MITOCHONDRIAL DYSFUNCTION

A. Jandová[*], *J. Pokorný*[†], *J. Kobilková*[‡], *S. Trojan*[§], *M. Nedbalová*[§], *A. Dohnalová*[§], *A. Čoček*[¶], *J. Mašata*[‡], *R. Holaj*[‖], *E. Tvrzická*[**], *P. Zvolský*[††], *M. Dvořáková*[††], *M. Cifra*[*]

Abstract: Cell-mediated immunity (CMI) response of healthy humans and cancer (Ca) patients to specific tumor antigen and nonspecific (LDV – lactate dehydrogenase virus) antigen, and of acute myocardial infarction (AMI) and schizophrenia (Sch) patients to nonspecific antigen was investigated. Large differences of CMI response of healthy humans in comparison with Ca, AMI, Sch patients were found. CMI response to antigens displays transferred information about cells under immune surveillance. LDV disturbs the oxidative energy production system. We assume that CMI response to LDV antigen monitors pathological states of mitochondrial energy production which results in disturbances of electromagnetic activity of living cells.

Key words: *Mitochondria, cell-mediated immunity, cancer, myocardial infarction, schizophrenia, LAI assay*

Received: February 24, 2009
Revised and accepted: June 3, 2009

[*]A. Jandová, M. Cifra
Institute of Photonics and Electronics v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic

[†]J. Pokorný – Corresponding author
Institute of Photonics and Electronics ASCR, v.v.i., Chaberská 57, 182 51 Praha 8, Czech Republic. Phone: (+420)266773432, Fax: (+420)284680222, E-mail: pokorny@ufe.cz

[‡]J. Kobilková, J. Mašata
Department of Gynaecology and Obstetrics of the First Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Czech Republic

[§]S. Trojan, M. Nedbalová, A. Dohnalová
Institute of Physiology of the First Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Czech Republic

[¶]A. Čoček
Department of Otorhinolaryngology of the Third Faculty of Medicine, Charles University in Prague, Czech Republic

[‖]R. Holaj
3rd Medical Department of the First Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Czech Republic

[**]E. Tvrzická
4th Medical Department of the First Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Czech Republic

[††]P. Zvolský, M. Dvořáková
Department of Psychiatry of the First Faculty of Medicine and General Teaching Hospital, Charles University in Prague, Czech Republic

©ICS AS CR 2009

1. Introduction

Cell-mediated immunity (CMI) is understood to correlate with adherence properties of leukocytes (from peripheral blood). LAI (leukocyte adherence inhibition) assay is a quantitative technique that was widely used to study and monitor the CMI [1-5]. The LAI assay is based on observation of *in vitro* adherence of leukocytes to surfaces of glass or some plastic materials in the presence and in the absence of antigens. CMI response to antigens is related to specific processes in cells which are under immune surveillance and, therefore, displays transferred information about their state and evolution. But the LAI assay method is complex and tedious, and unequivocal results are conditioned by high purity of antigens used (i.e. the most immunoactive components have to be separated), and by experienced visual microscopic evaluation. Antigen alters adherence after it binds to corresponding receptor in the cellular membrane. Not only chemical but also physical agents can affect CMI and change adherence of T lymphocytes.

Numerous experimental LAI assays were made for a quantitative examination of adherence of T lymphocytes to assess tumor-specific CMI [6-14]. We studied CMI in healthy humans and cancer (Ca) patients with gynecological and otorhinolaryngological (ORL) tumors using specific antigens prepared from corresponding cancer tissues and nonspecific antigen prepared from plasma of mice infected with lactate dehydrogenase-elevating virus (LDV) by high-pressure gel chromatography. As the purified antigens and other measures have made possible high reliability and reproducibility of the LAI assay results in our laboratory, we performed investigations for more than three decades and accumulated considerable amount of data on CMI in healthy humans and cancer patients [15-20].

Association of the CMI response to the nonspecific antigen with pathology of the cellular energy production was an original contribution published in the article "Disease" of the Cellular Energy System [15]. Therefore, our experimental investigations were underlain by a hypothesis that CMI response to the nonspecific antigen discloses defects of oxidative mitochondrial function which is a common ,,denominator,, of a group of diseases of the cellular energy production system. CMI response to nonspecific antigen was examined in acute myocardial infarction (AMI) and schizophrenia (Sch) patients too. The CMI data are presented in this paper with discussion of processes which are assumed to be monitored by the response to the nonspecific antigen.

2. Materials and Methods

2.1 Preparation of cells

T cells were separated from venous blood of healthy humans and cancer patients (with carcinoma of cervix, endometrium, mamma, ovary, melanoma, and with some other tumors). Histopathological verification of the cancer diagnosis was done. The method of preparation of cells is comprehensively described in [18, 21]. Using filtration with nylon absorbent wool, the filtrated pool contained about 90 % of T cells and the remaining part other cells [22]. T cells of AMI and Sch patients were prepared equally.

