

## RESEARCH ARTICLE



# Extremely low-frequency electromagnetic fields targeting spleen modifies the populations of immunocytes in the spleen

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## Abstract

Our study focused on investigating the bioeffects of extremely low-frequency electromagnetic fields (ELF-EMFs) on the immune function of the spleen. We designed an electromagnetic instrument that can locally target on spleen, the spleens of mice were locally exposed to the ELF-EMF (50 Hz, 30 mT) for 14 days (4 h/day). Parallely, the isolated splenic T cells were exposed to ELF-EMF (50 Hz, 15 mT) for 2 h. After the exposure, the splenocyte showed a reduced apoptosis rate. Among the splenocytes, the CD4<sup>+</sup> T cells and natural killer cells accumulated, the percentage of B cells decreased. In vitro study demonstrated that ELF-EMF induced the alteration of T cell subsets, showing an increased percentage of CD4<sup>+</sup> T cells and a decreased percentage of CD8<sup>+</sup> T cells. Within CD4<sup>+</sup> T cells, the population of T helper (Th) 17 cells increased, and the population of regulatory T cells (Treg) cells decreased. The enrichment of the nuclear factor (NF)-κB pathway in the splenic T cells was found to be reduced after exposure to ELF-EMF. Our findings suggest that ELF-EMF regulated the immune function of the spleen by changing the proportion of immune cells in the spleen. Specifically, the differentiation of spleen T cells was induced by ELF-EMF toward Th17 cells and inhibited by ELF-EMF into Treg cells. The NF-κB signaling pathway probably accounts for the effects of ELF-EMF on the spleen T cells.

## KEYWORDS

ELF-EMF, immune, spleen, T cells

Sijia Chen, Wei Wei, and Ziang Wang are first authors.

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## 1 | BACKGROUND

Electromagnetic fields (EMFs) exist everywhere in the modern environment, and the radiation of EMF acts on the tissues and cells of the organism, causing physiological and functional changes. This phenomenon is known as the biological effect of electromagnetic fields.

The wave of extremely low-frequency electromagnetic fields refers to 0–100 Hz, which belongs to nonionizing radiation. It has been reported that there is a potential link between exposure to extremely low-frequency (ELF)-EMF and the immune system (Mahaki et al., 2019). Previous studies have noted that ELF-EMF has bioeffects on the activities of immune cells. Short-term exposure to 50 Hz ELF-EMF enhanced the phagocytosis and upregulated the level of oxidative stress in bone marrow-derived macrophages (Frahm et al., 2010). The cytotoxic activity of splenic natural killer (NK) cells against *Candida stellatoidea* was suppressed by 50 Hz ELF-EMF (Canseven et al., 2006). Moreover, ELF-EMF has been found to influence the release of inflammatory cytokines. Fifty hertz ELF-EMF reduced the expression of peripheral interleukin (IL)-9 and tumor necrosis factor (TNF)- $\alpha$ , while increasing the level of IL-1 $\beta$ , IL-6, and IL-2 in the spleen, peripheral blood, and plasma (Mahaki et al., 2020; Salehi et al., 2012; Wyszowska et al., 2018).

As the largest peripheral immune organ, the spleen contributes significantly to the body's immune regulation network and is involved in both specific and non-specific cellular and humoral immune defensive responses. All peripheral blood immune cells will be filtered in the spleen before localization and recirculation. Normally there are a large number of immune cells localized in the spleen, including lymphocytes and monocyte-macrophages, which are responsible for eliminating pathogen and aged cells, regulating hemostasis, and adjusting specific immune responses (Bronte et al., 2013). The splenic cellular immunity participates in ameliorating multiple diseases. During ischemic myocardial injury, the motility of reservoir monocytes in the spleen was increased, and it was observed that massive monocytes left the spleen into the circulation and then accumulated in the ischemic myocardium to contribute to wound healing (Swirski et al., 2009). T cells in the spleen produce a cytotoxic effect on tumor cells (Ge et al., 1989). To facilitate the early immune response in malaria, monocytes were recruited from the bone marrow to the spleen to control the parasite burden (Antonelli et al., 2014). Nonetheless, the spleen has a two-way immunological function. In the early stage of acute pancreatitis, the spleen contributed to the systemic inflammatory response mainly by encouraging the secretion of inflammatory cytokines and the activation of amylase, thus accelerating the progression of severe acute pancreatitis and exacerbating the damage of the primary

### Highlights

- Locally electromagnetic field exposure could be targeted to the spleen for intervention.
- Extremely low-frequency electromagnetic field changes the immune status of the spleen.
- T cells play a key role in the process of electromagnetic field influencing spleen.

tissues and organs involved (Zhou et al., 2019). During sepsis, spleen-derived macrophages released type I interferon (IFN) to specifically inhibit the function of DC cell-induced cytotoxic T lymphocytes (Schwandt et al., 2012). Additionally, the splenic macrophage produced a significant amount of transforming growth factor (TGF)- $\beta$  to promote the progression of liver fibrosis (Akahoshi et al., 2002).

The spleen plays a critical role in the immune system, and a study used noninvasive ultrasound to stimulate the spleen to treat inflammatory arthritis (Zachs et al., 2019). However, it remains unclear whether extremely low-frequency electromagnetic fields can be employed to affect the spleen's immunological activities. In this work, the effects of ELF-EMF on the splenic immune cells were explored in healthy mice. We found a notable change in the spleen's immune cell population. Furthermore, the splenic T lymphocyte was sensitive to ELF-EMF and experienced differentiation toward T helper (Th17) cells. Moreover, ELF-EMF may inhibit the activation of the nuclear factor (NF- $\kappa$ B) signaling pathway in the spleen T cells, thereby reducing the differentiation of Treg cells in splenic T cells.

## 2 | METHODS

### 2.1 | Experimental electromagnetic fields

The coil form has to be carefully taken into consideration to produce the electromagnetic fields with the necessary flux density in the area of the spleen and cells. The most widely used varieties of excitation coils mainly include hollow coil, Helmholtz coil, eight-figure coil, C-type coil, and solenoid coil. Considering the subject is the mouse spleen, the instrument should be small and stable for placement, so the solenoid coil was utilized in the animal experiment. Since the cells in the culture dish would be close to the coil, the hollow coil was used in this study. The model of the planar hollow coil and iron core solenoid coil is established based on the simulated analysis. The detailed parameters are shown in Table 1.

**TABLE 1** Parameters of coils used in the study.

| Coil type                         | Number of the turns | Diameter (mm) |
|-----------------------------------|---------------------|---------------|
| Iron core solenoid coil (in vivo) | 8750                | 0.5           |
| Planar hollow coil (in vitro)     | 200                 | 1.0           |

The iron core coil used to establish ELF-EMF for animal experiments is a cube that measures 55 mm × 46 mm × 40 mm which was made of copper wires wrapped around a silicon steel sheet (Figure 1A,a)—the greater size guaranteed controllable temperature when the coils were operating. The size of the central exposure area of the coil was designed following the dimensions of the mouse spleen, which is a 25 mm × 12 mm rectangle. The remaining portions of the coil were covered with insulating materials in order to expose the mouse spleen to ELF-EMF locally. The planar spiral coil with a central hole, utilized in cell experiments, was created using the same materials (Figure 1A,c). To ensure that at least three wells could be included in the exposure region, the size of the coil used for cell exposure was designed considering the size of 12-well plates. The frequency was set to 50 Hz in our study, because, combined with previous research status, it can be seen that extremely low-frequency electromagnetic fields of 50 Hz were generally chosen for the studies and may have certain bioeffects (Mahaki et al., 2019; Piszczek et al., 2021). The magnetic field distribution of the coils was calculated using COMSOL Multiphysics® simulation software. The magnetic field for the coil used in the animal experiments was mostly distributed in the central area (Figure S1A,a). Aside from the core exposure area, there was little energy present, but the outer layers of the coil were insulated. We determined the magnetic flux density at a distance of 5 mm from the animal experiment coil since there was a specific distance between the coil and the spleen, it could be seen that the flux density of the exposure area was 29–30 mT (Figure S1A,b). The magnetic field distribution in the center of the coil used in the cell experiments was essentially uniform (Figure S1B,a). The distance between the coil and the cells was about 12.5 mm, and the magnetic flux density on the cell area was roughly 16 mT (Figure S1B,b). The intensity of the fields could be adjusted by a potentiometer and magnetic flux density was measured at the target site using a Gauss meter. The measurements are very close to the simulation results. These instruments were fabricated by the School of Electrical Engineering of Xi'an Jiaotong University. The identical instruments were used, respectively, in control mice and cells except without attaching to a potentiometer or electric current source. Four small high-power fans were positioned around the experimental table to provide a convection of cold air, which helped to control the temperature of the coils effectively.

