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Effects of Magnetic Field 0.1 and 0.05 mT on Leukocyte Adherence Inhibition

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T lymphocytes taken from healthy humans and cancer patients before and after medical treatment were exposed to the magnetic field 0.1 and 0.05 mT to study response of the cell-mediated immunity. Leukocyte adherence, which is considered to correlate with the cell-mediated immunity, was measured using an in vitro technique—leukocyte adherence inhibition (LAI) assay. Exposure to the magnetic field increases adherence of T lymphocytes especially those from cancer patients before medical treatment. The effects of exposure to the magnetic field 0.1 and 0.05 mT are similar to those of greater magnetic fields in the range of 0.5 to 10 mT. The effects of the AC and DC magnetic fields 0.05 mT do not display large differences attributable to the magnetic field.

Keywords Biological effects of magnetic field; Cell-mediated immunity; Effect of cancer on cell-mediated immunity.

Introduction

The main task of the cell-mediated immunity is reaction to foreign antigens on the surface of other cells. *T* cells, having thousands of receptor protein complexes in and at the plasma membrane, can respond to millions of antigens—ligands to receptors [1]. As a result, *T* cells secrete a variety of mediators called interleukins,

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lymphokines, or cytokines. The essential point of the majority of processes of immunity response is mediation of interactions between various surface receptors and their ligands [2]. Information on receptor-ligand interaction is transmitted inside the receptor cell and elicits a corresponding reaction. The main principles of signal transfer from receptor molecules of immune cells are the same as those of other cells, but the dominant signalling is performed by protein kinases or by *G* proteins associated with receptors, as well as by direct transfer of the hormone across the membrane and its intracellular activity, which is possible, for example, after activation of ion channels that are in conjunction with receptors [3]. Many receptors have a domain in their cytoplasmic part displaying enzyme protein-kinase activity [4]. Some receptors (such as Fc receptors) are noncovalently associated with cytoplasmic protein kinases. Intracelullar proteins altered by phosphorylation have direct or indirect effects on gene transcription, cytoskeleton formation, activity of ribosomes, and energy metabolism of the cell. Some receptors are associated with trimer *G* proteins and have a characteristic structure—their polypeptide chain crosses plasma membrane 7 times (Fig. 1). After joining a ligand to a receptor the guanosine diphosphate (GDP) molecule bound to associated *G* protein is exchanged for guanosine triphosphate (GTP) molecule and in this way activated *G* protein is detached from the receptor. The *G* protein is dissociated to subunit α (carrying GTP) and to solid dimer $\beta\gamma$. Both the parts are transferred to different cytoplasmic proteins and alter their activity [5]. They may change, for example, the transport efficiency of ion channels, gene expression, metabolism, or secretion. These processes are accumulated in cascades using large amount of Ca^{2+} ions.

We also should mention why especially phosphorylation, dephosphorylation, binding of Ca^{2+} ions, and G-protein/GTP association cause strong changes of enzyme activity (e.g., of lactic dehydrogenase [LDH] isozymes) and interactions between different proteins [6]. We assume that, in the reactions mentioned above, considerably charged groups (such as negative phosphate, positive Ca^{2+} ions, etc.) are attached to or detached from effector proteins. These processes change conformation of proteins, enzyme activity, and capability to form complexes with other proteins or lipids [7].

Calcium ions play important role in binding of *T* lymphocytes to other cells and very likely to solid state surfaces, too. Adherence to solid-state surfaces is a process that lasts about one hour. In a serum free medium, after gradual formation

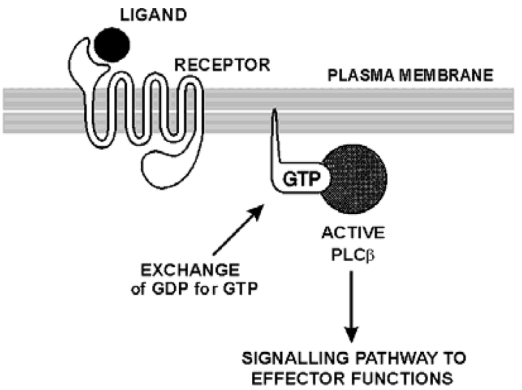


Figure 1. Schematic picture of a plasma membrane with a receptor, ligand, and signalling pathway from the membrane into the cell (PLCβ = phospholipase C-β).