2.2 The specific and the nonspecific antigen

Specific antigen was an immunoactive fraction prepared from a malignant tumor of the same type as that of the patient the blood was taken from. Organs were taken during surgery. Nonspecific antigen was an immunologically functional fraction prepared from the serum of inbred laboratory mice strain C3H infected with the LDV. High-pressure gel chromatography was used to separate the active antigen fractions and the columns were filled by Separon Gema Glc developed by O. Wichterle [23-24]. Description of the preparation methods of the antigens is given in [18] and some preparation details together with the gel chromatograms of the specific and nonspecific antigen fractions and their immunological effectivity measured by LAI assay are in [21]. The active part of the antigen was proved to be RNA [25-26].

3. Measurement and Evaluation

Experiments were performed double blind; origin of blood and diagnoses were imparted after LAI assay evaluation. Diagnoses of Ca patients were determined at the Department of Gynaecology and Obstetrics of the First Faculty of Medicine and at the Department of Otorhinolaryngology of the Third Faculty of Medicine, Charles University in Prague. Diagnoses of AMI and Sch were determined at the 3^{rd} Dept. of Medicine and at the Dept. of Psychiatry of the First Faculty of Medicine, respectively, where the patients were treated. In individual experiments the groups of patients and of healthy controls were matched.

To elicit the immune response the T cells prepared according to the method described in [18] were mixed with the antigen (specific or nonspecific) at a ratio of 1 part of the antigen to 4 parts of the T cell float. Antigen was mixed with the suspension just before 60 mins sedimentation process started. T lymphocytes in the suspension sedimented in the test tubes from green Sial glass. The same exposure conditions were used for T cells from healthy humans and from Ca, AMI, and Sch patients. Temperature during experiment was 22-28°C.

The results are expressed as a relative number $M = 100\ m/m_0$ (in %) of nonadherent cell, where m_0 and m is the number of cells in suspension before and after sedimentation-adhesion process, respectively. To simplify assessment of the LAI assays with respect to cancer patients data, the M values were normalized by the relation $IP = M/A$ where A=100/3 and some significant discrete levels were specified (IP – index of positivity). We use $IP = 1.3$ and $IP = 1.6$ to distinguish IP values of healthy humans and of Ca patients, respectively (using the nonspecific antigen a healthy human has $IP < 1.3$ with the probability about 0.967 and a Ca patient has $IP > 1.6$ with the probability about 0.974, which correspond to the data in [18]. Therefore, $IP < 1.3$ and $IP > 1.6$ denote states of negative and positive indications for cancer, respectively. The range $1.3 \leq IP \leq 1.6$ is a noise region, where the IP values may be indifferent or suspicious. Nonetheless, various events or agents can shift IP to the values higher than 1.3 or 1.6.

The relative frequency $p/N$of the cases of LAI assays (p is the absolute value of the frequency of cases, and N is the total number of cases) whose number of nonadhering T lymphocytes is in the range from M–$\Delta M/2$ to $M + \Delta M/2$ is evaluated ($\Delta M = 2\%$).

Evaluation of statistical significance was carried out. Nonparametric Mann-Whitney test was used.

4. Experimental Results

Data measured by the LAI assay method in healthy humans and Ca patients in the period from 1976 to 1992 were included in [18]. A more comprehensive set of data including also data from the period 1992-2000 (i.e. all the data measured in the period from 1976 to 2000) is presented in this paper. The relative frequencies p/N of cases as a function of the relative number M of non-adhering T lymphocytes of healthy humans and Ca patients are in Figs. 1a, 1b, and 1c. T lymphocytes from healthy humans with no antigen, and with specific and nonspecific antigens display IP values in the region of $IP < 1.3$ in 100, 95.9, and 96.68 % of cases, respectively (Tab. I). T lymphocytes from Ca patients with no antigen adhere ($IP < 1.3$ is in about 99.6 % of cases, Tab. I) and with specific and nonspecific antigens do not adhere ($IP > 1.6$ is in about 96.4 and about 97.4 % of cases, respectively, Tab. I). The numbers of examined healthy humans and Ca patients are large (thousands of cases – Tab. I). Differences between groups of healthy humans and Ca patients (with and without antigen) are statistically significant at the level 0.01 (Tab. IIa).

Healthy humans and patients	N			
	Total number of cases	$IP < 1.3$	$1.3 \leq IP \leq 1.6$	$IP > 1.6$
		(%)		
H	1721	100	0	0
H+A	3071	95.90	3.68	0.42
H+AV	1960	96.68	2.65	0.06
Ca	1100	99.55	0.46	0
Ca+A	1949	0.56	3.03	96.41
Ca+AV	2298	0.61	2.05	97.35
AMI	50	100	0	0
AMI+AV	50	0	0	100
Sch	583	100	0	0
Sch+AV	476	2.31	6.30	91.39

Tab. I *Number of cases and IP values of healthy humans (H), cancer (Ca), acute myocardial infarction (AMI) and schizophrenia (Sch) patients without antigen, with specific (A) and nonspecific (AV) antigens.*

Adherence of T lymphocytes from AMI patients is shown in Fig. 2. Without antigen and with nonspecific antigen all the IP values are smaller than 1.3 and greater than 1.6, respectively (preliminary data were published in [15, 27]). Similar CMI response is elicited by the antigen prepared from blood vessel wall afflicted with ateroma crusta (non published data).

