900 MHz Electromagnetic Fields Induce Microbiota Dysbiosis and Adaptive Immune System Disorders in Juvenile Rats

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Abstract

Although cell phones and electronic devices are now integral parts of modern life (especially for young people), the radiofrequency electromagnetic field (RF-EMF) emitted by these devices is a potential health hazard. The effects of RF-EMFs have been assessed in various fields, including epidemiology and neurology. However, there are few published data on the possible effects of RF-EMFs on immune cells; this is surprising, given the importance of the immune system's role in defending the body against infections and cancer. To assess whether chronic RF-EMF exposure has harmful effects on the immune system in juvenile rats, rats were exposed or not to 900MHz RF-EMF 23h/day during 5 weeks. Hematological, cytometric and bacteriological assays were used to probe differences between exposed and non-exposed tissues. Exposure of rats to 900 MHz RF-EMF was associated with differences in the innate immune system and even more marked in the adaptive immune system. An analysis of the intestinal microbiota revealed dysbiosis, with an over-representation of *Enterococcus*, *Clostridium* and *Bacteroides* spp. *Enterococcus* was found to have translocated into the spleen in 67% exposed rats. Exposure to a 900 MHz RF-EMF appeared to alter the immune system (and particularly the adaptive immune system) directly or via intestinal dysbiosis.

Introduction

The now widespread use of wireless technologies has increased the number of electromagnetic field (EMF) sources in our everyday environment. The most common technology-related EMFs include extremely low frequency (ELF) fields (<60 Hz) and radiofrequency (RF) fields (100 kHz–300 GHz). ELF fields are generated from electrical and electronic appliances, power lines and especially the household alternating current electrical power supply. Devices that emit RFs and microwaves are extensively used in industry (welding machines and induction heaters), telecommunications (TV and radio broadcast stations), medicine (NMR and diathermy), and everyday life (microwave oven and cell phones, now including "fifth generation" (5G) cellular networks). Cell phones emit and receive RFs at ultrahigh frequencies (from 300 MHz to 3 GHz) [1,2]. The International Commission on Non-Ionizing Radiation Protection caps the peak power emitted by cell phones at between 0.1 and 2 watts [3]. Some people have accused RF-EMFs (particularly those emitted by cell phones and base stations) of causing sleep disorders, tiredness, headaches, dizziness and/or balance disorders [4–6]. These accusations are subject to debate.

A number of preliminary epidemiologic studies assessed the relationship between EMF exposure (especially cell phone use) and the risk of developing various cancers (especially intracranial tumors)[7]. On the basis of these results, the International Agency of Research of Cancer classified RF-EMFs as possibly carcinogenic in humans (group 2B) [8]. Since then, further epidemiological studies have confirmed that long-term cell phone use (for more than 10 years) is associated with an increased risk of intracranial tumors [9,10].

In vivo models are important tools for understanding the effects of RF-EMFs; by limiting external confounding factors, researchers can study the effects of continuous or intermittent exposure in a specific population (e.g. juvenile rats). Some studies of exposure of rodents to 900 MHz fields (the frequency used by most cellphones) evidenced harmful effects on the brain, lymphoid organs, and kidney[11–14], whereas other studies of exposure to the same frequency did not [15–18].

In vitro cell culture experiments also gave conflicting results; some studies found that neurons, lymphocytes and monocytes were sensitive to EMF exposure, [19,20] while others found no effects of RF-EMFs on monocytes and epithelial cells[21].

The impact of RF-EMFs on the rodent immune system is also subject to debate[22], and appears to depend strongly on the exposure protocol and the animal's age. The underlying mechanisms are unclear but might involve histamine release[23] and/or calcineurin inhibition [24]. Some studies showed that chronic exposure of growing rats to a 900 MHz RF-EMF was associated with biochemical and oxidative damage to spleen and thymus cells [11,25]. However, other studies of acute or chronic exposure of rodents at 900 MHz did not evidence any changes in lymphocyte populations [26–28].

The body is vulnerable during early childhood, due to the ongoing development of physiological and neurologic functions[29] and the fact that RF-EMFs penetrate further into "juvenile" tissues (including the brain) than into adult tissues [30]. Studies of the impact of RF-EMF on juvenile populations are important because young people are intensive users of connected devices [31 32,33]. In 2014, Pelletier et al exposed juvenile rats to 900 MHz RF-EMF and described alterations in sleep patterns [34], thermal regulation[35], body weight, and food intake [36]. The objectives of the present study of juvenile rats were to evaluate (i) the effects of chronic 900 MHz RF-EMF exposure on the innate and adaptive immune systems and the intestinal microbiota [37–39] (since EMFs reportedly perturb bacterial growth, [40,41] which might induce gut dysbiosis), and (ii) the impact of any microbiota alterations on the immune system. We evaluated exposed vs. control differences in blood cell counts and (using flow cytometry) adaptive immune cell counts in the blood and lymphoid organs. Bacteria in feces and the spleen were characterized and compared with changes in immune parameters.

