± 0.2 °C by circulating water within the cavity. Sham-exposed samples were placed in an identical device in absence of RF EMF. In genome-wide analysis of gene expression (five independent

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experiments), 24-h RF EMF exposure at an SAR of 10 W/kg did not induce gene changes more than two-fold. Ten genes were identified with a fold-change greater than 1.3 and, among them, two cytokine receptor genes, chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated, but only the CXCR3 variation was statistically significant ($p<0.05$). These results were not confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR). The authors also reported that exposure did not produce significant changes in cell number and cell cycle distributions when assayed 24 h after exposures (as reported in Section 12.3.6). Positive controls were not included in the study design. [This study has been also discussed in sections 12.3.1, 12.3.2 and 12.3.3, where the results on DNA damage, signalling and gene expression are reported.]

Thorlin and co-workers (2006) exposed rat primary astroglial cells to 900 MHz RF EMF in a temperature-controlled waveguide. They applied GSM-modulated EMF at an SAR of 3 W/kg for 4, 8 and 24 h, or CW at 27 W/kg for 24 h. The temperature was kept constant at 37 ± 0.2 °C by means of a cooling water system and was measured throughout the exposure duration. The release into the extracellular medium of the two pro-inflammatory cytokines IL6 and tumour necrosis factor-alpha (TNFα) was analysed. Further, the levels of the astroglial cell-specific reactive marker glial fibrillary acidic protein (Gfap), whose expression dynamics is different from that of cytokines, were measured in astroglial cultures and in astroglial cell-conditioned culture medium after exposure to CW fields at SARs of 27 and 54 W/kg for 4 or 24 h. Moreover, microglial cell cultures were exposed to 900 MHz, GSM modulated, at an SAR of 3 W/kg for 8 h, and IL6, TNFα, total protein and the microglial reactivity marker ED1 (a macrophage activation antigen) were measured. No significant differences between EMF and sham-exposed samples were detected for any of the parameters studied at any time and for any of the exposure conditions tested, as assessed in three to eight independent experiments performed in blind. Cell cultures incubated at 38° or 42°C were used as positive controls and gave positive findings. [This study has also been described in Section 8.3 and 12.3.2.3, where the results on neurodegenerative disorders and cytokine expression are reported.]

Absence of effects was also reported by Hirose et al. (2010), who exposed primary rat microglial cells to W-CDMA 1950 MHz EMF at SARs of 0.2, 0.8 and 2.0 W/kg and assessed functional changes in immune reaction-related molecule expression and cytokine production. The duration of the RF exposure was 2 h and assay samples were processed 24 and 72 h later in a blind manner. Results showed that the percentage of cells positive for major histocompatibility complex (MHC) class II, which is the most common marker for activated microglial cells, did not differ between any of the EMF-exposed groups and the sham-exposed controls. Furthermore, no remarkable differences in the production of tumour necrosis factor-alpha (TNFα), interleukin-1b (IL1b), and interleukin-6 (IL6) were observed (three independent experiments). Treatments with lipopolysaccharide or interferon-Ɣ as positive controls gave positive findings. [This study has also been reported in Section 8.3 neurodegenerative disorders. The SAR distribution in the exposed sample was not very homogeneous (standard deviation 57%) and a temperature increase of 0.7 °C was recorded during exposure to an SAR of 2.0 W/kg.]

Kumar et al. (2011) excised femur and tibia bones from 11 rats and exposed them for 30 min to 900 MHz RF EMF, CW (SAR = 2 W/kg). The investigators were blinded to the exposure protocol. No significant changes in erythrocyte maturation rate were observed in bone marrow cells extracted from exposed bones compared to sham-exposed controls, as assessed by acridine orange fluorescence technique. Cell cultures treated with concanavalin-A, a mitogen, were used as positive controls and gave positive findings.[In this study, the effect of RF exposure on DNA strand breaks and cell proliferation was also investigated, as reported in Section 12.3.1 and 12.3.6.]

Studies not included in the analysis

Natarajan et al. (2002) investigated the capability of RF EMF to promote DNA-binding activity of nuclear factor kappa B (NF-κB), a protein complex involved in cellular responses to several stimuli, including inflammatory cytokine, chemokines and interferon. They exposed human monocyte Mono-Mac-6 cells to pulsed-wave radiation used in radar (8.2 GHz, 2.2 μs pulse width and pulse repetition rate of 1000 pulses/s, SAR = 10.8 ± 7.1 W/kg at the bottom of the culture flask), for 90 min at 37 °C. During exposure, cell cultures were maintained at 37.4 ± 0.4°C while sham-exposed cultures were kept at 37.2 ± 0.4°C. Cells were then re-incubated at 37 °C, and harvested 4 h post-exposure. Results showed a 3.6-fold increase in DNA-binding activity of NF-κB in exposed monocytes compared to the sham exposed ones (two independent experiments carried out in triplicate). Cell cultures treated with IL-1 were included in the study as positive control and gave the expected results. The authors also performed experiments aimed to investigate the effect of heating and observed a decrease in NF-κB DNA-binding activity at 43 °C. [This study has also been presented in Section 12.3.2.

Nevertheless, as also stated by the authors, a broad distribution of SAR levels in the samples cannot be excluded. The results of this investigation cannot be interpreted due to the scanty number of experiments; moreover, data are reported as fold-changes and statistical analysis has not been performed.]