of attachment microextensions, the cell spreads above the surface [8]. The role of negative surface charge at the solid surface in adherence [9] suggests a role of electromagnetic forces in adherence. Calcium ions in the cell may be bound to multipurpose intracellular receptor Calmodulin, mediating many Ca^{2+} regulated processes including protein phosphorylation. Cell adhesion molecules (cadherins, selectins, integrins) are dependent on the presence of Ca^{2+}. Integrins mediating the cell-cell as well as the cell-matrix adhesion are important for leukocyte adherence properties (e.g., leukocyte function associated integrin LFA-1 and macrophage integrin Mac-1). Concentration of Ca^{2+} ions inside a cell is several orders of magnitude smaller than in the extracellular medium ($<10^{-7}$ M in the cytosol). Flux through ionic channels can change Ca^{2+} cytoplasmic concentration. Ionic channels in plasma membranes may be open continuously, gated by the electric field, gated by a chemical activator (e.g., by receptors), gated by the electric field together with a chemical activator, or gated mechanically. We assume that for the function of *T* lymphocytes the channels gated by the electric field and by the electric field together with a chemical activator are the most important [10]. Increase of Ca^{2+}concentration in cytoplasm is caused by inositol, 4,5-triphosphate (IP_3) and diacylglycerol (DG). Increase of Ca^{2+} concentration stimulate protein kinase and phosphoprotein phosphatase activated by calmodulin and opens ionic channels. Opening of the channel gated by the electric field together with a chemical activator is caused by direct interaction with the *G* protein. The effect was proved by examination of 2 types of Ca^{2+} channels.

Signalling triggered by the receptor-antigen complex results in altered adherence properties of leukocytes (prepared from peripheral blood). The cell-mediated immunity is understood to correlate with adherence properties [11]. An in vitro quantitative technique used to monitor the cell-mediated immunity is the leukocyte adherence inhibition (LAI) assay based on observation of adherence of leukocytes to solid substrates in the presence or absence of antigens—ligands to receptors; (For an overview, see Pokorný et al. [12], Jandová et al. [13], Jandová et al. [14]). Adherence properties of leukocytes are sensitive to biological, chemical, and physical agents [15]. Adherence changes are caused by malignant process as well as by its medical treatment, by antigens of different kinds, and by magnetic field in a wide range of inductions. As the cellular chain transferring the initial signal from a receptor to final immune response is not yet adequately understood, it is difficult to determine the links that are the targets of particular agents [16, 17]. As the magnetic field is one of the physical agents that are capable of changing the leukocyte behavior [18], various mechanisms of magnetic field effects are studied, for example, changes of kinetics of the ion flux across the membrane and cyclotron resonance [19–21] or changes of quantum states of ions within the protein cavities and the field-dependent part of their dissociation probability [22, 23] but up to now the experimental findings have not elucidated their role in leukocyte adherence. For instance, the measured data display large changes of adherence of *T* lymphocytes taken from cancer patients before medical treatment caused by the magnetic field 0.5–10 mT [13, 14] but have not contributed to explanation of the mechanism. The changes of adherence caused by the magnetic field in this range of inductions are similar and no significant dependence on induction was observed. We asked a question whether the immune response to magnetic fields smaller than 0.5 mT and comparison of the effects of the AC and DC magnetic fields may help with understanding of the mechanisms of their action. We present measurements results of cell-mediated

immunity after exposure of T lymphocytes to 0.1 mT (AC) and to 0.05 mT (AC and DC) magnetic fields.

Materials and Methods

Specific antigen is an immunoactive fraction prepared from malignant tumor of the same type as that of the patient the blood was taken from. Organs with cancer were taken during surgery. Nonspecific antigen is an immunologically functional fraction prepared from the serum of inbred laboratory mouse strain C3H infected with the virus (LDV) enhancing the lactate dehydrogenase isozyme. Antigen was mixed with the suspension just before exposure to the magnetic field at a ratio of one part of antigen to 4 parts of T cell float. A comprehensive description of the preparation methods of the antigens is in Jandová et al. [13].

Suspension of T cells in the test tubes from green Sial glass was positioned in the center of a coil generating magnetic field. Exposure time was 60 min as we wanted to compare the results after exposure to the magnetic field with the standard LAI assay results. The test tubes of the sham experiments without exposure to the magnetic field was positioned in the same coil but with no magnetic field just after the magnetic field exposed experiment ended (i.e., after 60 min). The measurement method is described in detail in Jandová et al. [13].