Results

RF-EMF exposure induces changes in hematological variables

To assess the impact of RF-EMF exposure on the rat's immune system, we first measured a number of hematological variables in exposed and control animals (Table 1). The white blood cell (WBC) count was significantly lower in exposed rats than control rats ($p=0.0434$). The distribution of lymphocyte and neutrophil subsets was also modified. The proportion of neutrophils was significantly greater in the exposed group than in the control group ($p=0.0253$). In contrast, the proportion of lymphocytes was lower

in the exposed group than in the control group (p=0.007). The lymphocyte count was lower in the exposed group than in the control group (p=0.005).

Table 1
Blood counts (expressed as the mean ± SEM) in the control group (n=8) and the exposed group (n=9). NS: non-significant, *: p<0.05, **: p<0.01 in a Mann-Whitney test.

Variable	Control	RF-EMF	Intergroup comparison
WBC count (x10^9/L)	13.17 ± 0.380	9.99 ± 2.299	*
Lymphocytes (%)	70.86 ± 1.371	63.52 ± 2.313	**
Monocytes (%)	6.99 ± 0.299	8.87 ± 1.014	ns
Neutrophils (%)	22.15 ± 1.466	27.57 ± 2.087	*
Lymphocyte count (x10^9/L)	9.92 ± 0.147	6.93 ± 1.161	**
Monocyte count (*10^9/L)	0.81 ± 0.108	1.03 ± 0.206	ns
Neutrophil count (x10^9/L)	2.57 ± 0.374	3.594 ± 0.892	ns

RF-EMF exposure induces changes of B lymphocyte populations and IgG levels.

We used flow cytometry to analyze B lymphocyte subsets in the peripheral blood and the spleen of exposed and control rats (Fig. 1). In the blood, no significant intergroup differences were observed in the B-cell subsets, except for a higher absolute IgG1+ B lymphocyte count (p=0.009) in exposed rats than in control rats (Fig. 1A-B). In the spleen, the proportion of mature B lymphocytes was similar in the two groups (Fig. 1C). However, the proportion of IgG1+ B lymphocytes in exposed rats was higher than in controls (p=0.002). The proportion of memory B lymphocytes in spleen was significantly lower in exposed rats than controls (p=0.001) (Fig. 1C).

We measured the levels of Ig produced by B lymphocytes in the serum of exposed and control rats (Fig. 2). We observed significantly lower proportions of IgG1 (p=0.019) and IgG2c (p=0.003) in exposed rats compared to control. Taken as a whole, these data demonstrate that RF-EMF exposure induces an alteration of immune cells in the peripheral blood and impacts humoral immune response.

RF-EMF exposure induces major changes in T lymphocyte subsets

Both the proportion of T lymphocytes (CD45+ CD3+) and the absolute count in the blood were significantly lower in RF-EMF-exposed animals than in control animals (Fig. 3A, p=0.0012 and p=0.037 respectively). Although the percentages of helper (CD4+) T lymphocytes and cytotoxic (CD8+) T lymphocytes were similar, the absolute counts for these subsets were significantly lower in exposed rats than in control rats (Fig. 3B; p=0.008 (CD4+) and p=0.012 (CD8+), respectively).

As with our observations in the peripheral blood, the proportions of splenic total T lymphocytes (p=0.019) and CD4+ T lymphocytes (p=0.049) were also significantly lower in exposed rats than in control rats (Fig. 3C).

In the thymus, the proportion of total T lymphocytes was similar in the exposed and control groups (Fig. 3D). However, the CD4+ single-positive thymocyte count (but not the CD8+ single-positive thymocyte count) was significantly higher in the exposed group (p=0.031). The double-positive (CD4+/CD8+) thymocyte count was significantly lower in exposed rats (p=0.010; Fig. 3D). The thymic T lymphocyte precursor (CD4-/CD8-) count was also significantly higher in exposed rats than in control rats (p=0.033, Fig. 3D).

RF-EMF exposure induces changes in the composition of intestinal microbiota, together with dysbiosis.

To further characterize the immune changes observed in exposed rats, we analyzed the intestinal microbiota. The exposed and control groups differed significantly with regard to the microbial counts in the colon tissue (expressed in log CFU/g colon tissue) (Fig. 4). In the colon tissue, the beneficial microbiota was similar in exposed and control rats. In contrast, the pathogenic microbiota was significantly elevated in exposed rats, relative to control rats (Fig. 4A; p=0.024). The difference between the number of beneficial bacteria and the number of pathogenic bacteria was statistically significant in the exposed group (Fig. 4A; p=0.005) but not in the control group (p=0.79). Using conventional bacterial cultures, we identified five genera (*Enterococcus, Enterobacteria, Staphylococcus, Clostridium* and *Bacteroides*) in exposed or control rats. In exposed rats, we observed significantly higher counts of *Enterococcus* (p=0.011), *Clostridium* (p=0.002) and *Bacteroides* (p=0.041) (Fig. 4B), and significant translocation of *Enterococcus* to the spleen (Fig. 4C; p=0.001). These data demonstrate that RF-EMF exposure was associated with intestinal dysbiosis.

The Enterococcus **count in the gut is correlated with immune changes**

We next probed correlations between changes in the microbiota and changes in immune variables in the exposed and control groups. Significant correlations were only observed in the exposed group (Fig. 5). The *Enterococcus* count was negatively correlated with the total T lymphocyte count (Fig. 5A., p=0.046) and the helper T lymphocyte count (Fig. 5B; p=0.004) in blood. There were no other correlations with blood T lymphocyte subset counts (data not shown). In the spleen, the proportion of memory B lymphocytes was positively correlated with the *Enterococcus* count (Fig. 5C, p=0.028).