## 2.2 | Animal and experimental groups

In this study, 6–8-week-old male C57BL/6J mice were purchased from the Laboratory Animal Center of Xi'an Jiaotong University, PR China. Mice were divided into two groups for both short-term and long-term models. The mice in the EMF group ( $n = 18$ ) were subject to ELF-EMF, while the mice in the sham EMF group ( $n = 18$ ) were treated with sham ELF-EMF. The mice were anesthetized and exposed to either sham ELF-EMF or ELF-EMF (50 Hz, 30 mT) in a prone position (Figure 1A,b), respectively, for 30 min, 1 h, 4 h, and 14 days (4 h/day). The coils were placed against the surface location of the spleen which was decided according to the study which indicated the landmark should be the midpoint between the shoulder and hip joint of the left flank (Zachs et al., 2019). The mice were sacrificed 1 day after the end of the exposure. Then the spleens were collected for further testing. We calculated the spleen index (SI) according to the following formula: SI = spleen weight (mg)/body weight (g). All animal research protocols were approved by the Animal Care and Use Committee of Xi'an Jiaotong University and complied with the recommendations of guidelines published by the National Institutes of Health, China.

## 2.3 | Hematoxylin-eosin staining

Paraformaldehyde-fixed, paraffin-embedded spleens were cut into 4-μm-thick sections with a microtome. Dewaxing, dehydrating, staining, washing, differentiating, rinsing, and sealing were performed as previously described (Li et al., 2016). The pathological changes in the spleen were observed under an inverted microscope (ZEISS).

## 2.4 | Alexa Fluor 488 Annexin V/PI staining

The spleen tissue was ground and taken through a 70-mm-pore-size nylon cell strainer to obtain spleen single-cell suspension. The cells harvested from spleen single-cell suspension or in vitro were resuspended in 1X annexin-binding buffer. Five microliters Alexa Fluor 488 Annexin V and 1 μL PI were added to each 100 μL of cell suspension. After 15 min of incubation, the cells

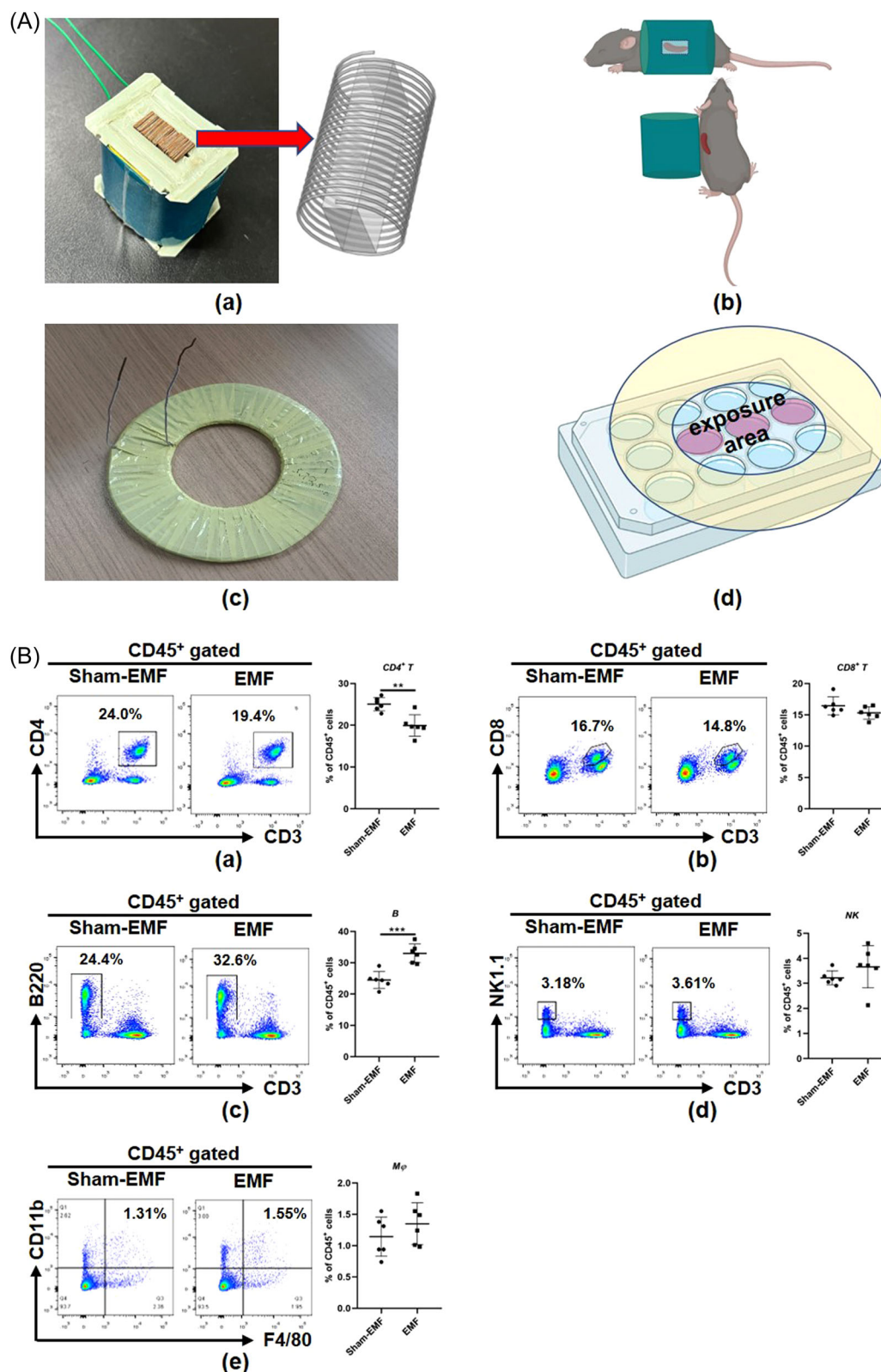


**FIGURE 1** The design of the ELF-EMF exposure system and the optimal daily exposure duration. (A ) The ELF-EMF exposure system: (a) The ELF-EMF instrument that can locally target the spleens of mice. (b) The pattern of mice being exposed to ELF-EMF. (c) The planar spiral coil that used for in vitro experiments. (d) The exposure area of electromagnetic fields used in cell experiments. (B) The frequencies of the mice's splenic immune cells changed after 4 h of the ELF-EMF exposure. The representative dot plots and percentages of mouse splenic immune cells: (a) CD4<sup>+</sup> T cells, (b) CD8<sup>+</sup> T cells, (c) B cells, (d) NK cells, and (e) macrophage cells. Each experimental group contains six animals. ELF-EMF, extremely low-frequency electromagnetic field; NK, natural killer. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

were detected by flow cytometry (Canto II; BD BioSciences, or DxFLEX; Beckman Coulter) and analyzed with FlowJo software (FlowJo LLC) or CytExpert (Beckman Coulter).

## 2.5 | Flow cytometric analysis

Erythrocytes of the prepared single-cell suspensions were lysed with erythrocyte lysis buffer (Cytelbio) and washed twice with phosphate-buffered saline (PBS). Cells (106) were then suspended in 100  $\mu$ L PBS and incubated at 4°C for 30 min with the following antibodies: anti-CD45, anti-CD3, anti-CD4, anti-CD8a, anti-NK1.1, anti-B220, anti-CD11b, and anti-F4/80. To quantify Tregs cells, a mouse True-Nuclear Transcription Factor Buffer Set (424401; BioLegend) was used. Splenic primary T cells were stained with CD4 and CD25. The cells were then fixed and permeabilized for intranuclear staining with Alexa Fluor 488-conjugated anti-Foxp3, according to the manufacturer's protocol (BioLegend). To detect Th1, Th2, and Th17 cells, every 1 ml of primary T cell culture (106) was added with 2  $\mu$ L leukocyte activation cocktail (550383; BD) and then was placed in a 37°C humidified 5% CO<sub>2</sub> incubator for 6 h. Following activation, cells were washed with PBS and stained with CD4. The cells were then fixed and permeabilized for intracellular staining with IFN- $\gamma$ , IL-4, and IL-17 according to the manufacturer's protocol (BioLegend). After 30 min, the cells were detected by flow cytometry (Canto II; BD BioSciences or DxFLEX, Beckman Coulter, USA) and analyzed using FlowJo software (FlowJo, LLC) or CytExpert (Beckman Coulter). The antibodies are shown in Supporting Information S1: Table 1.

