Electromagnetic Biology and Medicine, 28: 1–14, 2009
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ISSN 1536-8378 print/1536-8386 online
DOI: 10.1080/15368370802708868

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Cell-Mediated Immunity in Cervical Cancer Evolution

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Cell-mediated immunity (CMI) response to different antigens was examined in healthy women, in patients with cervical precancerous lesions, and in patients with cervical cancer. Cervical lesions were diagnosed by cytological (PAP) smears, from examination by colposcopy, and from "punch" biopsy material by histology. CMI response is related to specific processes in healthy and cancer cells. CMI was investigated by leukocyte adherence inhibition (LAI) assay using specific antigen (prepared from cervical carcinoma tissue) and non specific antigen (prepared from blood of mice infected by LDH—lactate dehydrogenase—virus). The CMI responses of healthy women and cancer patients to the antigens used are different: the majority of T lymphocytes display adherence and non adherence, respectively (but the CMI responses elicited by the antigens are not equal and small quantitative differences are observed). Regardless of the CIN (cervical intraepithelial neoplasia) grades, CMI responses correspond either to healthy women or to cervical carcinoma patients (at about similar ratio of cases in all the CIN groups). Effect of non specific antigen suggests that cervical carcinoma transformation may be connected with reduction of mitochondrial activity similar to processes in LDH virus infection.

Keywords Cell-mediated immunity; Cervical precancerous lesions; Cervical cancer evolution; Leukocyte adherence inhibition assay; Warburg effect, High-pressure gel chromatography.

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Introduction

The cell-mediated immunity (CMI) response to two different antigens was examined in healthy humans, in patients with cervical precancerous lesions, and in patients with cervical cancer. CMI response is related to specific processes in healthy and cancer cells and, therefore, displays transferred information about their state and evolution. CMI is understood to correlate with adherence properties of leukocytes from peripheral blood. Observation of adherence of T lymphocytes to surfaces of glass or some plastic materials in the presence or absence of antigens is the *in vitro* experimental measurement procedure (Grinnell, 1978; Witkowski and Brighton, 1972). The highly specific quantitative technique—the leukocyte adherence inhibition (LAI) assay—was widely used to study and to monitor CMI (Halliday and Miller, 1972; Holáň et al., 1974; Koppi et al., 1979). Numerous experimental LAI assays were made for quantitative examination of adherence of T lymphocytes to assess tumor-specific CMI (Thomson et al., 1979; Burger, 1979; Gipson and Martin, 1978; Grosser and Thomson, 1975; Hellstroem et al., 1976; Marti and Thomson, 1976; Noonan et al., 1977; Shani et al., 1978; Tataryn et al., 1978). We have performed the LAI assay investigations for three decades and accumulated considearble amount of data on CMI in healthy humans and cancer patients (Jandová et al., 1979, 1996, 1999, 2001, 2004, 2005).

Mechanism of adherence of cells includes attractive London dispersion forces, repulsive electrostatic forces (Curtis, 1964, 1973), and electrostatic double-layer forces (diffuse layers overlapping) (Claesson, 1994). Adherence depends on the surface charge of the substrate too (Maroudas, 1975, 1977). It is not quite clear whether adherence molecules, e.g., integrins (β_2) or selectins, which enable white blood cells to attach to and to cross the endothelial lining of blood vessels, are involved in adherence to solid state surfaces. But adherence of cells might not depend only on the described short-range mechanisms but also on a long-range mechanism (of electromagnetic origin) (Jandová et al., 1999) whose existence is supported by observation of interaction forces between T lymphocytes and formation of clusters (Jandová et al., 1996; Pokorný et al., 1996).

Chemical as well as physical agents (e.g., magnetic field; Jandová et al., 2001, 2004, 2005) can affect CMI and change adherence of T lymphocytes. Antigen alters adherence after it binds to the corresponding receptor in the cell membrane. After binding, a sequence of successive signaling processes is triggered resulting in changed adherence of the T lymphocyte cell. We studied CMI in healthy humans and cancer patients with gynecological (Jandová et al., 1996, 2001, 2004, 2005) and otorhinolaryngological (ORL) tumors (Jandová et al., 1999, 2001, 2004) using specific antigen prepared from corresponding cancer tissue and non specific antigen prepared from plasma of mice infected by LDH (lactate dehydrogenase) virus by high-pressure gel chromatography.