Discussion

Over the last few years, the use of RF-EMF-emitting devices (including cell phones, wi-fi devices, and satellite communication systems) has increased markedly – especially among young people. These devices are ubiquitous in everyday life (at home, at school, in public transportation, in the street, etc.) and thus continuously expose us to RF-EMFs. To model the chronic exposure of young organisms, we

exposed juvenile rats to a 900 MHz RF-EMF for 23 h/day over a 5-week period (as did Pelletier et al. [34]) and analyzed the exposure's impact on the immune system and the intestinal microbiota.

Although the first research data on the effects of RF-EMF were published over 20 years ago, there is still no consensus on the best model of exposure. Firstly, wireless technologies and thus exposure variables are changing constantly. Secondly, the intensity of RF-EMF exposure is not always clearly established. Some studies have attempted to quantify the intensity of EMFs present in the environment [42–44]. This variable might fluctuate markedly as a function of the geographical area and the number of connected devices close to the individual. In everyday life, the EMF intensity varies; however, the intensity had to be fixed in the present study.

Our results showed that the exposure of rats to an RF-EMF was associated with a lower WBC count, a lower total lymphocyte count, and a higher proportion of neutrophils in the peripheral blood. Similar changes in hematological variables had been observed in a study of juvenile rats exposed to 915 MHz frequencies for 8 h/day over a 2-week period [45]. In contrast to the results for short exposures[48], our data suggest that longer exposure to RF-EMF induces an alteration in the immune system in general and in the adaptive immune system in particular. Indeed, we observed differences in some B lymphocyte subsets ($IgG1^+$ B lymphocytes and memory B lymphocytes) and in T lymphocyte populations in blood, spleen, and thymus. Modifications in the secretion of Igs into the blood of exposed vs. controls rats reflect a profound change in the adaptive immune system. The present study is the first to have observed this phenomenon.

A study of rats exposed to 900 MHz RF-EMF for 2h/day over a 45-day period highlighted the presence of oxidative stress in secondary lymphoid organs [25]. The low lymphocyte counts in the exposed rats in our study might be explained by more frequent apoptosis; it has been shown that 900 MHz RF-EMF induces apoptosis through the mitochondrial pathway via a reactive-oxygen-species- and caspase-3-dependent mechanism [13,46].

Our analysis of the gut microbiota revealed dysbiosis in rats exposed to RF-EMF. This was characterized by elevated *Enterococcus, Clostridium* and *Bacteroides* counts in the gut tissue. Furthermore, *Enterococcus* had translocated into the spleen in 67% of the exposed rats and only 25% of the control rats.

These bacteria have already been identified in the literature as having an effect on the immune system. *Clostridium* was associated with food allergies [47,48], inducing an accumulation of intestinal T regulatory lymphocytes (Tregs) able to inhibit allergic inflammation [49] and metabolize short-chain fatty acids; this can have systemic anti-inflammatory effects and influence dendritic cell and T-cell responses [50]. The bacterium *Bacteroides fragilis* drives the differentiation of IL-10–secreting Tregs by producing an unusual capsular polysaccharide A. Daillère et al. have demonstrated immunomodulatory effects of *Enterococcus hirae* during cyclophosphamide therapy. This bacterium was associated with changes in the tumor

microenvironment, a low Treg count, and the stimulation of cognate antitumor cytotoxic T lymphocyte responses [51].

Our results showed that high *Enterococcus* counts in the gut tissue were correlated with low total T lymphocyte and CD4+ T lymphocyte counts in the blood of exposed rats. However, our present model did not able us to determine which mechanisms are involved. We hypothesize that the immune deregulations observed in peripheral blood and secondary lymphoid organs are caused (at least in part) by the intestinal dysbiosis. To explore this hypothesis and thus determine whether RF-EMFs have direct and/or indirect effects on the immune system, it would be interesting to exposure germ-free mice. This type of research would make it possible to more specifically target lymphocyte subsets (such as Th17 cells, Treg and innate immune cells) not studied in the present work.

This rat model of chronic exposure to RF-EMF demonstrated the dysregulatory effect of an RF-EMF on the immune system, and particularly an indirect effect through dysbiosis. The adaptive immune system appeared to be affected by the RF-EMF exposure, with major changes in B and T lymphocyte subsets in the blood and in secondary lymphoid organs.

Material And Methods

Animals

All animal experiments were approved by the local animal care and use committee and by the French Ministry of Research (registration number: APAFIS 3735-2016012017118094). Experiments were conducted on 17 male Wistar rats (Janvier Labs, Le Genest Saint Isle, France) weighing between 55 and 85 g and aged 3 weeks at the time of their arrival in our animal facility. The RF-EMF-exposed and control (non-exposed) groups comprised 9 and 8 animals, respectively. Each group was housed in a separate anechoic chamber with a 12 hr:12 hr dark/light cycle and controlled thermoneutral air temperature (24 ± 1°C), relative air humidity (mean ± standard deviation (SD): 39±12%) and air velocity (<0.2 m/s). Rats were housed individually in plastic cages (425 mm x 266 mm x 185 mm) within the anechoic chamber. Standard chow (3436EXF12, Serlab, Montataire, France) and drinking water were available *ad libitum*.