The coil producing magnetic field is 30 cm in diameter and 33 cm in length. The axis of the coil is oriented vertically. Magnetic induction was measured by the magnetometer (Gauss/Teslameter) model 7030, Sypris (F. W. Bell, Orlando, FL, USA) with Hall probes (measured by Department of Magnetism, Institute of Physics, ASCR, Prague).

The number of nonadherent T cells in suspension was counted after exposure and with no exposure to the magnetic field. The results of the LAI assay are expressed as a relative number M (in percent) of nonadherent cells, as an index of positivity IP (a normalized value of $M - \mathrm{IP} = M/33.3$), and as a nonadherence index $\mathrm{NAI} = 100 \times (M_S - M_N)/M_N$ representing difference in adherence with the specific and with the nonspecific antigen (M_S and M_N are corresponding numbers of nonadherent cells, respectively).

Statistical significance was evaluated using ANOVA method (analysis of variance), 2 groups t-test (TGt), and matched t test (Mt).

Experimental Results

We investigated effects of exposure to the AC magnetic field 50 Hz/0.1 and 0.05 mT and to the DC magnetic field 0.05 mT on human T lymphocytes. Figs. 2 and 3 show the mean values of the relative number M of nonadherent cells and of the index of positivity IP (a), and of the nonadherence index NAI (b) for the AC magnetic fields 0.1 and 0.05 mT, respectively. T lymphocytes were taken from healthy humans (H1 and H2) and from cancer patients: from the same patients before (Ca before = CaB) and after (Ca after = CaA) medical treatment (about 3–4 months after treatment). The groups of healthy humans (H1 and H2) were measured together with the groups of patients before and after medical treatment, respectively. The differences between the mean values of M (and of NAI) for H1 versus CaB, H2 versus CaA, and CaB versus CaA with no exposure and with exposure to the magnetic field 0.1 mT are statistically significant except for H2 versus CaA for 0.1 mT (after medical treatment behavior of T lymphocytes from a cancer patient is similar to that of a healthy

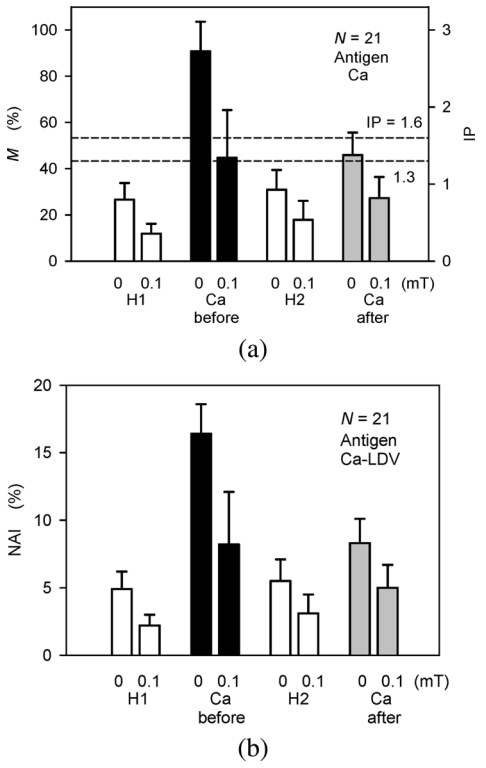


Figure 2. Effect of exposure of *T* lymphocytes to the AC magnetic field 0.1 mT. Number *M* of nonadherent cells and index of positivity IP (a), and nonadherence index NAI (b) for *T* lymphocytes from healthy humans (H1, H2) and cancer patients before (CaB) and after (CaA) medical treatment.

human; Table 1). After exposure to 0.05 mT field there are no statistical significant differences between H2 versus CaA, CaB versus CaA—magnetic field effect on adherence of *T* lymphocyte before medical treatment is similar to the effect of medical treatment—Table 2.

Effects of DC magnetic field 0.05 mT were measured using the same coil where AC experiments were carried out. Fig. 4 shows *M* and IP (a), and NAI (b) measured on *T* lymphocytes taken from healthy humans and cancer patients before and after medical treatment. Generally, the results are similar to those for exposure to the AC magnetic field 0.05 mT. The values of statistical significance in Table 3 correspond to those in Table 2 for AC magnetic field 0.05 mT (except for H2 versus CaA for Ca and Ca-LDV antigens and 0.05 mT). Comparison of the results for exposure to the AC and to the DC magnetic field given in Fig. 5 reveals statistically significant differences for *T* lymphocytes taken from cancer patients after medical treatment too (Table 4). But, as follows from comparison of *M* (and NAI) values for *T* lymphocytes from cancer patients after medical treatment with no exposure to the magnetic field (Figs. 3 and 4), the medical treatment is less effective in the group exposed to the DC magnetic field than in the group exposed to the AC magnetic field. It seems that the effect of the DC magnetic field on adherence is not smaller than the effect of AC magnetic field.