## 2.6 | Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA of the spleen tissue, or cultured cells was extracted with Trizol (Takara) according to the manufacturer's instructions, and then reverse transcription was performed by using a Primescript<sup>TM</sup> RT reagent kit (Takara). qRT-PCR was then performed using SYBR Premix Ex Taq II and an ABI7500 fast instrument (ABI Life Technologies), with GAPDH as an internal control. The primers are shown in Supporting Information S1: Table 2.

## 2.7 | Primary cell isolation

Primary T lymphocyte isolation was performed using the Mouse Spleen Mononuclear Cell Isolation Kit (Tianjin Haoyang Biological Manufacture). Three

milliliters separation medium was added into a 15 mL centrifuge tube, and 3 mL spleen single-cell suspension was slowly added to the surface of the separation medium. After 30 min of centrifuge at 500g, the layer of opalescent mononuclear cells was collected and transferred into a new 15 mL centrifuge tube. Following washing, the cells were resuspended in 100  $\mu$ L sterile PBS and then incubated with antibodies against the cell surface markers: anti-CD45 and anti-CD3 at 4°C for 30 min. Then the T lymphocytes were isolated by fluorescence-activated cell sorting (Moflo Astrios EQ; Beckman Coulter)

## 2.8 | Cell culture and exposure

The isolated spleen T cells were cultured in Roswell Park Memorial Institute 1640 medium (Hyclone; Thermo Fisher Scientific) supplemented with 10% fetal calf serum (Hyclone) and 1% penicillin/streptomycin. The cells were cultured at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. The primary T cells were placed in a 12-well plate and underwent exposure (50 Hz, 15 mT) with the coil on the top of the plate for 2 h (Figure 1A,d).

## 2.9 | RNA sequencing and data analysis

The total RNA of primary splenic T cells was extracted with TRIzol (Takara) according to the manufacturer's instructions. The sequencing libraries were constructed by reverse-transcribing the RNA to complementary DNA (cDNA) and randomly amplifying primed cDNA molecules. Sequencing was performed with Illumina Sequencing Platform (Illumina) according to the manufacturer's instructions. The raw sequencing data was evaluated by FastQC and the low-quality bases were trimmed using Trimmomatic to get relatively accurate and effective data. The processed reads were aligned to a reference genome and mapped to specific genomic locations using HISAT2. The expression level of the genes subsequently was quantified by the StringTie software. The gene expression differences were analyzed by DESeq2 and results were visualized. Finally, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and KOG enrichment analysis were performed using clusterProfiler software.

## 2.10 | Statistical analysis

Results were presented as the mean  $\pm$  standard errors of the means. Comparisons were performed using Student's *t* test. *p* < 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism 8 (GraphPad Software Inc.).

### 3 | RESULTS

#### 3.1 | The optimal daily exposure duration of the ELF-EMF exposure system

It is necessary to determine the suitable daily exposure period because the mice were exposed to the local ELF-EMF while under anesthesia, making a 24-h exposure impractical. Therefore, we investigated the bioeffect of ELF-EMF on the spleen at various intervals of short-term exposure. After 30 min and 1 h of exposure, there were no significant differences in the frequencies of spleen immunocytes between the sham-EMF group and the EMF group (Figure S2). However, when the exposure period was extended to 4 h, the percentage of splenic CD3<sup>+</sup> CD4<sup>+</sup> T lymphocytes of the EMF group decreased compared with the sham-EMF group ( $25.07 \pm 0.6484$  vs.  $19.95 \pm 1.0480$ ) (Figure 1B,a), while the B cells population was higher in the EMF group than that in the sham-EMF group ( $24.48 \pm 1.1000$  vs.  $32.98 \pm 1.2070$ ) (Figure 1B,c), other immune cells did not show significant changes (Figure 1Bb,d,e). Since the profile of splenic immunocytes of mice changed during 4 h of ELF-EMF exposure, we tended to prefer 4 h as the daily exposure time. We measured the temperatures at various times while taking into account the heat production of iron core coils. The temperature was 27.2°C at first, and it progressively increased from 31.4°C at the 30-min mark to 33.1°C at the 1-h mark, until it stabilized at 33.3°C after 3 h of exposure (Figure S3). Given that the body temperature of the mouse is approximately 37°C, the temperature fluctuation range was satisfied. Consequently, we decided that the daily exposure period for the ensuing long-term experiments would be 4 h.

#### 3.2 | The bioeffects of ELF-EMF on the immune function of the spleen

Following the determination of the daily exposure time, it was first examined the changes in healthy mice spleen brought on by ELF-EMF exposure. After 14 days (4 h/day) of exposure, no difference in SI was observed between the sham-EMF group and the EMF group ( $3.500 \pm 0.1386$  vs.  $3.232 \pm 0.1656$ ) (Figure 2a). Spleen hematoxylin and eosin (H&E) staining did not show an obvious difference in red and white pulp between the sham-EMF group and the EMF group (Figure 2b). When we compared spleen cell apoptosis with or without ELF-EMF, the results indicated that the rate of apoptosis during the early stages of spleen cells significantly declined after ELF-EMF exposure ( $12.950 \pm 0.8441$  vs.  $9.638 \pm 0.7251$ ), while there was no significant difference for the late apoptosis rate of splenic cells ( $10.320 \pm 1.938$  vs.  $7.095 \pm 0.4477$ ) (Figure 2c). We then

analyzed the changes of inflammatory cytokines levels in the spleen of healthy mice, IL-4 and IL-17 were upregulated and IL-10 were downregulated after ELF-EMF exposure while TGF- $\beta$  and IFN- $\gamma$  showed no significant changes (Figure 2d). We then investigated the profiles of spleen immune cells. The CD3<sup>+</sup> CD4<sup>+</sup> T lymphocytes ( $16.93 \pm 1.309$  vs.  $21.08 \pm 1.056$ ) and NK cells populations ( $4.425 \pm 0.3940$  vs.  $6.332 \pm 0.4296$ ) were higher in the EMF group than those in the sham-EMF group (Figure 3Aa,c), whereas the expression of B cells decreased after the EMF exposure ( $78.53 \pm 0.8620$  vs.  $72.93 \pm 1.083$ ) (Figure 3A,c), meantime there were no significant differences in the frequencies of CD3<sup>+</sup> CD8<sup>+</sup> T lymphocytes ( $9.42 \pm 0.6339$  vs.  $11.02 \pm 0.4236$ ) and macrophages ( $2.055 \pm 0.1986$  vs.  $2.030 \pm 0.1735$ ) between the EMF group and sham-EMF group (Figure 3A,b,d).

#### 3.3 | Effects of ELF-EMF on splenic T cell: In vitro

Since the CD3<sup>+</sup> CD4<sup>+</sup> T lymphocytes and several T-cell-related cytokines significantly changed after ELF-EMF exposure, we assumed that ELF-EMF had bioeffects on the splenic T cells. Consequently, the primary T cells were isolated from the mouse spleen and used to verify whether ELF-EMF may have a direct impact on the differentiation of splenic T cells. Even though the spleen is adjacent to the body surface, there is still a certain distance between them. Besides, the structure of the spleen is complex. Thus, even if 30 mT of intensity was applied externally, the amount of energy that the splenic T cells could absorb was limited. Therefore, for the in vitro studies, 15 mT was used instead of 30 mT. Similar but different from the animal model, ELF-EMF could affect cells apoptosis, the rate of apoptosis during the late stages of primary T cells significantly declined ( $1.995 \pm 0.0301$  vs.  $1.735 \pm 0.0556$ ), while no obvious difference was found for early apoptosis rate between the control group and EMF group ( $2.578 \pm 0.1301$  vs.  $2.460 \pm 0.1758$ ) (Figure 3b). We then analyzed the changes in inflammatory cytokines levels in primary T cells, the production of IL-4 and IL-17 were increased, while IL-10 was decreased after ELF-EMF exposure (Figure 3c). After 2 h of exposure to ELF-EMF, the CD3<sup>+</sup> CD4<sup>+</sup> T lymphocytes population was increased compared with the control group ( $43.90 \pm 0.3090$  vs.  $60.12 \pm 0.2399$ ), whereas the CD3<sup>+</sup> CD8<sup>+</sup> T lymphocytes population was decreased ( $42.82 \pm 0.2193$  vs.  $25.33 \pm 0.3378$ ) (Figure 4a). We further investigated the polarization of CD3<sup>+</sup> CD4<sup>+</sup> T cells after ELF-EMF exposure. The results showed that the frequency of Treg which is an immunosuppressive T cell was significantly reduced in the EMF group compared with the control group ( $7.227 \pm 0.7666$  vs.  $2.617 \pm 0.2918$ ), while the frequency of Th17 cells was elevated after ELF-EMF exposure ( $0.3899 \pm 0.0285$  vs.