As a response to nonspecific antigen T lymphocytes taken from Sch patients display the nonadherence properties similar to those of Ca patients (Fig. 3). T lym-

Healthy humans and cancer patients					
H – H		Ca – Ca		H – Ca	
H x H+A	0.01	Ca x Ca+A	0.01	H x Ca	0.01
H x H+AV	0.01	Ca x Ca+AV	0.01	H+A x Ca+A	0.01
H+A x H+AV	NS	Ca+A x Ca+AV	0.01	H+AV x Ca+AV	0.01

a)

Healthy humans and AMI patients			
H – AMI		AMI – AMI	
H x AMI	NS	AMI x AMI+A	0.01
H+AV x AMI+AV	0.01	AMI+A x AMI+AV	0.01

Healthy humans and Sch patients			
H – Sch		Sch – Sch	
H x Sch	0.01	Sch x Sch+AV	0.01
H+AV x Sch+AV	0.01	—	—

b)

Tab. II *Statistical significance of differences between M values of healthy humans and of patients with Ca (a), AMI, and Sch (b). (Statistical significance of a data group is tested against another data group, e.g. H × Ca) NS – non significant. All the symbols have the same meaning as in Tab. I.*

phocytes with no antigen and with nonspecific antigen have $IP < 1.3$ in 100% and $IP > 1.6$ in about 91.4 % of cases, respectively (Tab. I). Preliminary data were published in [28-31].

Statistical significance of the differences between groups of healthy humans and of patients with AMI (or with Sch) is at the level of 0.01 (Tab. IIb). We have to mention that simultaneously with T lymphocytes from Ca, AMI, and Sch patients T lymphocytes from healthy humans were measured.

The similarity of adherence properties of T lymphocytes from Ca, AMI, and Sch patients poses a question what processes are monitored by the CMI response to the nonspecific (LDV) antigen.

5. Discussion

Serum activity of LDH isoenzyme is increased in association with certain disease conditions such as Ca and myocardial infarction [32] and can be revealed by the CMI response to the nonspecific antigen [15]. The CMI response to the nonspecific antigen is associated with Sch too [15]. Riley and Wróblewski [33] and Rowson and Mahy [32] analysis of combined effect of infection with LDV and tumor growth revealed a complex response following tumor implantation. Correlation of the five phases of LDH isoenzyme activity in the plasma with progressive growth of the solid Ehrlich Ca in mice was found – the LDH 2 and 3 appear in the initial phase after tumor implantation, the isoenzyme 4 in the fast growing phase and then the isoenzyme 5.