RF-EMF exposure

The RF-EMF exposure protocol was the same as that used by Bosquillon de Jenlis et al. [36]. After 4 days of acclimatization, the RF-EMF exposure (23 hours per day, over a 5-week period) was initiated. The climatic chambers were equipped with RF-EMF antennae powered by a generator (model RFS 900–64, RFPA, Artigues-près-Bordeaux, France) emitting a continuous-wave 900 MHz EMF. Antennae (model 800–10465, KATHREIN-Werke KG, Rosenheim, Germany) were placed horizontally in the climatic chamber, 80 cm above the exposed rats' boxes. The generator's power was set to obtain a field intensity of 1.8 V/m. The animals' estimated specific absorption rate was 30 mW/kg. The dose of RF-EMF exposure was checked with a radiofrequency dosimeter (PMM EP600, Narda Safety Test Solutions, Hauppauge, NY)

and monitor on computer software (Win EP 600, Narda Safety Test Solutions). To allow animal care, the generator was turned off for one hour a day.

Exposure, sacrifice, and sample collection

After 5 weeks of RF-EMF exposure, the rats were sacrificed by heart puncture under isofluorane 2.5% anesthesia (Iso-Vet 1000 mg/g, Piramal Healthcare UK Ltd, Morpeth, United Kingdom; airflow: 1 L/min). The blood from the heart puncture was collected. Parts of the spleen and thymus were collected postmortem and placed in phosphate buffer saline (PBS, Corning, New York, NY, USA). Splenic or thymic cells were dissociated with the gentleMACS^{TM} Octo Dissociatior (Miltenyi Biotec, Bergisch Gladbach, Germany), using a spleen program. The samples were passed through a 100 µm sieve and then frozen at -150°C in a solution composed of 9:1 v/v fetal bovine serum (FBS): dimethylsulfoxide (DMSO).

Microbiological analyses

Culture methods

All sample preparation steps (collection, centrifugation, dilution, and inoculation into culture media) were performed on the same day for anaerobe and aerobe assays. Anaerobic conditions were applied first; to protect the anaerobic bacteria, all steps were performed as quickly as possible.

After weighing, thawed sample of intestine and other organs were mashed in an equal weight of cysteine broth and homogenized in a sterile blender in order to maintain adherent bacteria. Next, 1 mL samples of organ homogenate (intestine and organs) were diluted and plated on various selective and non-selective media for qualitative and quantitative cultures of aerobic and anaerobic microbiota (100 µL of diluted medium per plate). Standard microbiological techniques and media were used, as described elsewhere [52] [53]. All aerobic culture plates were incubated at 37°C for 48 h. All anaerobic culture plates were incubated in an anaerobic chamber (Bactron Anaerobic, Sheldon Manufacturing, Cornelius, OR, USA) and examined after 4 days of incubation at 37°C. The culture plates were evaluated by a researcher who was blinded to the samples' group assignment. Isolated colonies grown on Petri dishes were counted using an automatic colony counter (Scan® 500, INTERSCIENCE, Saint-Nom-la-Bretèche, France) and expressed as $\log^{10}$ colony-forming units per gram of tissue (log(CFU)/g).

After macroscopic and microscopic observations (Gram staining procedures), biochemical assays were performed. A number of specific chromogenic media were also used. Again, the results were read by a researcher who was blinded to the samples' group assignment.

Bacterial translocation to the spleen

The spleen was removed for the culture of translocated organisms. Bacteria were counted and identified according to their characteristic colonies and microscopic features and by using matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (as described by Joly et al. [54]) under aseptic

conditions. Thawed tissue samples were placed in sterile, preweighed glass containers. On the day of inoculation, organs were weighed, placed in sterile lab blender bags, and then homogenized in Ringer's saline solution (Bio-Rad, Marnes-la-Coquette, France). Lastly, plates were inoculated with the resulting homogenate. The conventional media have been described elsewhere [53]. After incubation, bacteria were identified and counted using standard microbiological techniques by a researcher who was blinded to the samples' group assignment [52]. The lactobacilli and bifidobacterial were considered to be beneficial microbes, whereas enterococci, enterobacteria, clostridia, Bacteroides and staphylococci were considered to be pathogenic.

Data acquisition and analysis

Blood analyses

On the day after sacrifice, a complete blood count was determined on a KT-6400 analyzer (Genrui Biotech Inc., Shenzhen, China). Next, peripheral blood mononuclear cells (PBMCs) from non-exposed or exposed animals were separated using gradient separation in Ficoll Pacques Plus© (GE-Healthcare, Little Chalfont, United Kingdom). The top Ficoll phase (i.e. the plasma sample) was frozen at -150°C. The PBMC band was collected, washed in PBS, pelleted at 300 x g, and then frozen at – 150°C in a solution composed of 9:1 v/v FBS:DMSO.