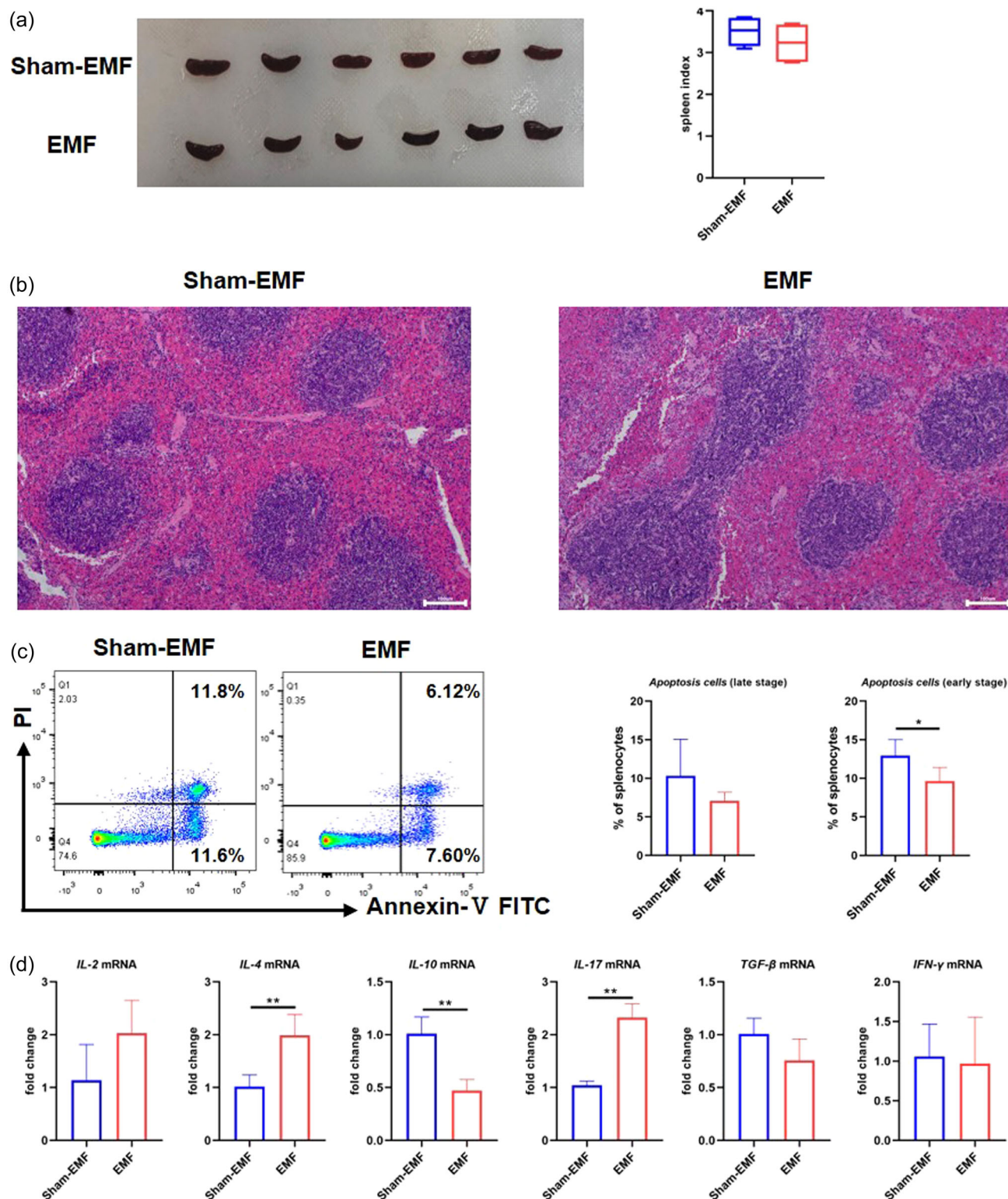


**FIGURE 2** Changes of SI, histology, and apoptosis rate after 14 days (4 h/day) of exposure. (a) SI = spleen weight (mg)/body weight (g). (b) Spleen H&E staining. Magnification for all images is  $\times 100$ . (c) the apoptosis rate of splenic cells in the early and late stages. (d) The mRNA expression of cytokines in spleens of healthy mice. Each experimental group contains six animals. EMF, electromagnetic field; H&E, hematoxylin and eosin; IFN- $\gamma$ , interferon- $\gamma$ ; IL-2, interleukin-2; mRNA, messenger RNA; SI, spleen index; TGF- $\beta$ , transforming growth factor- $\beta$ . \* $p < 0.05$ , \*\* $p < 0.01$ .

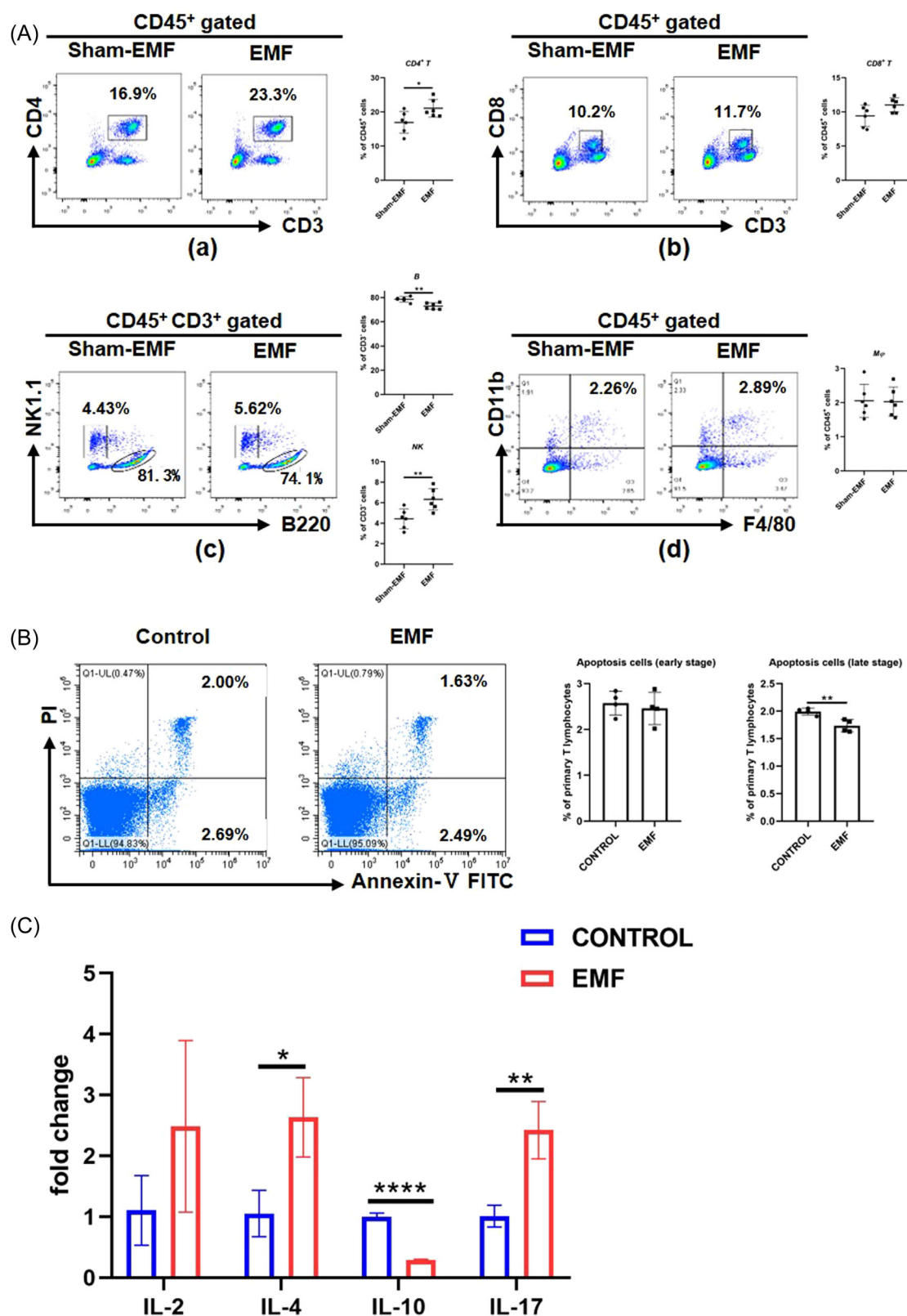


**FIGURE 3** ELF-EMF has bioeffects on the frequencies of splenic immune cells in healthy mice and isolated splenic T cells. (A) After 14 days (4 h/day) of exposure to ELF-EMF, the representative dot plots and percentages of immunocytes in the spleen: (a) CD4<sup>+</sup> T cells, (b) CD8<sup>+</sup> T cells. (c) B cells and NK cells, (d) macrophage cells. (B) Apoptosis rates of isolated splenic T cells in the early and late stages after 2 h of exposure. (C) The mRNA expression of cytokines in isolated splenic T cells after 2 h of exposure. Each experimental group contains six animals. ELF-EMF, extremely low-frequency electromagnetic field; IL-2, interleukin-2; mRNA, messenger RNA; NK, natural killer. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.0001$ .