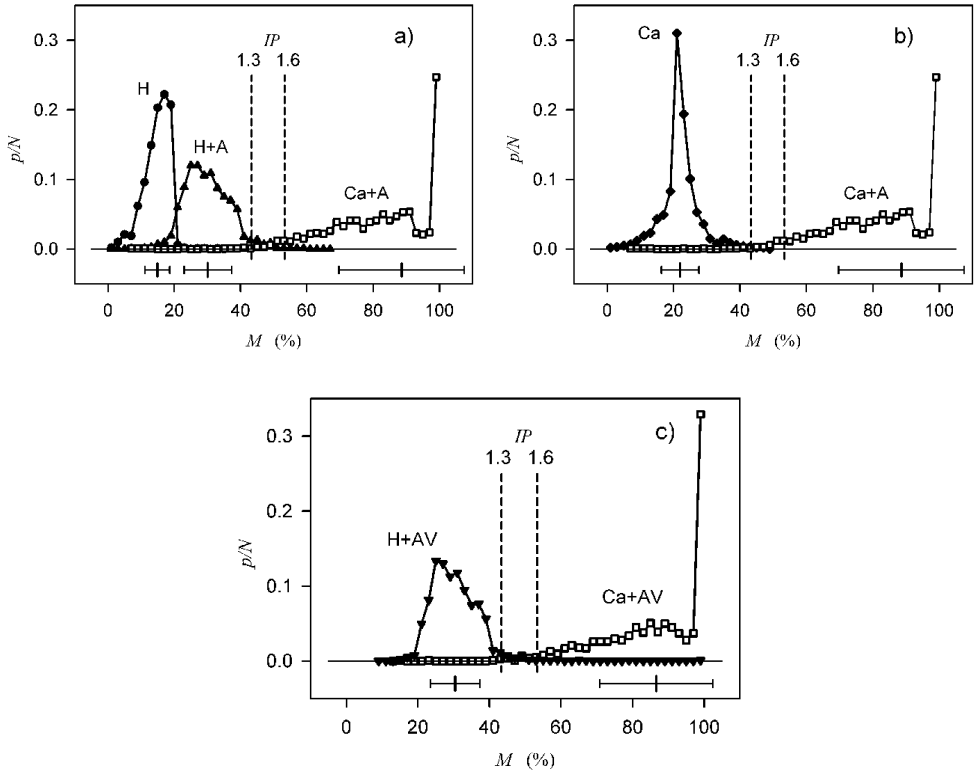


Fig. 1 *The relative frequency p/N of cases as a function of the number M of nonadherent T lymphocytes (the experimental values connected with lines). T lymphocytes were taken from 1) healthy humans and examined without antigen – H, $N_H = 1721$ (a), with specific antigen – H+A, $N_{H+A} = 3071$ (a), and with nonspecific (LDV) antigen – H+AV, $N_{Ca+AV} = 1960$ (c); 2) cancer patients and examined without antigen – Ca, $N_{Ca} = 1100$ (b), with specific antigen – Ca+A, $N_{Ca+A} = 1949$ (a, b), and with nonspecific antigen – Ca+AV, $N_{Ca+AV} = 2298$ (c). The dashed vertical lines denote the principle normalized values of M (i.e. IP = 1.3, and IP = 1.6). Circles, triangles up, triangles down, diamonds, and squares denote the evaluated values of H, H+A, H+AV, Ca, and Ca+A (or Ca+AV), repectively. The short vertical lines connected with horizontal lines under the p/N curves denote their mean values and standard deviations. The data plotted in Figs. 1a, 1b, and 1c represent LAI experimental results measured and evaluated from 1976 to the end of 2000.*

LDV belongs to a new family of positive strand RNA viruses (presently classified as genus Arterivirus). The genom organization and the replication strategy of this virus are similar to those of the coronaviruses, but the size of the genom is smaller than that of the coronaviruses [34]. The virion possesses an envelope and a spherical nucleocapsid which are 50-60 nm in diameter [35]. A more detailed description of LDV is in [19] too.

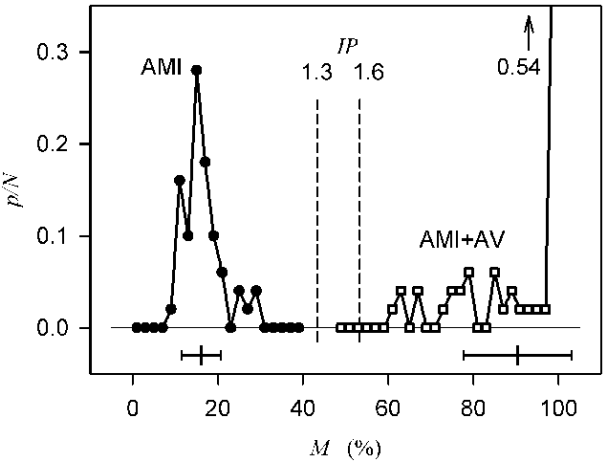


Fig. 2 *The relative frequency p/N of cases as a function of the number M of nonadherent T lymphocytes (the experimental values connected with lines). T lymphocytes were taken from patients with acute myocardial infarction and investigated without antigen – AMI, $N_{AMI} = 50$, and with nonspecific antigen $AMI + AV$, $N_{AMI+AV} = 50$. Circles and squares denote the evaluated values of AMI and $AMI + AV$, respectively.*

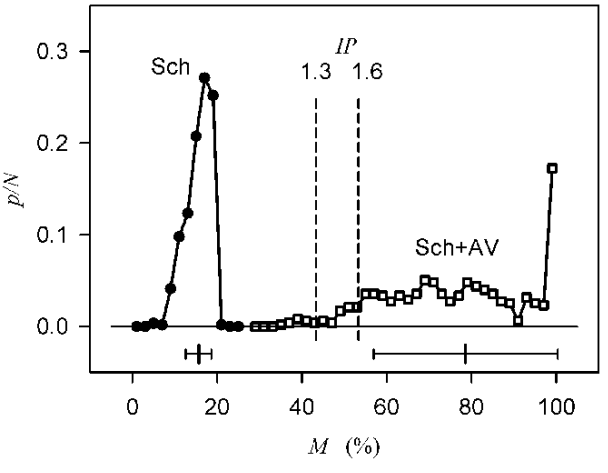


Fig. 3 *The relative frequency p/N of cases as a function of the number M of nonadherent T lymphocytes (the experimental values connected with lines). T lymphocytes were taken from patients with schizophrenia and examined without antigen – Sch, $N_{Sch} = 583$, and with nonspecific antigen Sch+AV, $N_{Sch+AV} = 476$. Circles and squares denote the evaluated values of Sch and Sch+AV, respectively.*

Infection with LDV is manifested by a large amount of LDH (lactate dehydrogenase enzyme now clasified as NAD 1.1.1.27 Oxidoreductase) in plasma (five- to tenfold elevation of LDH levels but no significant harm to the host). LDH performs catabolic activity in cellular energy production chain. It catalyses the conversion of pyruvate to lactate with concomitant conversion of NADH to NAD^+ (reduced to oxidised nicotinamide adenine dinucleotide) or vice versa [32]. When oxygen is absent or in short supply LDH converts pyruvate, the final product of glycolysis and the input compound for mitochondrial energy production, to lactate. In this way, energy rich pyruvate is removed from energy production process. Other parasitic impacts of LDV on energy production are still not published. Changes of immune activity in the acute phase of infection and LDV presence in murine neuroblastoma are given in [36]. Escape from all host defense mechanisms might be caused by defective T cell functions [37] and antigen presenting macrophages [38]. Working with experimental mice tumors, we disclosed that antigens prepared from the serum of mice infected with LDV elicits CMI response in humans. LDV antigen susceptibility to immune response is described – for instance - in [19]. LDV – "ideal" persistent virus – establishes a life-long viremic infection in mice, might be considered as a parasite causing disturbances of the cellular energy system, and is very likely observed as a dark particle at mitochondria.