Flow cytometry analyses

Single-cell suspensions prepared from the spleen, thymus or PBMCs were thawed in RPMI (Roswell Park Memorial Institute) medium (PAN-Biotech, Aidenbach, Germany) supplemented with 10% FBS (Eurobio, Courtaboeuf, France), 2 mM L-glutamine (Sigma Aldrich, Saint-Louis, Missouri, USA) and 10 U/mL penicillin-streptomycin (Sigma Aldrich) for three hours. The cells were then stained with fluorochrome-conjugated antibodies (Abs). The following Abs were used for flow cytometry experiments: PE-anti-rat-CD3 (clone REA223), APC-anti-rat-CD4 (clone REA482), FITC-anti-CD8a (clone REA437), FITC-anti-rat-immunoglobulin (Ig)M (clone ES26-13D3.4), APC-anti-rat-CD45R (clone REA450), APCVio770-anti-rat-CD45 (clone REA504), FITC-anti-rat-CD161 (clone REA227), APC, anti-rat-IgG1 (clone RG11/39.2) (Miltenyi Biotec). Lymphoid cell populations were analyzed after gating (1 x 10^5 viable cells), according to their forward scatter and side scatter. B lymphocytes (CD45+/CD3-/CD45R+), active B lymphocytes (CD45+/CD3-/IgG1+), immature B lymphocytes (CD45+/CD3-/IgM+), switched B lymphocytes (CD45+/CD3-/IgG1+), memory B lymphocytes (CD45+/CD3-/CD45R+/CD27+), T lymphocytes (CD45+/CD3+), helper T lymphocytes (CD45+/CD3+/CD4+CD8a-) and cytotoxic T lymphocytes (CD45+/CD3+/CD4-CD8a+) subset were detected according to a previously described gating strategy [55–57]. All sample were acquired using a MACSQuantify (Miltenyi Biotec). The flow cytometry data were analyzed using FlowJo software (version 10.2).

Serum analyses

Immunoglobulin concentrations in plasma samples were determined using an Antibody Isotyping 6-Plex Rat ProcartaPlex© assay (Invitrogen, Carlsbad, CA). Plasma samples were first diluted 4000-fold, as recommended by the manufacturer. Bead immunoassay results were read on a MagPix Milliplex Map system (Merck, Fontenay-sous-Bois, France) and analyzed with ProcartaPlex© Analysis software (Invitrogen).

Statistical analyses

The normality of the data distribution was checked with the Agostino-Pearson test. In order to compare control and exposed groups and probe correlations between the two, we applied Mann Whitney, Wilcoxon, chi-squared and Pearson's r tests, as appropriate (see the figure legends). Statistical analyses were performed with GraphPad Prism software (version 8.4.0, GraphPad Software, San Diego, CA, USA). Quantitative variables were expressed as the median (range) in the figures and as the mean ± SEM in the table. The threshold for statistical significance was set to $p<0.05$.

Declarations

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Author Contributions

J-P.M., L.C., A.P. contributed to the design of the study. A.B.D.J. helped with A.C. and S.D. in conducting of the in vivo study. L.C., H.O-H. performed hematological and cytometric experiments. N.D. and H.K-C. performed microbiological experiments. H.G., L.C., M.N. and J-P.M. analyzed the data. M.N. and L.C. wrote the manuscript. L.G., H.K-C., V.B., A.P. and J-P.M. critically evaluated the results and enhanced the manuscript.