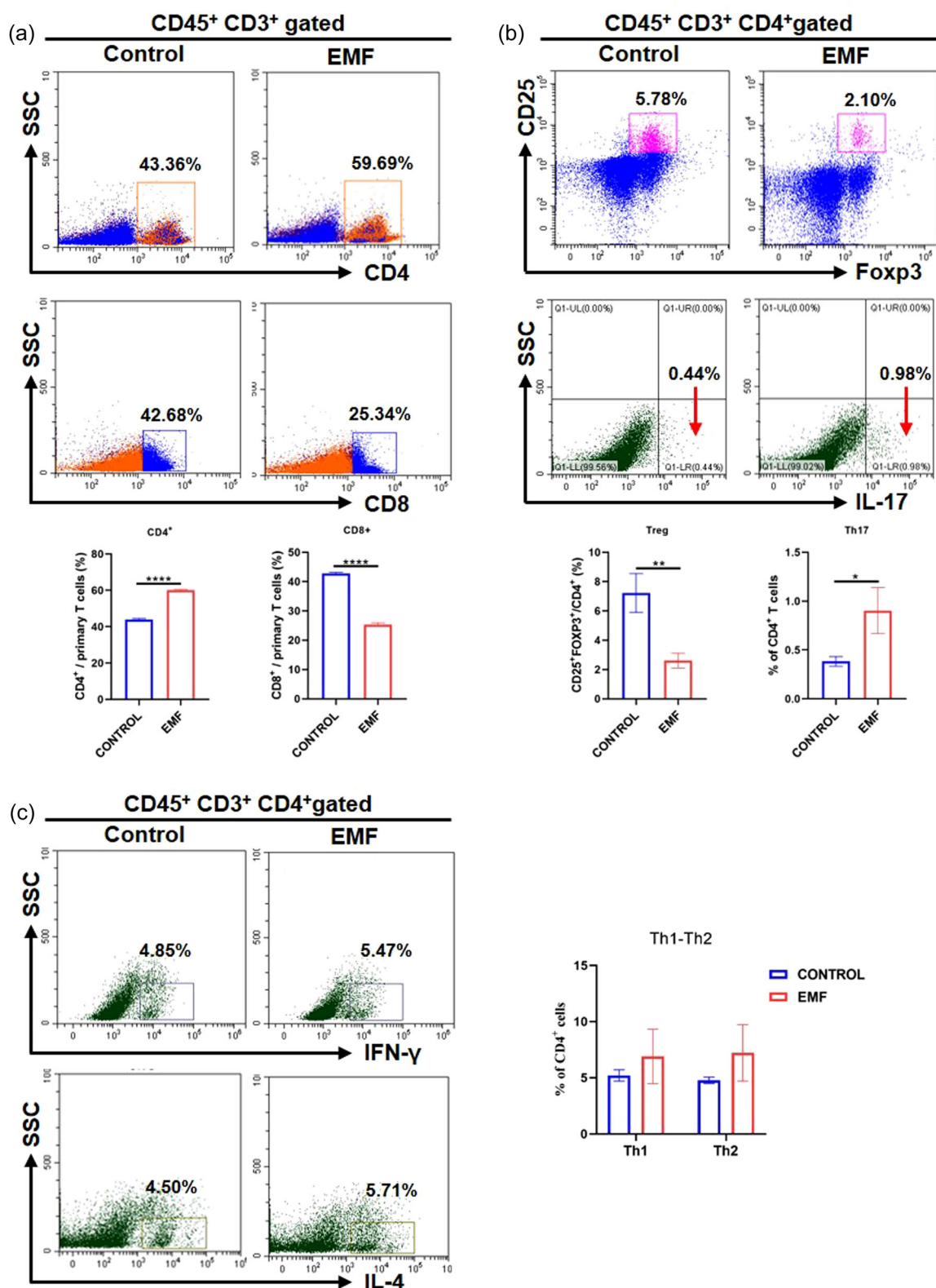


**FIGURE 4** ELF-EMF influences the differentiation of isolated splenic T cells. (a) Representative dot plots and percentages of CD4<sup>+</sup> and CD8<sup>+</sup> T cells after 2 h of exposure. (b) Representative dot plots and percentages of Treg and Th17 cells after 2 h of exposure. (c) Representative dot plots and percentages of Th1 and Th2 cells after 2 h of exposure. ELF-EMF, extremely low-frequency electromagnetic field; IFN- $\gamma$ , interferon- $\gamma$ ; IL- $\beta$ , interleukin- $\beta$ ; Th17, T helper 17 cells; Tregs, regulatory T cells. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ .

$0.9033 \pm 0.1354$ ) (Figure 4b). Although no significant difference was detected for the populations of both Th1 ( $5.210 \pm 0.2883$  vs.  $6.897 \pm 1.402$ ) and Th2 cells ( $4.773 \pm 0.1650$  vs.  $7.217 \pm 1.452$ ) in the two groups, both of them showed increased trend after ELF-EMF exposure (Figure 4c).

### 3.4 | ELF-EMF inhibits the enrichment of the NF- $\kappa$ B pathway in the spleen immune

To further investigate the mechanism of bioeffects of ELF-EMF on the spleen, messenger RNA-sequencing analysis was used to measure the gene expression of spleen primary T cells in the control and EMF group. Significantly changed genes were selected in the EMF group, compared with the control group (Figure 5b). KEGG analyses revealed that the NF- $\kappa$ B pathway is inhibited after ELF-EMF exposure (Figure 5a). According to the previous study, the canonical NF- $\kappa$ B pathway is activated by the results of stimulation with IL-1 and TNF- $\alpha$ , we then investigated the effect of ELF-EMF on IL-1 $\beta$  and TNF- $\alpha$  expression. The results showed decreased expression of IL-1 $\beta$  and TNF- $\alpha$  in the mouse model after ELF-EMF exposure (Figure 5c).

## 4 | DISCUSSION

Recently, the research on the biological effects of ELF-EMF on immunity has gained more attention, aiming at clarifying the influence of low-frequency electromagnetic fields on the immune system. Nevertheless, the electromagnetic devices employed in these researches mostly addressed the whole body. Electromagnetic fields affect various organs and different cells to varying degrees throughout the body (Piszczyk et al., 2021). Since most cells and organs are in communication with one another, it is challenging to explain the role and mechanism of electromagnetic fields for specific immune organs. In the present study, we designed an electromagnetic instrument that can locally target the spleen which is the largest immune organ, and observed the changed profiles of T, B, NK, and macrophage cells in the mouse spleen.

The bioeffects of EMF on the immune system are considered to have both protective and harmful aspects. It was proposed that EMF suppressed the immunological response to infection by inhibiting the activity of intracellular calcineurin and inducing reactive oxygen species (ROS) production. (Doyon et al., 2017). Additionally, EMF had an adverse effect on homeostasis and probably was related to cancer, leukemia, or other diseases (Zhang et al., 2017). Conversely, EMF can benefit immunity without causing ionizing radiation, thermal effects, or breaking down chemical bonds (Mahaki et al., 2019).