In a follow-up study, Natarajan et al. (2006) exposed the same cell type (Mono-Mac-6) for 90 min intermittently (30 min on, 30 min off, 30 min on) to 0.79 ns long pulses with average peak electric field of 1 kV/cm (100 kV/m), pulse repetition rate of 250 Hz and carrier frequency ranging from 0 Hz to 2 GHz. Cells were incubated and harvested at 10 min, 3 h, 8 h and 24 h post exposure. No difference in the levels of NF-kB DNA-binding activity was detected in cells harvested at 10 min, 3h and 8 h, while 24 h incubation after exposure resulted in a 3.5-fold increase in NF-κB-binding activity in exposed cultures compared to sham controls. Such an increase disappeared at 48 h incubation post exposure. However, the exposure did not significantly affect the expression of the κB-dependent gene expression profiles, measured at 8 and 24 h post exposure. In cultures exposed to gamma rays as positive control, positive findings were found. [This study has also been discussed in Section 12.3.2 signal transduction. The validity of the results remains unclear since the number of independent experiments carried out is not reported. Moreover, data are reported as fold-changes, although the authors claimed that statistical analysis has been performed.]

Table 10.4.1. In vitro studies assessing effects of RF-EMF exposure on immune system and haematology

Cell type Number of independent experiments	Biological endpoint	Exposure conditions	Results	Comment	Reference
Murine cytolitic T lymphocytes n=2–4	Cell proliferation (^{3}H-thymidine incorporation) immediately or 24 h after exposure	2450 MHz, CW SAR 5–50 W/kg PCS: average SAR 5 W/kg 2 h RF exposure in presence or in absence of 20 U/ml IL-2 and treatment with different IL-2 concentrations after RF exposure	CW: Reduced proliferation at SAR=50 W/kg in cells exposed in presence of IL-2 and tested immediately after or 24 h post exposure, for all the IL-2 concentrations added post exposure. Increased proliferation in cells exposed at 25 W/kg in presence of IL-2, assayed immediately after exposure. Decreased proliferation in cells assayed 24 h after exposure. PCS: Increased proliferation in cells RF exposed in absence of IL-2 and no effect in cells exposed in presence of IL-2, assayed 2 h after exposure. No influence of IL-2 concentration added after RF exposure.	CW: Data not reported for cultures exposed at 50 W/kg in absence of IL-2. For proliferation see Section 12.3.6.1 No information on blinding of staff .	Cleary et al. (1996)
Human lymphocytes n=20	Analysis of CD25, CD95 and CD28 molecules in young and elderly donors	1800 MHz GSM Average SAR 2 W/kg 44 h (10 min on/20 min off cycles)	Age-related down-regulation of CD95 in stimulated $CD4^+$ T cells. No effect in non-stimulated lymphocytes.		Capri et al. (2006)

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Human peripheral blood mononuclear cells n=15–22	mRNA: variety of cytokine and immune-relevant genes	1950 MHz GSM Average SAR 1 W/kg 8 h (5 min on/10 min off cycles)	No effect	For intracellular and intercellular signalling see Section 12.3.2	Tuschl et al. (2006)
Human-derived immune cell lines (TK6, HL-60, Mono-Mac-6) n=5	Inflammatory cytokine release (TNFα, IL1B, IL6, IL8, IL10, IL12)	1900 MHz, pulsed Average SAR 1 and 10 W/kg 6 h (5 min on/10 min off cycles)	No effect after exposure and 18 h later.	For apoptosis and cell cycle see Section 12.3.4 and 12.3.6 No information on blinding of staff .	Chauhan et al. (2007)
Human lymphoblastoid Jurkat T cells n=3–6	Genome-wide analysis of gene expression	1763 MHz, CDMA Average SAR 2 and 10 W/kg 1h/day for 3 days	Down- regulation of CXCR3 not confirmed by RT-PCR. No validation of the other genes	For DNA damage, cell signaling, gene expression and cell proliferation see Section 12.3.1, 12.3.2, 12.3.3 and 12.3.6 No information on blinding of staff	Huang et al. (2008)
Rat primary astroglial and microglial cells n=3–8 n=3–4	Release of TNFα and IL6; cellular content of Gfap and ED-1	900 MHz GSM Average SAR 3 W/kg 4, 8 and 24 h 900 MHz CW SAR 27 and 54 W/kg 24 h	No effect No effect	For neurodegenerative disorders and cytokine expression see Section 8.3 and 12.3.2.3	Thorlin et al. (2006)
Primary microglial cell cultures n=3	Changes in immune reaction-related molecule expression Cytokine production	1950 MHz, WCDMA Average SAR 0.2, 0.8, 2 W/kg 2 h	No effect 24 and 48 h post exposure.	SAR not homogeneous. For neurodegenerative disorders see section 8.3	Hirose et al. (2010)
Rat bone marrow lymphocytes n=11	Erythrocyte maturation rate	900 MHz, CW SAR 2 W/kg 30 min	No effect	For DNA damage and cell proliferation see Section 12.3.1 and 12.3.6	Kumar et al. (2011)

"No effect" means no statistically significant effect.

Abbreviations: CD: cluster differentiation antigen; CDMA: code division multiple access; CW: continuous wave; Gfap: glial fibrillary acidic protein; GSM: Global System for Mobile Communication; ^{3}[H]thymidine: tritiated thymidine; IL: interleukin; PCS: personal communication system; RT-PCR: Reverse transcriptase-polymerase chain reaction; SAR: specific absorption rate; TNF: tumor necrosis factor; W-CDMA: Wideband Code Division Multiple Access.

Excluded papers

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