First of all we will discuss the results of the study of the CMI response of healthy humans and Ca patients to the specific and to the nonspecific (LDV) antigen examined by LAI assay. A part of the data was published in [18]. CMI response of healthy humans and of Ca patients to the specific as well as to the nonspecific antigen displays statistically significant differences. For Ca patients CMI responses to the specific and to the nonspecific antigen seem to be similar (as might be assessed from Figs. 1a, 1b, and 1c), but the quantitative difference is statistically significant (Tab. IIa) – the antigens need not monitor the same process. Oxidative energy production is suppressed in Ca cells, as was disclosed in [39-40]. Only about one half of the ATP production is supplied by mitochondria. Contemporary research confirms the pathogenic role of damaged mitochondria oxidative processes in Ca cells [41-49]. Suppression of mitochondrial activity is considered to be a general "signature" of Ca [44]. Description of bioenergetic phenotype of Ca cells is in [50], multiple regulation pathways in [51], stimulation of glycolytic gene expression in [52], inactivation of *VHL* genes in [53], repression of β-F1-ATPase expression in [54], and the role of cytochrome c oxidase in the Warburg effect in [55].

Functional mitochondria form zones of strong static electric field around their outer membrane [56]. The static electric field and the released energy not utilized for ATP production (about 60 % of energy input into mitochondria) create conditions for excitation of Fröhlich's coherent oscillations. Therefore, mitochondrial dysfunction may result in disturbances of coherence of Fröhlich's oscillations and electromagnetic field with consequences in the cell behavior [57-58]. Therefore, besides synthesis of ATP and reactive oxygen species, mitochondria have essential role in electromagnetic activity of the cell. Restoration of normal mitochondria function converts a Ca cell to a normal cell or triggers apoptosis, if the aberration of the cell is too large [59]. Biophysical mechanism should be included into description of a more comprehensive Ca transformation pathway which is now mainly based on biochemical changes. We assume that cancer problem cannot be solved without

understanding the electromagnetic activity of living cells.

LDV antigen elicits CMI response of T lymphocytes taken from AMI patients as follows from Fig. 2. Myocardial mitochondrial dysfunction may be involved in the pathogenesis of cardiac contractile dysfunction [60]. Multiple defects in mitochondria are central and primary to heart failure progression [61]. Mitochondria have been implicated in the loss of cells in various cardiac pathologies, including ischaemia/reperfusion injury, cardiomyopathy, and congestive heart failure [62].

CMI response to LDV antigen in Sch patients is shown in Fig. 3. The information is transferred across the blood-brain barrier to the immune system. Susceptibility gene for schizophrenia modulates mitochondria function promoting mitochondrial fragmentation in neurons [63]. Evidence suggests that there is mitochondrial dysfunction in schizophrenia [64].

Regardless of the generally accepted view that LDV is solely a mice pathogen, we have to admit that LDV infection may be associated with the diseases examined or with the same or similar pathological processes triggered by different agents may be monitored by CMI response to the nonspecific (LDV) antigen. Nevertheless, if LDV is a parasite on the oxidative energy production system (i.e. on the mitochondrial production) of infected cells, the process monitored by the nonspecific antigen might be connected with disturbances of this system. Mitochondria dysfunction in Ca cells is experimentally proved. CMI response of T lymphocytes of AMI and Sch patients to nonspecific antigen is consistent with diagnostic findings of mitochondria defects and dysfunction too. The type, extent, and locus of mitochondria damage and its role in and connection to harmed biochemical processes have to be analyzed by direct investigation of the mitochondrial function.

6. Conclusion

CMI responses to the nonspecific (LDV) antigen and to specific tumor antigens in cancer patients are similar – the majority of T lymphocytes do not adhere. Nonspecific antigen elicits nonadherence response of T lymphocytes taken from acute myocardial infarction and schizophrenia patients too. As LDV is connected with disturbances of cellular mitochondrial energy system, we assume that the response to the nonspecific antigen might monitor transferred information about mitochondrial dysfunction which has to be followed by defects of the electromagnetic activity of living cells. Mitochondial dysfunction – suppression of oxidative energy production - may be associated with diverse diseases such as cancer, acute myocardial infarction. schizophrenia, and very likely with many others too.

Abbreviations

CMI – cell-mediated immunity, LAI – leukocyte adherence inhibition, LDV – lactate dehydrogenase virus, LDH – lactate dehydrogenase isoenzyme (now classified as NAD 1.1.1.27 Oxidoreductase), *IP* – index of positivity, ORL – otorhinolaryngological, Ca – cancer, AMI – acute myocardial infarction, Sch – schizophrenia

Acknowledgement

This work was partly supported under Grant OC 281.001 of the Ministry of Education, Youth, and Sports of the Czech Republic in the framework of COST 281, under research project MSM 0021620820 of the Ministry of Education, Youth, and Sports of the Czech Republic, and under Grant NR 9441-3, 2007 of the Ministry of Health Care of the Czech Republic.