Through the NADPH oxidase pathway and the production of ROS, EMF promoted the development of neutrophils extracellular traps which could degrade pathogenic substances and kill bacteria (Golbach et al., 2015). Acute inflammation was involved in wound healing, it was demonstrated that EMF had a stimulatory effect on the release of anti-inflammatory cytokines and angiogenic factors, which could be useful in promoting tissue repair (Saliev et al., 2014). This bioeffect is the outcome of the interaction between electromagnetic fields and organisms, which involves multiple variable parameters, including strength, direction, and frequency of electromagnetic fields, as well as the types of cells, organs, and electromagnetic susceptibility in organisms. The various combinations of these parameters can potentially lead to significantly different or even opposite effects (Piszczyk et al., 2021). The disparities in the aforementioned studies could be attributed to the various electromagnetic field parameters, cell types, and animal models.

Based on the above situation and our research purpose, we first explored the ELF-EMF parameters that ought to be used in this study. Electromagnetic fields produce heat; an excessively high temperature will not only make it difficult to observe the biological effects of electromagnetic fields but will also limit its use in therapeutic settings. Therefore, the ideal temperature for heat production of ELF-EMF should not be higher than the body temperature of mice. We found that the populations of splenic immune cells changed after 4 h of exposure. Following 3 h of radiation, the temperature stabilized at 33.3°C, and the findings of simulative computation indicated that the air temperature surrounding the mice's body surface was approximately 31°C, while the normal body temperature of the mice was approximately 37°C. Thus, the thermal effects of the electromagnetic coil in this experiment are minimal. Because of the biological effects of this exposure situation at a controlled temperature, we ultimately decided to set the daily exposure period at 4 h.

It was discovered that 50 Hz EMF at a flux density of 30 mT did not change the SI after 14 days of exposure. H&E staining of the spleen showed no discernible differences. The spleen cells displayed a reduced apoptosis rate. Since the ELF-EMF density in our study was mild, the spleen may benefit from exposure to ELF-EMF and not suffer any mechanical harm from it. According to earlier research, electromagnetic fields have a broad impact on all types of immune cells, influencing both innate and adaptive immunity (Mahaki et al., 2019; Rosado et al., 2018). NK activity was suppressed by 60 Hz EMF at a flux density of 1 mT after 13 weeks of exposure (House et al., 2000). According to Kim et al. (2017), the mouse macrophage cell line (RAW 264.7) was exposed to 50 Hz, 0.8 mT EMF for 8 or 20 h, and this resulted in an increase in NO production as well as proinflammatory cytokines including IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , which activated the immune system (Kim et al., 2017). We were able

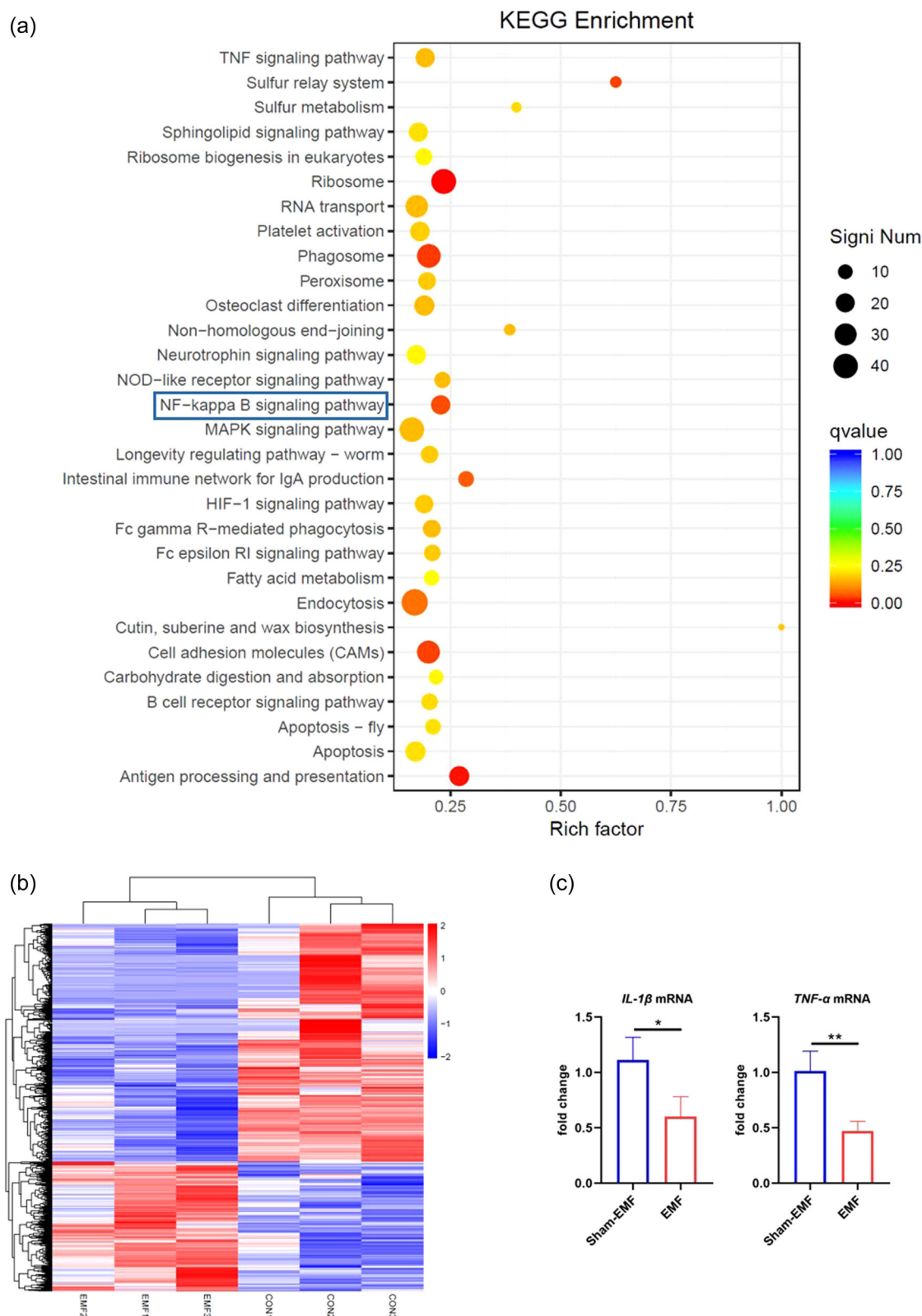


**FIGURE 5** Comprehensive gene expression analysis of the splenic primary T cells from control and EMF groups. (a) Changed pathways in the primary T cells with EMF exposure were determined via KEGG pathway analysis mice. (b) Heatmap of differentially expressed genes. (c) The mRNA expression of *IL-1 $\beta$*  and *TNF- $\alpha$*  in the spleen of healthy mice. CAM, cell adhesion molecule; EMF, electromagnetic field; HIF-1, hypoxia-inducible factor; *IL-1 $\beta$* , interleukin- $\beta$ ; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; mRNA, messenger RNA; NF- $\kappa$ B, nuclear factor- $\kappa$ B; *TNF- $\alpha$* , tumor necrosis factor- $\alpha$ . \* $p < 0.05$ , \*\* $p < 0.01$ .

to confirm in this study that the splenic immune cell profile was altered by ELF-EMF. The spleens of mice experienced increased percentages of CD4<sup>+</sup> T cells and NK cells while decreased B cell population. The bioeffects of EMF can vary over time, and continuous exposure and intermittent exposure could lead to different biological outcomes. Wyszowska et al. (2018) exposed rats to (50 Hz, 7 mT) EMF and found that the continuous exposure for 24 h caused elevated levels of leukocytes, lymphocytes, hemoglobin, and hematocrit. However, no such significant differences were observed following repeated exposure (1 h/day, 7 days) (Wyszowska et al., 2018). The contrasting changes in CD4<sup>+</sup> T cells observed in our data between the 4-h single exposure and the 4 h/day recurrent exposure for 14 days could be attributed to the various exposure duration types. Furthermore, the disparity in the outcomes could be explained by the distinct total cumulative bioeffects of the two exposure situations.

T lymphocytes are the most important effector in adaptive immunity, splenic T cells mediate cellular immunity, assist humoral immunity, active immune response, and induce immune tolerance. Humoral immunity is mediated by B lymphocytes, and splenic B cells express surface receptors, secrete cytokines, and generate antibodies to sustain immune homeostasis. NK cells are involved in innate immunity; they are the first line of defense for immune surveillance, able to identify antigens and kill tumor cells and virus-infected cells (Golub et al., 2018; Lewis et al., 2019). Thus, by altering the frequencies of splenic immune cells, ELF-EMF regulates the immunological function of the spleen.