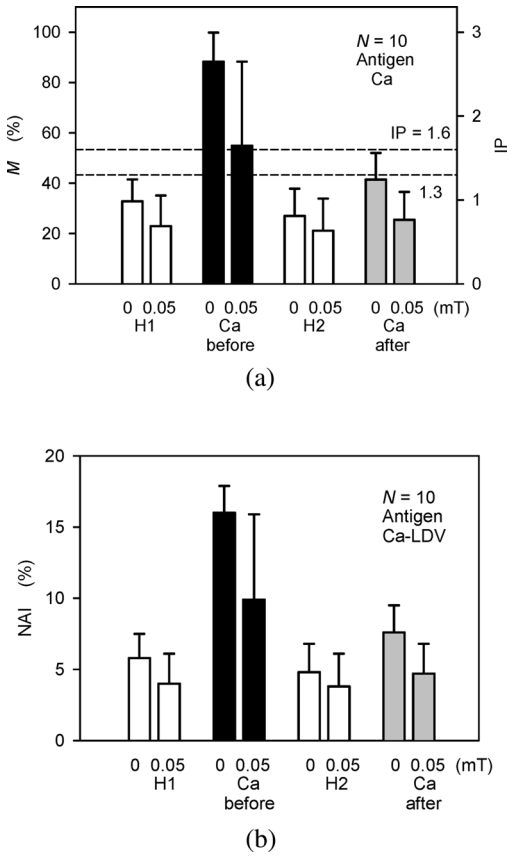


Figure 3. Effect of exposure of *T* lymphocytes to the AC magnetic field 0.05 mT. *M* and IP (a), and NAI (b) values.

Discussion and Conclusions

Cell-mediated immune response to malignant process and to exposure to the magnetic field was studied. The physical mechanisms of the effects of the magnetic field may be based on change of the space orientation of the magnetosome grains and of the free radicals, on the Larmor precession of moving charges creating

Table 1
Statistical significance of the results of exposure to AC magnetic field 0.1 mT (NS = no significance)

Antigen	Mag. field	H1 × CaB × H2 × CaA ANOVA	H1 × CaB TG*t*	H2 × CaA TG*t*	CaB × CaA TG*t*
Ca	No	0.0001	0.001	0.001	0.001
	0.1 mT	0.0001	0.001	0.1-NS	0.01
Ca-LDV	No	0.0001	0.001	0.001	0.001
	0.1 mT	0.0001	0.001	0.05	0.001

Table 2
Statistical significance of the results of exposure to AC magnetic field 0.05 mT (NS = no significance)

Antigen	Mag. field	H1 × CaB × H2 × CaA ANOVA	H1 × CaB TG*t*	H2 × CaA TG*t*	CaB × CaA TG*t*
Ca	No	0.01	0.01	0.05	0.001
	0.05 mT	NS	0.05	NS	NS
Ca-LDV	No	0.01	0.01	0.05	0.001
	0.05 mT	NS	0.05	NS	NS

magnetic moment, on the cyclotron resonance of ions, on the changes of charge transfer etc. The final biological effect may be connected, for exmaple, with altered transport of Ca^{2+} ions through channels across the membrane or with dissociation of protein-ion complexes. As the whole signalling process from the receptor at the membrane inside the cell and back to control adherence is still not adequately