References

[1] Halliday W. J., Miller S.: Leukocyte adherence inhibition: a simple test for cell-mediated immunity and serum block factors. Int. J. Cancer, **9,** 1972, pp. 477-483.

[2] Holáň V., Hašek M., Bubeník J., Chutná J.: Antigen mediated macrophage adherence inhibition. Cell. Immunol., **13,** 1974, pp. 107-116.

[3] Koppi T. A., Maluish A. E., Halliday W. J.: The cellular mechanism of leukocyte adherence inhibition. J. Immunol., **123**, 1979, pp. 2255-2260.

[4] Grinnell F.: Cellular Adhesiveness and Extracellular Substrata. In: Int. Rev. Cytol., eds. Bourne G. H., Danielli J. F., Academic Press, New York, **53**, 1978, pp. 65-144.

[5] Witkowski J. A., Brighton W. D.: Influence of serum on attachment of tissue cells to glass surfaces. Exp. Cell. Res., **70**, 1972, pp. 41-48.

[6] Bugger H., Abel E. D.: Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome. Clin. Sci., **114**, 2008, pp. 195-210.

[7] Gipson T. G., Martin W. J.: In vivo Anti-Tumor Immunity Detected by Leukocyte Adherence Inhibition. Cancer Immunol. Immunother., **3**, 1978, pp. 201-205.

[8] Grosser N., Thomson D. M. P.: Cell-mediated Antitumor Immunity in Breast Cancer Patients Evaluated by Antigen-induced Leukocyte Adherence Inhibition in Test Tubes. Cancer Res., **35**, 1975, pp. 2571-2579.

[9] Hellstroem I., Hellstroem K. E., Shantz G.: Demonstration of Tumor-Associated Immunity with a Leukocyte Adherence Inhibition (LAI) Assay. Int. J. Cancer, **18**, 1976, pp. 354-361.

[10] Marti J. H., Thomson D. M. P.: Anti-Tumour Immunity in Malignant Melanoma Assay by Tube Leucocyte Adherence Inhibition. Brit. J. Cancer, **34**, 1976, pp. 116-133.

[11] Noonan F. P., Halliday W. J., Wall D. R., Clunie G. J. A.: Cell-mediated Immunity and Serum Blocking Factors in Cancer Patients during Chemotherapy and Immunotherapy. Cancer Res., **37**, 1977, pp. 2473-2480.

[12] Shani A., Ritts R. E., Thynne G. S., Weiland L. H., Silvers A., Moertel Ch. G., Go V. L. W.: A Prospective Evaluation of the Leukocyte Adherence Inhibition Test in Colorectal Cancer and Its Correlation with Carcinoembryonic Antigen Levels. Int. J. Cancer, **22**, 1978, pp. 113-119.

[13] Tataryn D. N., Macfarlane J. K., Thomson D. M. P.: Leukocyte Adherence Inhibition for Detecting Specific Tumour Immunity in Early Pancreatic Cancer. Lancet, **311** (8072), 1978, pp. 1020-1022.

[14] Thomson D. M. P., Tataryn D. A., Schwartz R., MacFarlane J. K.: Abrogation of the Phenomenon of Leukocyte Adherence Inhibition by Excess Circulating Tumour Antigen. Europ. J. Cancer, **15**, 1979, pp. 1095-1106.

[15] Jandová A., Motyčka K., Čoupek J., Sanitrák J., Heyberger K., Laurová L., Mejsnarová B., Novotná J., Pezlarová J., Macholda F., Šimonová J., Dostál C., Kubát Z.: "Disease" of the Cellular Energy System. Sborník lékařský, **81**, 1979, pp. 321-327 (in Czech).

[16] Jandová A., Kobilková J., Nedbalová M., Šorfová J., Dohnalová A., Trojan S., Pokorný J.: Study of interaction forces in human CD4Ly. Bioelectrochem. Bioenerg., **41**, 1996, pp. 181-185.

[17] Jandová A., Hurych J., Nedbalová M., Trojan S., Dohnalová A., Čoček A., Pokorný J., Trkal V.: Effects of sinusoidal magnetic field on adherence inhibition of leukocytes: preliminary results. Bioelectrochem. Bioenerg., **48**, 1999, pp. 317-319.

[18] Jandová A., Hurych J., Pokorný J., Čoček A., Trojan S., Nedbalová M., Dohnalová A.: Effects of Sinusoidal Magnetic Field on Adherence Inhibition of Leukocytes. Electro- Magnetobiol., **20**, 2001, pp. 397-413.

[19] Jandová A., Pokorný J., Čoček A., Trojan S., Nedbalová M., Dohnalová A.: Effects of Sinusoidal Magnetic Field 0.5 mT on Leukocyte Adherence Inhibition. Electromag. Biol. Med., **23**, 2004, pp. 81-96.

[20] Jandová A., Mhamdi L., Nedbalová M., Čoček A., Trojan S., Dohnalová A., Pokorný J.: Effects of Magnetic Field 0.1 and 0.05 mT on Leukocyte Adherence Inhibition. Electromag. Biol. Med., **24**, 2005, pp. 283-292.

