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Short communication

Effects of sinusoidal magnetic field on adherence inhibition of leucocytes: preliminary results

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Abstract

The leucocyte surface properties manifest the cell-mediated immunity. The response of the cell-mediated immunity to external magnetic field was examined by observing leucocyte adherence to solid state surfaces. In the presence of antigen, leucocytes taken from cancer patients exhibit decreased adherence in contrast with adherence of leucocytes from healthy humans. After 1 h exposure to a sinusoidal magnetic field of 50 Hz and of 1 mT or 10 mT, adherence of leucocytes taken from cancer patients is strongly increased. The 1 mT magnetic field has stronger effect than the 10 mT field. © 1999 Elsevier Science S.A. All rights reserved.

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1. Introduction

Adherence of leucocytes (peripheral white blood cells) to solid state surfaces (e.g., of glass or some plastic materials) is used to monitor the cell-mediated immunity. Leucocytes taken from immunized humans have diminished adherence if corresponding antigen is present in the suspension. The adherence properties may be examined by the LAI (Leucocyte Adherence Inhibition) test [1,2]. Description of the adherence process may be found, e.g., in Refs. [3,4]. Experimental findings showed a gap between the adherent cell and the substrate [5] and Curtis explained adherence on the basis of electromagnetic interactions (London dispersion forces) [6]. We assume that adherence of cells includes two different mechanisms: the long-range and short-range mechanisms. The long-range mechanism depends on the electromagnetic field around the cell and we suppose that the field is generated by the Fröhlich coherent vibrations [7–10]. The short-range mechanism is a domain of chemical binding. Theory of the long-range mechanism of interaction is supported by observation of interaction forces between leucocytes (CD4Ly) and formation of clusters [11]. Assumptions that the cancer process can alter the leucocyte-mediated immunity led to attempts to use the LAI test for detection of malignancies in humans [12]. The LAI test, with increased sensitivity and specificity, enables to disclose reliably adherence inhibition of leucocytes taken from cancer patients [7–9].

To alter the adherence properties, the antigen has to bind to the corresponding receptor in the cell membrane. After binding, a sequence of successive signaling processes is triggered resulting in changed adherence of the leucocyte cell. We will examine the effects of sinusoidal magnetic field on cell-mediated immunity of leucocytes by investigating their adherence. The experimental findings may help us to understand the signaling mechanism in leucocytes as well as the nature of adherence.

2. Materials and methods

CD4Ly cells were separated from venous blood of healthy humans and cancer patients (orthinolaryngological tumors—CA pharynx). The blood was heparinized, incu-

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bated at 37°C for 30 min, and then the plasma with leucocytes was placed in saline solution. After centrifugation, the supernatant was filtered through nylon absorbent wool resulting in separation of the CD4Ly cells (helper T cells). CD4Ly cells are the main components of the filtered pool.

The LDH virus antigen was used. This antigen was prepared from the blood of mice infected with the LDH virus. Antigen was added to suspension just before exposure to the magnetic field.

The suspension containing the CD4Ly cells was given in the test tube and PVC dishes ($\Phi = 4.5$ cm) and positioned in the exposure chamber in the gap of a magnet with the magnetic induction 0, 1, and 10 mT for 60 min. The frequency of the sinusoidal magnetic field is 50 Hz. The same exposure conditions were used for CD4Ly cells from healthy humans and cancer patients.

Leucocyte-mediated immunity was examined by the LAI test to assess the effects of the magnetic field. Index of positivity (IP) was evaluated from the number of nonadhering leucocytes. The index of positivity is given by the relation $IP = M/33$ where M is the relative number of nonadhering leucocytes (in percentage). The IP value is negative, positive, or suspicious if $IP < 1.3$, $IP > 1.6$, or $1.3 \leq IP \leq 1.6$, respectively.

Evaluation of statistical significance was carried out using the Friedman test, the *t*-test for two independent ensembles, and the *t*-test for two dependent ensembles.

3. Experimental results

IPs for CD4Ly cells taken from healthy humans are negative (below the limit IP = 1.3 indicated by the dotted lines) as follows from Figs. 1–3 which show the test tube and dish experiments. The leucocytes show negative response to the action of the antigen without magnetic field. The magnetic field causes a small increase of the IP values. The changes of the IP values caused by the magnetic field have predominantly the statistical significance of 5% (IP_0 against IP_B where the indexes 0 and B denote $B = 0$ and $B \neq 0$, respectively).