LDH virus antigen susceptibility to immune response is described in Jandová et al. (2004). Changes of immune activity in acute phase of infection and LDH virus presence in murine neuroblastoma are given in Leclercq et al. (1978). Escape from all host defense mechanisms might be caused by defective T cell functions (Michaelides and Simms, 1980) and antigen presenting macrophages (Isakov et al., 1982). Binding to macrophages and expression of proteins is analyzed in Cafruny et al. (2003). Infection is manifested by a large amount of LDH (now classified as NAD 1.1.1.27 Oxidoreductase) enzymes in plasma (five- to tenfold elevation of plasma LDH enzyme levels but no significant harm to the host). LDH virus—"ideal" persistent virus—establishes a life-long viremic infection in mice, parasites on energy system, and may be observed as a dark particle at mitochondria. (Our experimental investigation

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revealed that about 1/3 of human population in the Czech Republic was infected by LDH virus; the data in other countries may be similar.) The virus properties and possible connection with cancer were studied in Riley and Wroblewski (1960) and Rowson and Mahy (1975).

Adherence of T lymphocytes taken from healthy women, from patients with cervical precancerous lesions and with cervical cancer diagnosed cytologically, colposcopically, and histologically was experimentally investigated. Cervical precancerous lesions can be accurately diagnosed and serve as an adequate model. Genital cancer is the outcome of persistent human papillomavirus (HPV) infection and failure of the host immune system to eliminate the virus (Van Poelgeest et al., 2006). HPV is the name of a group of viruses that includes more that 100 different genotypes. With respect to capability of cancer development HPV's are denoted as HR or LR types (high-risk or low-risk, i.e. with high or low oncogenic potential, respectively, presumably reflecting the potency of their oncoproteins). For instance, the HR group includes HPV 16, 18, 31, 35, 39, 45, etc. (more than 50% of cervical cancer cases harbor HPV 16 type). One of the risk factors of HPV infection is impaired cell-mediated immunity (CMI) (Ferenczy et al., 2003). Immunological abnormalities were tested in precancerous and carcinoma patients (Das et al., 2007). The immunological basis for recovery from HPV-associated CIN (cervical intraepithelial neoplasia) is not clear (Sarkar et al., 2005; Bungener et al., 2006; Lee et al., 2006; Passmore et al., 2006). It is well known that the E4 HPV 16 protein induces the collapse of the cytokeratin filaments (Roberts et al., 1993). The schemes of HPV action do not seem to contain mitochondrial dysfunction which may be a general defect in cancer cells (Carew and Huang, 2002; Cuezva et al., 2002; Rossignol et al., 2004; Kroemer, 2006; Brandon et al., 2006; Chatterjee et al., 2006; King et al., 2006; Mathupala et al., 2006; Lill and Mühlenhoff, 2005). Analysis of the CMI in the period of transformation of healthy humans to cancer patients could contribute to understanding of critical steps of malignant process.

Materials and Methods

Diagnostic Methods

Cervical lesions were diagnosed by cytological (PAP) smears, from examination by colposcopy, and from colposcopically directed "punch" biopsy material by histology. Cytology and colposcopy revealed suspect cervical lesions which were exactly classified by biopsy. The grades of development of precancerous lesions LSIL and HSIL (low- and high-grade squamous intraepithelial lesions, respectively) were determined by cytology, and CIN1, CIN2, and CIN3 (cervical intraepithelial neoplasia) grades by histology. Cytology and colposcopy diagnostic results in HSIL grade correspond to CIN2 and CIN3 grades. Persisting LSIL with HPV infection indicates increased probability for development of CIN2 or CIN3 lesions (Solomon et al., 2001).

Preparation of T Cells

The T lymphocytes were prepared from venous blood of healthy women, and of patients with cervical precancerous lesions and cervical cancer.

Venous blood (8 ml) was taken from the patient and transferred into a test tube with 500 U of heparin, and incubated in a thermostat for 30 minutes at 37°C. A Pasteur pipette was used to transfer the plasma rich in white blood cells into a new

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test tube. The contents were diluted with a saline solution or phosphate buffered saline (PBS), minimum essential medium (MEM), medium to its original volume, and centrifuged at 1,800 rpm. The supernatant was poured off and the sediment was layered with 1 ml of distilled water, bubbled through a Pasteur pipette, and lysis of erythrocytes was achieved. Thereafter, dilution to volume with some of the above-mentioned solutions according to an option was done. Thus, the lysis of erythrocytes was stopped. The contents was centrifuged once again for 10 min at 1,800 rpm. The rinsing solution was poured off, the sediment of white blood cells was diluted with a selected medium, filtered through nylon absorbent wool (resulting in enrichment of the T cells), and diluted again so as to make available 4×10^6 cells per 1 ml for the test, as counted in the Bürker chamber. The method of preparation is described in Jandová et al. (2001).