Ohtani et al. (2015) investigated the effect of EMF on T lymphocytes and found that there were no alterations in the distribution of Th cells, cytotoxic CD4/CD8 T cells, and activated CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cell populations following exposure. We believed that ELF-EMF changed the subsets of splenic T cells from our findings that showed an increase in the expression of IL-4, which is secreted by Th2 cells, and IL-17, which is secreted by Th17 cells, while reduced production of IL-10, which is produced by Treg cells (He et al., 2011; Ruterbusch et al., 2020). By stimulating B cell proliferation and promoting the production of IgE, IL-4 contributes to type 2 immune response and exerts immune-modulating effects in asthma, atopic dermatitis, and urticaria (Ranasinghe et al., 2014). Under normal conditions, IL-10 can maintain tissue homeostasis by acting as an anti-inflammatory cytokine and reducing the overreaction of the immune system during infection (Saraiva et al., 2010). On the other hand, IL-10 is considered to down-regulate the activation of immune cells in the tumor microenvironment, hence inhibiting antitumor immunity (Kevin et al., 2001). IL-17 is closely related to multiple disorders and has a variety of biological functions. In addition to promoting protective immunity against bacterial infections, viruses,

parasites, and fungal diseases, IL-17 also drives harmful inflammatory pathology during the development of autoimmune diseases (McGeachy et al., 2019). The balances of Treg/Th17 and Th1/Th2 cytokines levels in the spleen were modified by ELF-EMF, which might trigger the alteration in the immune status of the spleen. This implies that ELF-EMF may regulate the spleen's immune function by influencing T cell differentiation. In addition, to fully comprehend the bioeffects of ELF-EMF on the spleen, more research was necessary as the altered NK and B cell populations were not explicitly examined. Additionally, in order to investigate the effect of ELF-EMF itself on T cell differentiation, only basal medium was used in the in vitro study without the addition of any stimulus-activating reagents, which reduced the amount of time that the cells could be cultured in vitro and limited the exposure time. In future study, we will make more efforts to address the issue of the culture cycle to optimize the exposure duration and achieve maximum consistency between in vivo and in vitro exposure time.

According to previous studies, T cells can differentiate into CD4<sup>+</sup> T, CD8<sup>+</sup> T, and NKT cells. Of these, the CD4<sup>+</sup> T cells can be polarized into multiple subsets with specific functions, and NF- $\kappa$ B was involved in practically every stage of the T cell differentiation process (Gerondakis et al., 2010; Hovelmeyer et al., 2022; Voisin et al., 2021). Meanwhile, Zhang et al. (2017) demonstrated that ELF-EMF induced decreased plasma levels of NF- $\kappa$ B, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. However, whether local ELF-EMF targeting the spleen has a bioeffect on the NF- $\kappa$ B pathway in the spleen to change T cell subset differentiation remains unclear. Our in vitro study demonstrated that ELF-EMF regulated the T cell profile, resulting in an increase in the CD4<sup>+</sup> T cell frequency and a decrease in CD8<sup>+</sup> T cell frequency. The percentage of Th 17 cells increased and the percentage of Treg cells decreased within CD4<sup>+</sup> T cells. Though not statistically significant, the frequencies of Th1 and Th2 cells displayed an inclination trend. The alteration of cytokines, including IL-4, IL-10, and IL-17, provided more proof about the differentiation of T cells. CD4<sup>+</sup> T cells are engaged in pathogen clearance, autoimmune disease, and tumor immunity response through various complicated subgroups. Th1 cells participate in the immune response to intracellular pathogens and are related to the pathogenesis of several autoimmune diseases. In cancer, high expression of Th1 cells generally represents a favorable prognosis (Barnes et al., 2017; Gerondakis et al., 2014). Th2 cells have a major role as mediators in many allergic inflammatory disorders and are implicated in the body's defense against helminth infection. (Walker et al., 2017). In addition to being protective against extracellular infections, Th17 cells are detrimental in the pathological process of various inflammatory and autoimmune diseases (Sandquist et al., 2018). Treg cells are essential

for peripheral immune tolerance and can perform harmful functions by blocking pathogen clearance and suppressing antitumor immunity (Josefowicz et al., 2012). Thus, the altered Th17/Treg aisle in the splenic CD4<sup>+</sup> T cells indicated the immune function of the spleen may be stimulated by the ELF-EMF. Meanwhile, it was discovered that ELF-EMF decreased the enrichment of the NF- $\kappa$ B pathway in the spleen and reduced the production of IL-1 $\beta$  and TNF- $\alpha$ , which were believed to be important inducers of the canonical pathway of NF- $\kappa$ B (Gerondakis et al., 2014; Hayden et al., 2011). It suggests that the effect of ELF-EMF on T-cell differentiation could be attributed to the NF- $\kappa$ B pathway. However, the specific mechanism still needs to be further investigated and discussed.

## 5 | CONCLUSIONS

In summary, it was demonstrated that ELF-EMF directly regulates the immune function of the spleen. The changed frequencies of immune cells in the spleen after local exposure to ELF-EMF were newly observed, including the increased proportions of CD4<sup>+</sup> T cells and NK cells, and the decreased percentage of B cells. In vitro, it was detected that ELF-EMF induced the differentiation of splenic T cells toward Th17 cells and inhibited the differentiation of splenic T cells into Treg cells. This effect was probably brought about by ELF-EMF reducing the enrichment of the NF- $\kappa$ B pathway. The effects of extremely low magnetic fields on the spleen were tentatively explored in this preliminary study. Despite the limited results, one may be certain that the extremely low-frequency electromagnetic fields are effective and feasible for “regulating the spleen.” It may be inferred from the results of this experiment and the relevant literature that the clinical application will be promising. However, there is still more to be done in that regard, and we are addressing it.

## AUTHOR CONTRIBUTIONS

**Sijia Chen:** Conceptualization; methodology; validation; formal analysis; investigation; resources; data curation; writing—original draft. **Wei Wei:** Conceptualization; methodology; resources; funding acquisition. **Ziang Wang:** Methodology; validation; formal analysis; investigation; writing—review and editing. **Jiazhen Zhu:** Formal analysis. **Hailong Zhang:** Formal analysis; investigation; data curation. **Guihu Wang:** Formal analysis; data curation. **Ni Guo:** formal analysis. **Jun Li:** resources; writing—review and editing, project administration. **Yanhua Mu:** Resources. **Naming Zhang:** Conceptualization; methodology; writing—review and editing; supervision; project administration. **Zongfang Li:** Conceptualization; writing—review and editing; supervision; project administration; funding acquisition.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

This study was approved by the Administration Committee of Experimental Animals, Shaanxi Province, China. All of the experimental procedures involving animals were conducted in accordance with the Institutional Animal Care guidelines of Xi'an Jiaotong University, China and approved by the Administration Committee of Experimental Animals, Shaanxi Province, China. There are no human subjects in this article and informed consent is not applicable.

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## REFERENCES

- Akahoshi, T., Hashizume, M., et al. Role of the spleen in liver fibrosis in rats may be mediated by transforming growth factor  $\beta$ -1. *J. Gastroenterol. Hepatol.* 17(1), 59–65 (2002). <https://doi.org/10.1046/j.1440-1746.2002.02667.x>
- Antonelli, L.R., Leoratti, F.M., et al. The CD14+CD16+ inflammatory monocyte subset displays increased mitochondrial activity and effector function during acute *Plasmodium vivax* malaria. *PLoS Pathog.* 10(9), e1004393 (2014). <https://doi.org/10.1371/journal.ppat.1004393>
- Barnes, T.A., Amir, E.HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer. *Br. J. Cancer* 117(4), 451–460 (2017). <https://doi.org/10.1038/bjc.2017.220>
- Bronte, V., Pittet, M.J.: The spleen in local and systemic regulation of immunity. *Immunity* 39(5), 806–818 (2013). <https://doi.org/10.1016/j.immuni.2013.10.010>
- Cansever, A.G., Seyhan, N., et al. Suppression of natural killer cell activity on *Candida stellatoidea* by a 50 Hz magnetic field. *Electromagn. Biol. Med.* 25(2), 79–85 (2006). <https://doi.org/10.1080/15368370600697453>
- Doyon, P.R., Johansson, O. Electromagnetic fields may act via calcineurin inhibition to suppress immunity, thereby increasing risk for opportunistic infection: Conceivable mechanisms of action. *Med. Hypotheses* 106, 71–87 (2017). <https://doi.org/10.1016/j.mehy.2017.06.028>
- Frahm, J., Mattsson, M.-O., et al. Exposure to ELF magnetic fields modulate redox related protein expression in mouse macrophages. *Toxicol. Lett.* 192(3), 330–336 (2010). <https://doi.org/10.1016/j.toxlet.2009.11.010>