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Figures

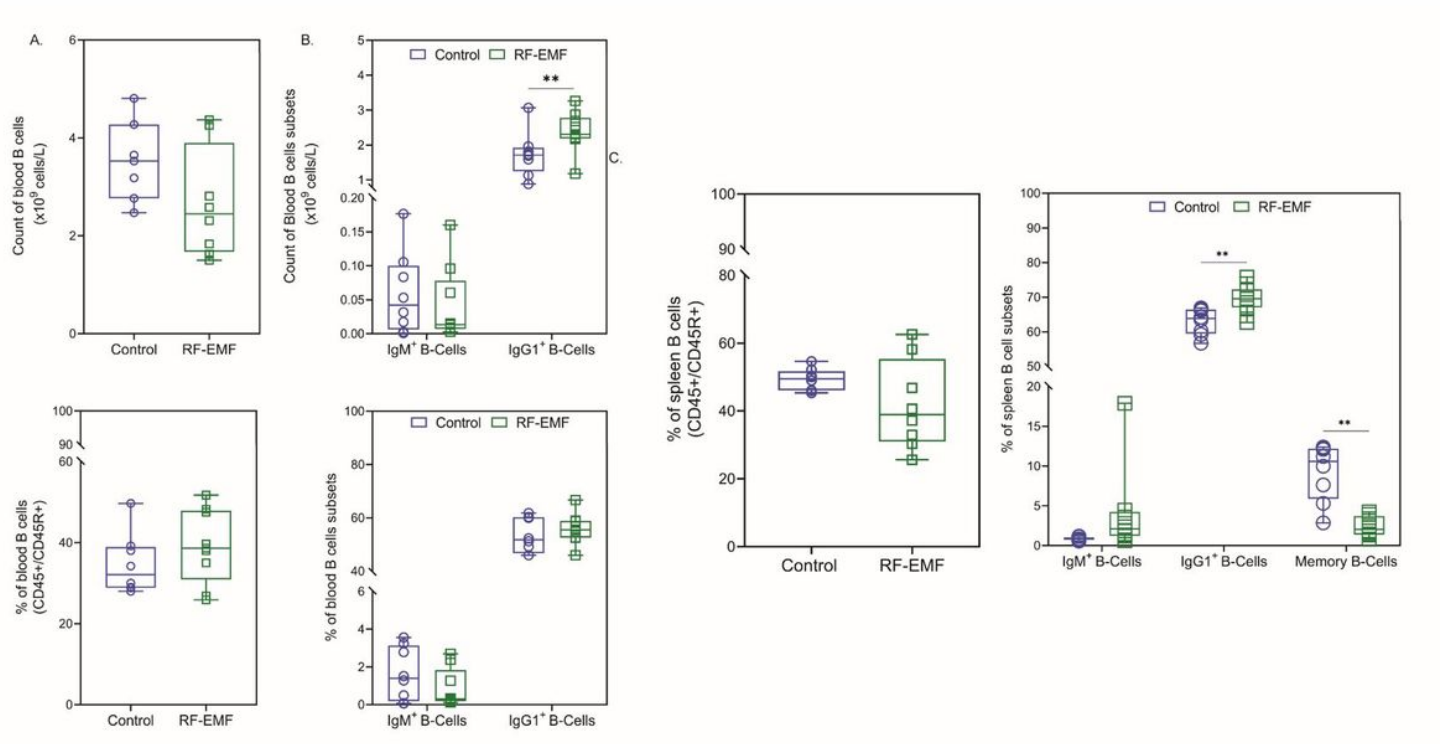

Figure 1

The distribution of B lymphocyte subsets in peripheral blood and spleen samples from control and exposed rats

Frequencies or counts of active B lymphocytes ($CD45^+/CD3^-/IgG1^+$), B lymphocytes (CD45+/CD3-/CD45R+), immature B lymphocytes (CD45+/CD3-/IgM+), switched B lymphocytes (CD45+/CD3-/IgG1+), and memory B lymphocytes (CD45+/CD3-/CD45R+/CD27+) were analyzed by flow cytometry in peripheral blood (A-B) and spleen (C) from exposed (n=8) and control (n=9) rats. The mean proportions were calculated with reference to the total CD45+ cell count. NS: non-significant, *: p<0.05, **: p<0.01 in a Mann-Whitney test.

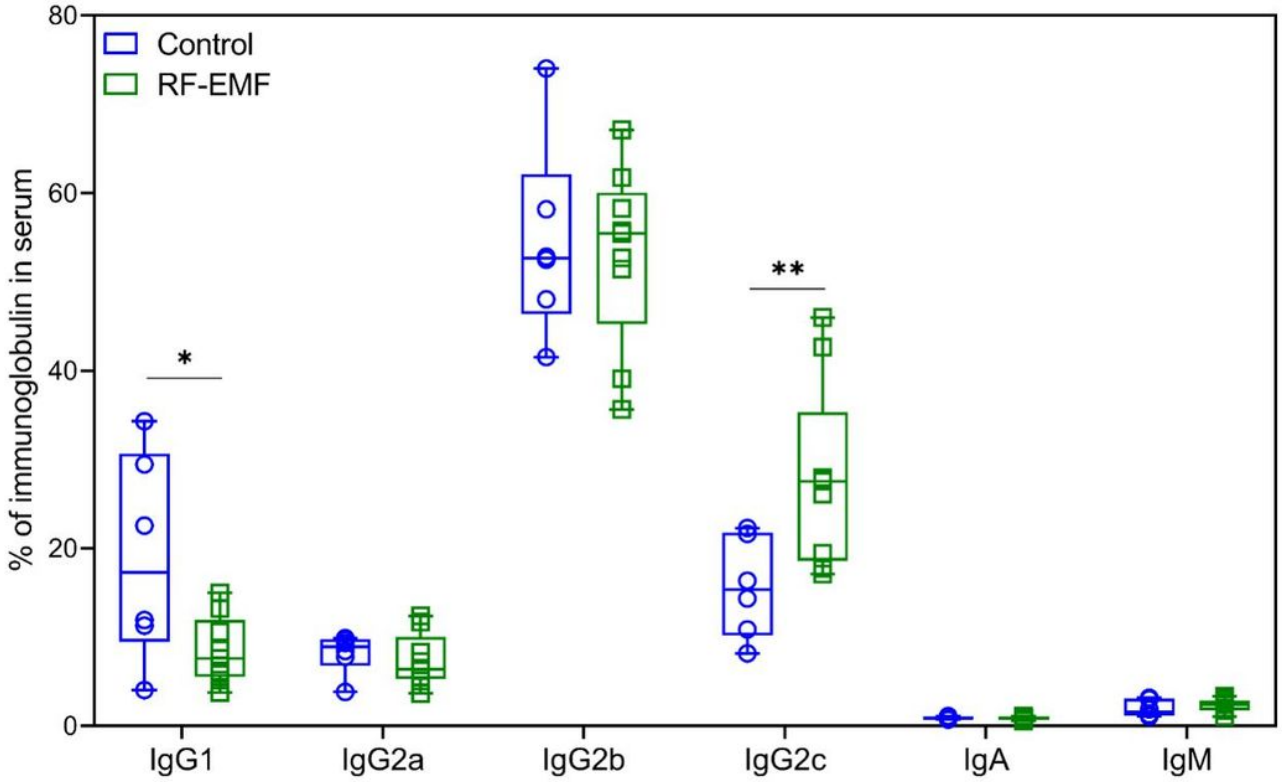

Figure 2

The distribution of serum Ig levels in control and exposed rats

Serum IgG1, IgG2a, IgG2b, IgG2c, IgA and IgM levels in exposed (n=9) and control (n=8) rats were measured in a Luminex© bead assay. *: p<0.05 in a Mann-Whitney test.