[21] Jandová A., Pokorný J., Kobilková J., Janoušek M., Mašata J., Trojan S., Nedbalová M., Dohnalová A., Beková A., Slavík V., Čoček A., Sanitrák J.: Cell-Mediated Immunity in Cervical Cancer Evolution. Electromag. Biol Med., **28**, 2009, pp. 1-14.

[22] Tlaskalová-Hogenová H., Čoupek J., Pospíšil M., Tučková L., Kamínková J., Mancal P.: Affinity chromatography of human lymphocytes on Spheron immunoadsorbent columns. J. Polymer Sci.: Polymer Symp., **68**, 1980, pp. 89-95.

[23] Jandová A., Sanitrák J., Motyčka K., Pezlarová J., Čoupek J.: Isolation of Immunoreactive Components from Experimental and Human Tumor Tissues and Serums by High-Performance Gel Chromatography. J. Chromatography, **146**, 1978, pp. 253-260.

[24] Jandová A., Čoupek J., Sanitrák J., Motyčka K., Mach O., Pekárek J.: Preparation of Immunologically Functional Fractions from Serum of Inbred Mice of Strain C3H Infected with the LDH Virus. CS Patent PV 452-84, January 19, 1984.

[25] Jandová A., Kobilková J., Pilecká E., Dienstbier Z., Pokorný J., Hraba T.: Tumor RNA Effected Leukocyte Activity in Cancer Patients. Abstracts 14th Int. Cancer Congress, Budapest, August 21-27, 1986.

[26] Jandová A., Bendl J., Nedbalová M., Trojan S.: The Inhibition Adherence of Leukocytes in Relation to Specific Rabbit Antibody and to RNA Polymerase. Physiol. Res., **44**, 4, 1995, 23P.

[27] Jandová A., Tvrzická E., Hrabák F.: Immunological Aspects of Aterosclerosis. Sympozium Pathogenetic Aspects of Aterosclerosis, June 16-17, 1988, Mantín, Czechoslovakia (in Czech).

[28] Dvořáková M., Zvolský P., Jandová A.: Immunological Findings in the Parents of Schizophrenics with Positive LAI test. 9^{th} National Psychiatric Meeting, Prague, Czechoslovakia, April 24-27, 1984, Abstracts of Presentations, p. 13 (in Czech).

[29] Dvořáková M., Zvolský P., Jandová A.: Some Immunological Findings in the Parents of Schizophrenic Patients. A Preliminary Report. Sborník lékařský, **87**, 1985, pp. 216-222 (in Czech).

[30] Dvořáková M., Jandová A., Zvolský P., Vinglerová M., Herzog P., Sanitrák J., Motyka K.: Immunological Contribution to Psychiatric Patients Research. Čs psychiatrie, **81**, 1985, pp. 29-31 (in Czech).

[31] Zvolský P., Dvořáková M., Jandová A.: Some Immulologic Findings in the Parents of Schizophrenics. IVth World Congress of Biol. Psychiatry, Philadelphia U.S.A., September 8-13, 1985. Abstracts, p. 347.

[32] Rowson K. E. K., Mahy B. W. J.: Lactic Dehydrogenase Virus. Springer, Wien – New York, 1975.

[33] Riley V., Wróblewski F.: Serial Lactic Dehydrogenase Activity in Plasma of Mice with Growing or Regressing Tumors. Science, **132** (3420), 1960, pp. 151-152.

[34] Plagemann P. G. W., Moenning V.: Lactate-Dehydrogenase-Elevating Virus, Equine Arteritis Virus, and Simian Hemorrhagic Fever Virus: A New Group of Positive-Strand RNA Viruses. Advan. Virus Res., **41**, 1992, pp. 99-192.

[35] Li K., Chen Z., Plagemann P. G. W.: The neutralization epitope of lactate dehydrogenase-elevating virus is located on the short ectodomain of the primary envelope glycoprotein. Virology, **242**, 1998, pp. 239-245.

[36] Leclercq G., Willems J., Heremans H., Coutelier J.-P., Billiau A., Joniau M.: Cellular immunity changes caused by LDH virus: Analogy with observations on neuroblastoma-bearing mice. Int. J. Cancer, **40**, 1978, pp. 669-675.

[37] Michaelides M. C., Simms E. S.: Immune responses in mice infected with lactic dehydrogenase virus. III. Antibody response to a T-dependent and a T-independent antigen during acute and chronic LDV infection. Cell. Immunol., **50**, 1980, pp. 253-260.

[38] Isakov N., Feldman M., Segal S.: Acute infection of mice with lactic dehydrogenase virus (LDV) impairs the antigen-presenting capacity of their macrophages. Cell. Immunol., **66**, 1982, pp. 317-332.

[39] Warburg O., Posener K., Negelein E.: Über den Stoffwechsel der Carcinomzelle. Biochem. Z., **152**, 1924, pp. 309-344 (in German).

[40] Warburg O.: On the Origin of Cancer Cells. Science, **123**, 1956, pp. 309-314.

[41] Brandon M., Baldi P., Wallace D.C.: Mitochondrial mutations in cancer. Oncogene, **25**, 2006, pp. 4547-4662.