- Ge, Y., Gao, G., et al. Changes of peripheral T-cell subsets in asplenic W256 tumor-bearing rats. *J. Surg. Oncol.* 42(1), 60–68 (1989). <https://doi.org/10.1002/jso.2930420113>
- Gerondakis, S., Fulford, T.S., et al. NF- $\kappa$ B control of T cell development. *Nat. Immunol.* 15(1), 15–25 (2014). <https://doi.org/10.1038/ni.2785>
- Gerondakis, S., Siebenlist, U. Roles of the NF-kappaB pathway in lymphocyte development and function. *Cold Spring Harb. Perspect. Biol.* 2(5), a000182 (2010)
- Golbach, L.A., Scheer, M.H., et al. Low-frequency electromagnetic field exposure enhances extracellular trap formation by human neutrophils through the NADPH pathway. *J. Innate Immun.* 7(5), 459–465 (2015). <https://doi.org/10.1159/000380764>
- Golub, R., Tan, J., et al. Origin and immunological functions of spleen stromal cells. *Trends Immunol.* 39(6), 503–514 (2018). <https://doi.org/10.1016/j.it.2018.02.007>
- Hayden, M.S., Ghosh, S. NF- $\kappa$ B in immunobiology. *Cell Res.* 21(2), 223–244 (2011). <https://doi.org/10.1038/cr.2011.13>
- He, X., Li, X., et al. Down-regulation of Treg cells and up-regulation of TH1/TH2 cytokine ratio were induced by polysaccharide from *Radix Glycyrrhizae* in H22 hepatocarcinoma bearing mice. *Molecules* 16(10), 8343–8352 (2011). <https://doi.org/10.3390/molecules16108343>
- House, R.V., McCormick, D.L. Modulation of natural killer cell function after exposure to 60 Hz magnetic fields: confirmation of the effect in mature B6C3F<sub>1</sub> mice. *Radiat. Res.* 153(5), 722–724 (2000). [https://doi.org/10.1667/0033-7587\(2000\)153%5B0722:MONKCF%5D2.0.CO;2](https://doi.org/10.1667/0033-7587(2000)153%5B0722:MONKCF%5D2.0.CO;2)
- Hovelmeyer, N., Schmidt-Supprian, M., et al. NF-kappaB in control of regulatory T cell development, identity, and function. *J. Mol. Med.* 100(7), 985–995 (2022).
- Josefowicz, S.Z., Lu, L.F., et al. Regulatory T cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* 30, 531–564 (2012). <https://doi.org/10.1146/annurev.immunol.25.022106.141623>
- Kevin, W., M.M., Rene de Waal, et al. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19, 683–765 (2001). <https://doi.org/10.1146/annurev.immunol.19.1.683>
- Jang, Y.W., Kim, S.J., et al. Extremely low-frequency electromagnetic field exposure enhances inflammatory response and inhibits effect of antioxidant in RAW 264.7 cells. *Bioelectromagnetics* 38(5), 374–385 (2017). <https://doi.org/10.1002/bem.22049>
- Williams, A., Lewis, S.M., et al. Structure and function of the immune system in the spleen. *Sci. Immunol.* 4(33), eaau6085 (2019). <https://doi.org/10.1126/sciimmunol.aau6085>
- Li, B., Zhang, S., et al. Dynamics of the spleen and its significance in a murine H22 orthotopic hepatoma model. *Exp. Biol. Med.* 241(8), 863–872 (2016). <https://doi.org/10.1177/1535370216638772>
- Jabarivasal, N., Mahaki, H., et al. Effects of various densities of 50 Hz electromagnetic field on serum IL-9, IL-10, and TNF- $\alpha$  Levels. *Int. J. Occup. Environ. Med.* 11(1), 24–32 (2020). <https://doi.org/10.15171/ijoem.2020.1572>
- Mahaki, H., Tanzadehpanah, H., et al. A review on the effects of extremely low frequency electromagnetic field (ELF-EMF) on cytokines of innate and adaptive immunity. *Electromagn. Biol. Med.* 38(1), 84–95 (2019). <https://doi.org/10.1080/15368378.2018.1545668>
- McGeachy, M.J., Cua, D.J., et al. The IL-17 family of cytokines in health and disease. *Immunity* 50(4), 892–906 (2019). <https://doi.org/10.1016/j.immuni.2019.03.021>
- Ohtani, S., Ushiyama, A. The effects of radio-frequency electromagnetic fields on T cell function during development. *J. Radiat. Res.* 56(3), 467–474 (2015). <https://doi.org/10.1093/jrr/rtr126>
- Piszczyk, P., Wojcik-Piotrowicz, K., et al. Immunity and electromagnetic fields. *Environ. Res.* 200, 111505 (2021). <https://doi.org/10.1016/j.envres.2021.111505>
- Ranasinghe, C., Trivedi, S., et al. IL-4 and IL-13 receptors: roles in immunity and powerful vaccine adjuvants. *Cytokine Growth Factor Rev.* 25(4), 437–442 (2014). <https://doi.org/10.1016/j.cytogfr.2014.07.010>
- Rosado, M.M., Simko, M., et al. Immune-modulating perspectives for low frequency electromagnetic fields in innate immunity. *Front. Public Health* 6, 201885 <https://doi.org/10.3389/fpubh.2018.00085>
- Ruterbusch, M., Pruner, K.B., et al. In vivo CD4(+) T cell differentiation and function: revisiting the Th1/Th2 paradigm. *Annu. Rev. Immunol.* 38, 705–725 (2020). <https://doi.org/10.1146/annurev-immunol-103019-085803>
- Salehi, I., Sani, K.G., et al. Exposure of rats to extremely low-frequency electromagnetic fields (ELF-EMF) alters cytokines production. *Electromagn. Biol. Med.* 32(1), 1–8 (2012). <https://doi.org/10.3109/15368378.2012.692343>
- Saliev, T., Mustapova, Z., et al. Therapeutic potential of electromagnetic fields for tissue engineering and wound healing. *Cell Prolif.* 47(6), 485–493 (2014). <https://doi.org/10.1111/cpr.12142>
- Sandquist, I., Kolls, J. Update on regulation and effector functions of Th17 cells. *F1000Research* 7, 205 (2018). <https://doi.org/10.12688/f1000research.13020.1>
- Saraiva, M., O'Garra, A. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10(3), 170–181 (2010). <https://doi.org/10.1038/nri2711>
- Schwandt, T., Schumak, B., et al. Expression of type I interferon by splenic macrophages suppresses adaptive immunity during sepsis. *EMBO J.* 31(1), 201–213 (2012). <https://doi.org/10.1038/emboj.2011.380>
- Swirski, F.K., Nahrendorf, M., et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science* 325(5940), 612–616 (2009). <https://doi.org/10.1126/science.1175202>
- Voisin, A., Grinberg-Bleyer, Y. The many-sided contributions of NF-kappaB to T-cell biology in health and disease. *Int. Rev. Cell. Mol. Biol.* 361, 245–300 (2021)
- Walker, J.A., McKenzie, A.N.J. TH2 cell development and function. *Nat. Rev. Immunol.* 18(2), 121–133 (2017). <https://doi.org/10.1038/nri.2017.118>
- Wyszowska, J., Jędrzejewski, T., et al. Evaluation of the influence of in vivo exposure to extremely low-frequency magnetic fields on the plasma levels of pro-inflammatory cytokines in rats. *Int. J. Radiat. Biol.* 94(10), 909–917 (2018). <https://doi.org/10.1080/09553002.2018.1503428>
- Zachs, D.P., Offutt, S.J., et al. Noninvasive ultrasound stimulation of the spleen to treat inflammatory arthritis. *Nat. Commun.* 10(1), 951, 2019. <https://doi.org/10.1038/s41467-019-08721-0>
- Zhang, D., Zhang, Y., et al. Resveratrol may reverse the effects of long-term occupational exposure to electromagnetic fields on workers of a power plant. *Oncotarget* 8(29), 47497–47506 (2017). <https://doi.org/10.18632/oncotarget.17668>
- Zhou, R., Zhang, J., et al. A new role for the spleen. *Am. J. Pathol.* 189(11), 2233–2245 (2019). <https://doi.org/10.1016/j.ajpath.2019.07.008>

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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