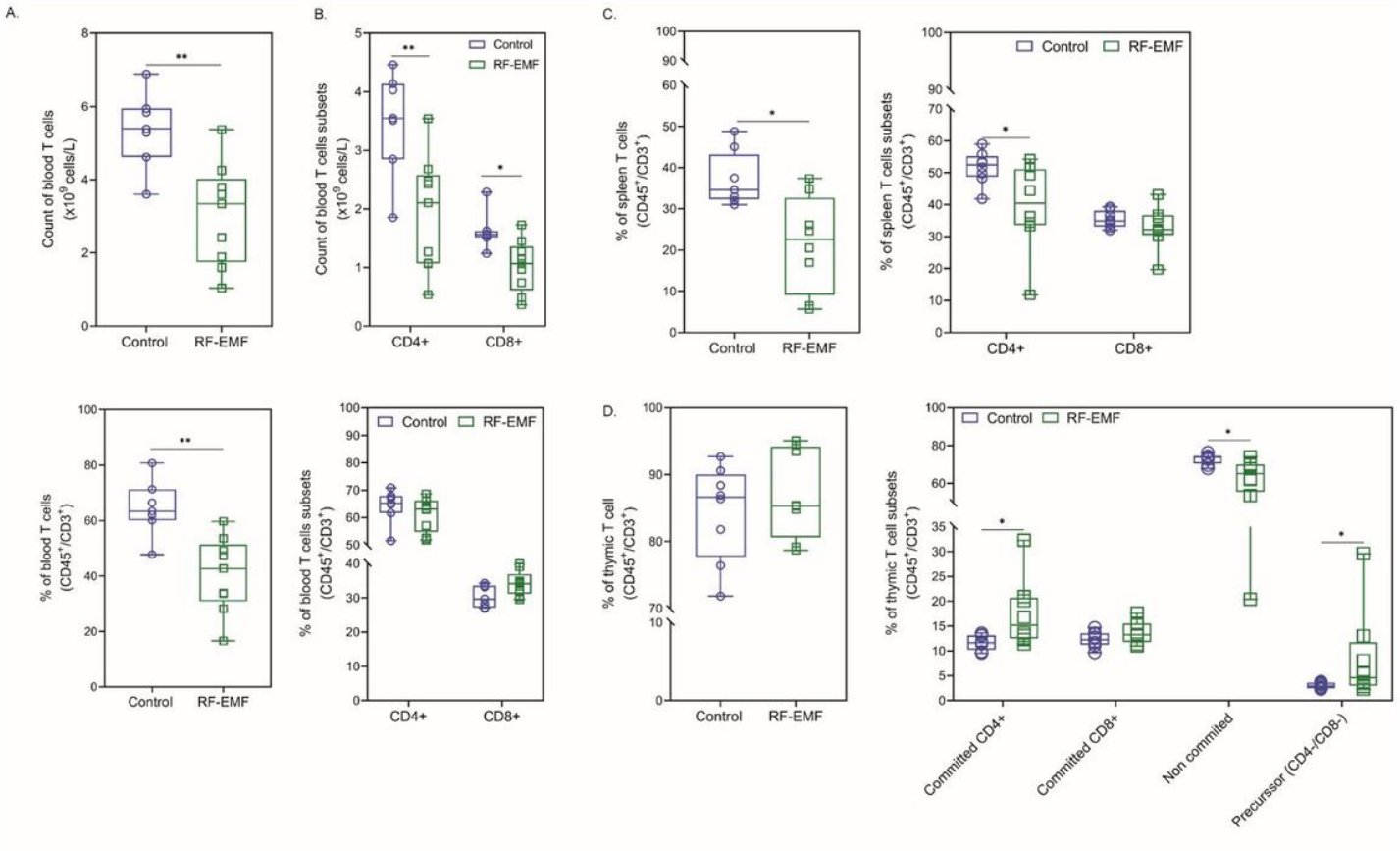

Figure 3

The distribution of T lymphocyte subsets in peripheral blood, spleen and thymus samples from control and exposed rats.

Frequencies or counts of total T lymphocytes (CD45+/CD3+), helper T lymphocytes ($CD45^+/CD3^+/CD4^+CD8a^-$) and cytotoxic T lymphocytes ($CD45^+/CD3^+/CD4^-CD8a^+$) were analyzed (using flow cytometry) in peripheral blood (A-B), spleen (C) and thymus (D) samples from control (n=9) and exposed (n=8) rats. The mean proportion was calculated with reference to the total CD45+ cell count. NS: nonsignificant, *: $p<0.05$, **: $p<0.01$ in a Mann-Whitney test.