[42] Carew J. S., Huang P.: Mitochondrial defects in cancer. Mol. Cancer, **1**, 2002, pp. 9-20.

[43] Chatterjee A., Mambo E., Sidransky D.: Mitochondrial DNA mutations in human cancer. Oncogene, **25**, 2006, pp. 4663-4674.

[44] Cuezva J. M., Krajewska M., López de Heredia M., Krajewski S., Santamaria G., Kim H., Zapata J. M., Marusawa H., Chamorro M., Reed J.C.: The Bioenergetic Signature of Cancer: A Marker of Tumor Progression. Cancer Res., **2**, 2002, pp. 6674-6681.

[45] King A., Selak M. A., Gottlieb E.: Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene, **25**, 2006, pp. 4675-4682.

[46] Kroemer G.: Mitochondria in cancer. Oncogene, **25**, 2006, pp. 4630-4632.

[47] Lill R., Mühlenhoff U.: Iron-sulfur-protein biogenesis in eukaryotes. Trends Biochem. Sci., **30**, 2005, pp. 133-141.

[48] Mathupala S. P., Ko Y. H., Pedersen P. L.: Hexokinase II: Cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. Oncogene, **25**, 2006, pp. 4777-4786.

[49] Rossignol R., Gilkerson R., Aggeler R., Yamagata K., Remington S. J., Capaldi R. A.: Energy Substrate Modulates Mitochondrial Structure and Oxidative Capacity in Cancer Cells. Cancer Res., **64**, 2004, pp. 985-993.

[50] Pedersen P. L.: Warburg, me, and Hexokinase 2: Multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen. J. Bioenerg. Biomembr., **39**, 2007, pp. 211-222.

[51] Ma W., Sung H. J., Park J. Y., Matoba S., Hwang P. M.: A pivotal role for p53: balancing aerobic respiration and glycolysis. J. Bioenerg. Biomembr., **39**, 2007, pp. 243-246.

[52] Godinot C., de Laplanche E., Hervouet E., Simonnet, H.: Actuality of Warburg's views in our understanding of renal cancer metabolism. J. Bioenerg. Biomembr., **39**, 2007, pp. 235-241.

[53] Semenza G. L.: HIF-1 mediates the Warburg effect in clear cell renal carcinoma. J. Bioenerg. Biomembr., **39**, 2007, pp. 231-234.

[54] Cuezva J. M., Sánchez-Aragó M., Sala S., Blanco-Rivero A., Ortega Á. D.: A message emerging from development: the expression of mitochondrial β-F1-ATPase expression in cancer. J. Bioenerg. Biomembr., **39**, 2007, pp. 259-265.

[55] Herrmann P. C., Herrmann E. C.: Oxygen metabolism and a potential role for cytochrome c oxidase in the Warburg effect. J. Bioenerg. Biomembr., **39**, 2007, pp. 7-250.

[56] Tyner K. M., Kopelman R., Philbert M. A.: ,,Nanosized Voltmeter,, Enables Cellular-Wide Electric Field Mapping. Biophys. J., **93**, 2007, pp. 3-1174.

[57] Pokorný J., Hašek J., Vaniš J., Jelínek F.: Biophysical aspects of cancer – Electromagnetic mechanism. Indian J. Exper. Biol., **46**, 2008, pp. 310-321.

[58] Pokorný J.: Biophysical Cancer Transformation Pathway: Electromag. Biol. Med., 2009 (in print).

[59] Bonnet S., Archer S. L., Allalunis-Turner J., Haromy A., Beaulieu Ch., Thompson R., Lee Ch. T., Lopaschuk G. D., Puttagunta L., Bonnet S., Harry G., Hashimoto K., Porter Ch. J., Andrade M. A., Thebaud B., Michelakis E. D.: Mitochondria-K^+ Channel Axis Is Suppressed in Cancer and Its Normalization Promotes Apoptosis and Inhibits Cancer Growth. Cancer Cell, **11**, 2007, pp. 37-51.

[60] Burger D. R.: Leukocyte Adherence Inhibition. In: Immunodiagnosis Cancer, eds. Herberman R. B., McIntire K. R., Dekker, New York., 1979, pp. 1124-1145.

[61] Marin-Garcia J., Goldenthal M. J.: Mitochondrial centrality in heart failure. Heart Fail. Rev., **13**, 2008, pp. 137-150.

[62] Gustafsson A. B., Gottlieb R. A.: Heart mitochondria: gates of life and death. Cardiovasc. Res., **77**, 2008, pp. 334-343.

[63] Kvajo M., Dhilla A., Swor D. E., Karayiorgou M., Gogos J. A.: Evidence implicating the candidate schizophrenia/bipolar disorder susceptibility gene G72 in mitochondrial function. Mol. Psychiatry, **13**, 2008, pp. 685-696.

[64] Shao L., Martin M. V., Watson S. J., Schatzberg A., Akil H., Myers R. M., Jones E. G., Bunney W. E., Vawter M. P.: Mitochondrial involvent in psychiatric disorders. Ann. Med., **40**, 2008, pp. 281-295.