The CD4Ly cells taken from cancer patients show highly positive value of IP (2.81) for $B = 0$, negative value for $B = 1$ mT, and positive value for $B = 10$ mT (Fig. 1—the test tube experiments). A similar behaviour was found in the dish experiments (Fig. 2); only the increase of adherence at $B = 10$ mT is greater than in the previous case. The magnetic field diminishes the effect of antigen and increases the adherence of CD4Ly from cancer patients. The statistical significances of the changes of IP values caused by the magnetic field are 0.01% and 1% for IP_0 against IP_B and IP_{B1} against IP_{B10}, respectively. The index $B1$ ($B10$) denotes $B = 1$ mT ($B = 10$ mT).

Comparison of the IP values of CD4Ly from cancer patients and healthy persons is shown in Figs. 2 and 3.

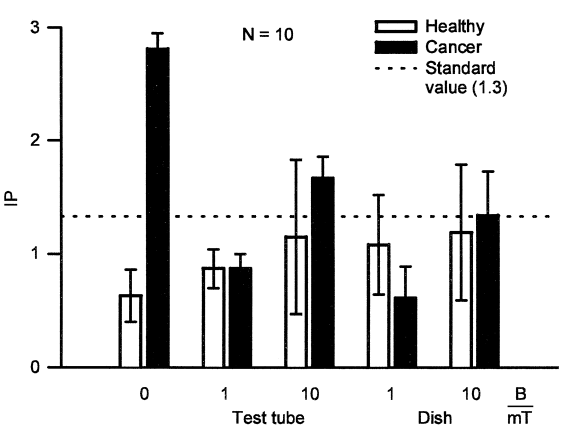


Fig. 1. Index of positivity (IP) of CD4Ly cells from healthy humans and cancer patients for control groups and groups exposed to magnetic field. The test tube and dish experiments.

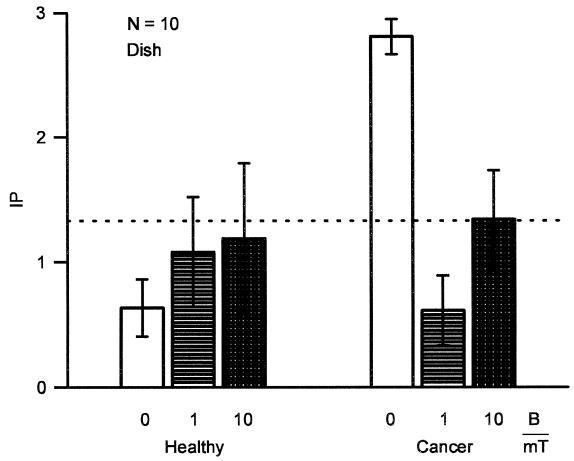


Fig. 2. Index of positivity (IP) of CD4Ly cells from healthy humans and cancer patients for control groups and groups exposed to magnetic field. The PVC dish experiments.

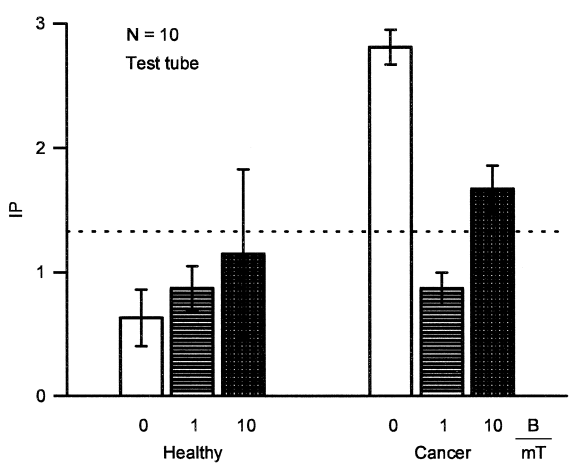


Fig. 3. Comparison of the IP values of CD4Ly cells taken from healthy humans and cancer patients.