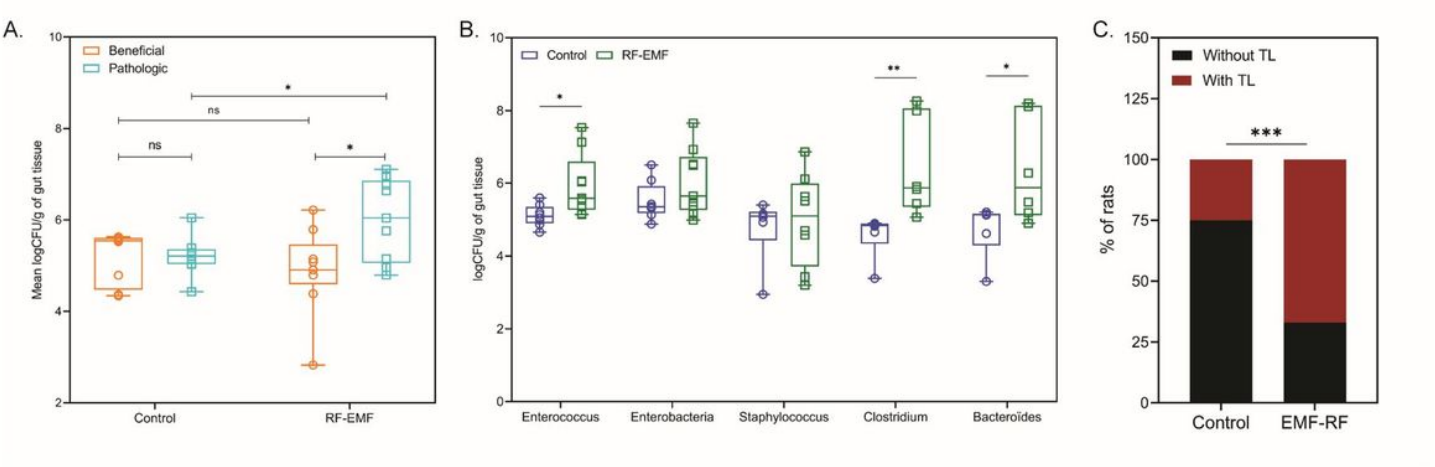

Figure 4

The microbiota in gut tissue samples from control and exposed rats

Beneficial and pathogenic microbiotas were determined for exposed (n=8) and control (n=9) rats. The results were expressed in CFUs per gram of colon tissue (A). Each pathologic genus (Enterobacteria, *Enterococcus*, *Staphylococcus*, *Clostridium*, and *Bacteroides*) was individually quantified in colon tissue from exposed (n=8) and control (n=9) rats (B). The proportion exposed or control animals in which *Enterococcus* had translocated to the spleen (w. = with; w/ = without) is indicated (C). The threshold for statistical significance in Mann-Whitney and chi-squared tests was set to $p<0.05$.

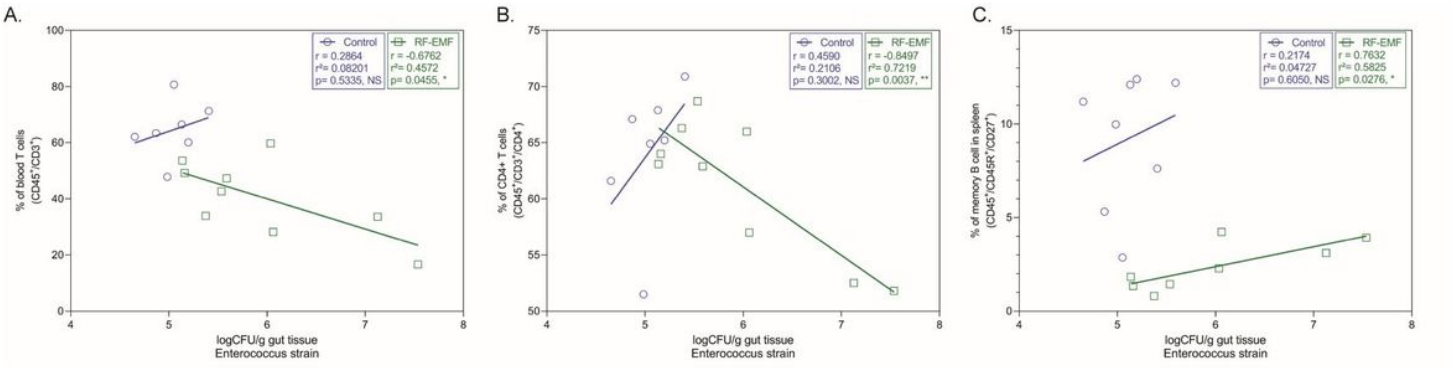

Figure 5

Relationships between the Enterococcus count in the gut and hematological variables in control and exposed rats.

The Enterococcus count was significantly correlated (according to Pearson's correlation coefficient) with the proportions of total T lymphocytes (A), helper T lymphocytes (B), and memory B lymphocytes (C) in the blood in the exposed group only. The threshold for statistical significance was set to $p<0.05$.