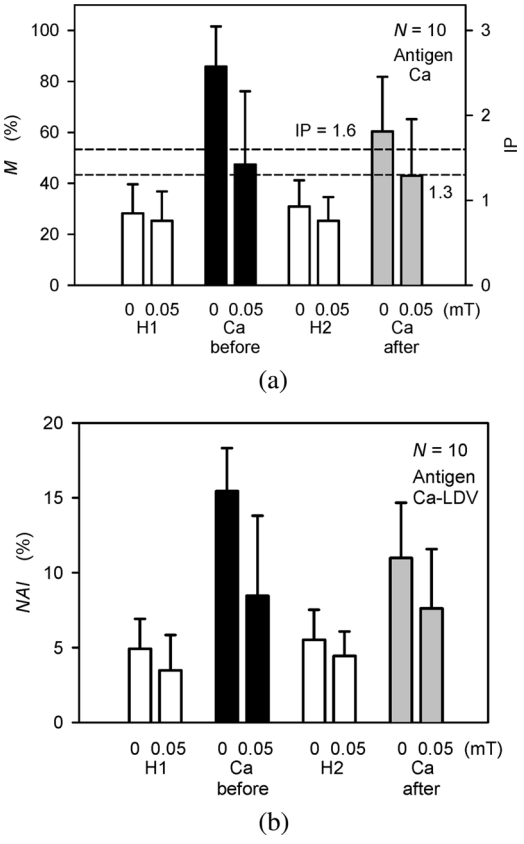
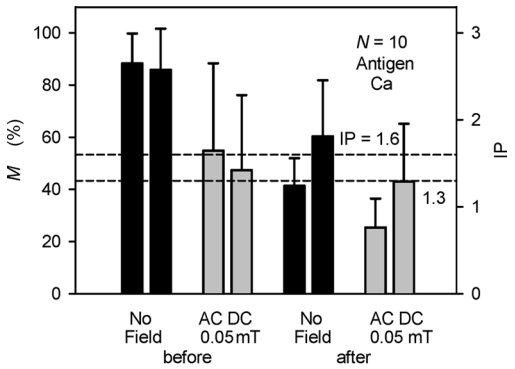


Figure 4. Effect of exposure of *T* lymphocytes to the DC magnetic field 0.05 mT. *M* and IP (a), and NAI (b) values.

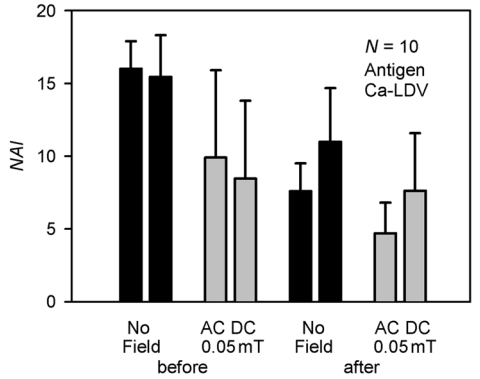
Table 3
Statistical significance of the results of exposure to DC magnetic field 0.05 mT (NS = no significance)

Antigen	Mag. field	H1 × CaB TG*t*	H2 × CaA TG*t*	CaB × CaA M*t*
Ca	No	0.000	0.001	0.006
	0.05 mT	0.044	0.032	NS
Ca-LDV	No	0.000	0.001	0.005
	0.05 mT	0.019	0.030	NS

Antigen	Group	0 × 0.05 mT M*t*
Ca	CaB	0.005
	CaA	0.012
Ca-LDV	CaB	0.005
	CaA	0.006



(a)



(b)

Figure 5. Comparison of the effect of exposure of *T* lymphocytes to the AC and to the DC magnetic field (*T* lymphocytes from cancer patients). *M* and IP (a), and NAI (b) values.

Table 4
Statistical signicance of the effects of exposure to the AC magnetic field versus exposure to the DC magnetic field (0.05 mT) on *T* lymphocyte taken from Ca patients (NS = no significance)

Antigen	Mag. field	Before med. treat. TG*t*	After med. treat. TG*t*
Ca	No	NS	0.022
	0.05 mT	NS	0.038
Ca-LDV	No	NS	0.020
	0.05 mT	NS	0.056 – NS

understood the links in the signalling pathway and the mechanism that changes adherence properties (and the immune function) is not determined yet. Experimental research should accumulate results to elucidate the mechanism of immunity changes connected with malignant process and with exposure to the magnetic field.

In this contribution we present experimental study of the effect of magnetic field with induction smaller than 0.5 mT. Exposure of *T* lymphocytes to the magnetic field 0.1 and 0.05 mT elicits effects that do not differ from those for the field 0.5–10 mT. The AC and DC fields 0.05 mT have similar effects on adherence of *T* lymphocytes taken from healthy humans and from cancer patients. The greatest effect has the magnetic field on *T* lymphocytes taken from cancer patients before medical treatment. The measured adherence changes support suggestions that a weak magnetic field comparable with the earth magnetic field can change the immune function in humans.

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