First of all, we have to emphasize the big difference between the adherence of leucocytes from healthy humans and cancer patients with no magnetic field ($B = 0$). The statistical significance of the results is on the level of 0.01%. The big difference in adhesion properties at $B = 0$ decreases under the influence of the magnetic field. Exposure of the CD4Ly to the magnetic field diminishes the difference of IP values in such a way that the statistical significance is on the level of 5% or worse.

4. Discussion

CD4Ly cells are helper T cells whose activation is a complicated process that is still incompletely understood [13]. An antigen-presenting cell must provide at least two signals to activate a helper T cell. The basic signal is provided by a foreign peptide bound to a class II MHC (major histocompatibility complex) molecule on the surface of antigen-presenting cell which activate the T cell CD4 receptor complex. The additional signal is provided either by secreted molecules, such as interleukin-1, or by binding of co-receptor CD28 to the B7 molecule on the surface of presenting cell. The activation results in proliferation and secretion of interleukins. If the CD4Ly cell receives only the basic signal without the additional signal, it is altered and cannot be activated later. The present incomplete knowledge of activation of T cells does not enable us to analyse the point of interaction with the external magnetic field and to determine the link or links which are affected by the field.

5. Conclusions

Magnetic field exposure strongly increases adherence of CD4Ly taken from cancer patients, but has only small effects on adherence of CD4Ly from healthy humans. The IP values of CD4Ly from cancer patients depend on the induction of the magnetic field. IP for $B = 1$ mT is smaller than IP for $B = 10$ mT. We measured only at two values of magnetic induction. We cannot, therefore, assess the dependence of adherence on the induction.

We did not analyze the mechanism of the action of the magnetic field on the adherence of leucocytes yet. We do not know the target point of the interaction.

Acknowledgements

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References

[1] W.J. Halliday, S. Miller, Leucocyte adherence inhibition: a simple test for cell-mediated immunity and serum block factors, Int. J. Cancer 9 (1972) 477–483.
[2] V. Holáň, M. Hašek, J. Bubeník, J. Chutná, Antigen mediated macrophage adherence inhibition, Cell Immunol. 13 (1974) 107–116.
[3] F. Grinnell, in: G.H. Bourne, J.F. Danielli (Eds.), Cellular Adhesiveness and Extracellular Substrata, Int. Rev. Cytol., Vol. 53, Academic Press, New York, 1978, pp. 65–144.
[4] J.A. Witkowski, W.D. Brighton, Influence of serum on attachment of tissue cells to glass surfaces, Exp. Cell Res. 70 (1972) 41–48.
[5] A.S.G. Curtis, The mechanism of adhesion of cells to glass, J. Cell Biol. 20 (1964) 199–215.
[6] A.S.G. Curtis, in: T.A.V. Butler, D. Noble (Eds.), Cell Adhesion, Prog. Biophys. Mol. Biol., Vol. 27, Pergamon, Oxford, 1973, pp. 317–386.
[7] J. Pokorný, J. Fiala, A. Jandová, J. Kobilková, Fröhlich electromagnetic field and adherence of leucocytes, 2nd Int. Sci. Meet. on Microwaves in Medicine, Rome, 11–14 October, 1993, Conf. Proc., Congress Centre, University of Rome La Sapienza, 1993, pp. 39–42.
[8] A. Jandová, J. Kobilková, J. Pokorný, Leucocyte adherence in ovarial cancer patients, Neural Network World 3 (1994) 367–374.
[9] J. Pokorný, A. Jandová, J. Šorfová, J. Kobilková, S. Trojan, M. Costato, M. Milani, Leucocytes of cancer patients give evidence of fundamental physical forces: a pathway for a new view, Laser and Technology 6 (1996) 15–23.
[10] J. Pokorný, T.-M. Wu, Biophysical Aspects od Coherence and Biological Order, Academia, Prague, 1998.
[11] A. Jandová, J. Kobilková, M. Nedbalová, J. Šorfová, A. Dohnalová, S. Trojan, J. Pokorný, Study of interaction forces in human CD4Ly, Bioelectrochem. Bioenerg. 41 (1996) 181–185.
[12] D.R. Burger, in: R.B. Herberman, K.R. McIntire (Eds.), Leucocyte Adherence Inhibition, Immunodiagnosis of Cancer, Dekker, New York, 1979, pp. 1124–1145.
[13] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J.D. Watson, Molecular Biology of the Cell, Garland Publ., New York, 1994.