Some data on selection and purification of T cells pool for measurement have to be mentioned. The flow cytometry is not usable for cell separation as the medium used (citrate puffer and other components) has negative impact on the properties of T cells with respect to LAI assay investigation. A filtration column consisting of a $5\,cm^3$ syringe filled with nylon absorbent wool was used (a new syringe with new absorbent wool has to be used for each individual case). The pool passing through the column contained more than 90% of T cells and the remaining part other cells (Tlaskalová-Hogenová et al., 1980). Evaluation of the LAI assay was performed by an experienced laboratory specialist educated for differential diagnosis of white blood cells.

The Specific and the Non Specific 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. Non specific antigen was an immunologically functional fraction prepared from the serum of inbred laboratory mice strain C3H infected with the LDH virus. High-pressure gel chromatography was used to separate the antigen fractions. The detection was carried out by means of an R 043 differential refractometer and a UV flow-through spectrophotometer with variable wavelengths. Saline solution was used as a mobile phase. Immunologically active fractions of antigen was obtained at the wavelength of 340 nm. Figure 1 shows a gel chromatogram of the

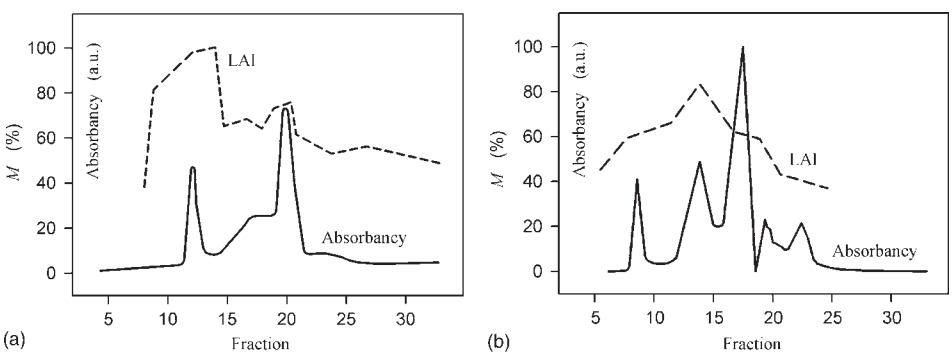


Figure 1. Gel chromatogram of antigen fractions prepared from (a) human cervical carcinoma (Jandová et al., 1978) and (b) from serum of inbred mice of strain C3H infected with the LDH virus (Jandová et al., 1984)—the full curves, and immunological effectiveness of the fractions verified by LAI assay—the dashed curves.

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specific and non specific antigen fractions and their immunological effectivity measured by LAI assay (Jandová et al., 1978, 1984).

A comprehensive description of the preparation methods of the antigens is given in (Jandová et al., 2001).

Measurement and Evaluation

Suspension with the T cells prepared according to the method described above was mixed with antigen at a ratio of 1 part (20 µl) of antigen to 4 parts (80 µl) of T cell float. The mass of antigen per one T cell is of the order of magnitude of 10 ag. For instance, the mass of the non specific antigen is 28.8 ag (about 1,233 molecules) per one T cell. The suspension with T lymphocytes sedimented in the test tubes from green Sial glass. Temperature during sedimentation was in the range 22–28°C. After 60 min of sedimentation-adhesion process the number m of non adherent cells was counted.

The results are expressed as a relative number $M_i = 100\,m/m_0$ (in %) of non adherent cells, where m_0 is the number of cells in suspension before sedimentation-adhesion process. Mean M values are evaluated from M_i. To simplify assessment of the LAI assays with respect to cancer patients data, we normalize M values by the relation $IP = M/A$ where $A = 100/3$ (IP is Index of Positivity) and specify some significant discrete levels. We use $IP = 1.3$ and $IP = 1.6$ to distinguish IP values of healthy humans and cancer patients, respectively. A healthy human has $IP < 1.3$ with the probability of about 0.953. A cancer patient has $IP > 1.6$ with the probability of about 0.956 (Jandová et al., 2001). 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 non adhering T lymphocytes is in the range from $M-\Delta M/2$ to $M+\Delta M/2$ ($\Delta M = 5\%$) is evaluted too.

Statistical Evaluation

Evaluation of statistical significance was carried out. Mann-Whitney rank-sum test, paired samples statistics and quantity p from nonparametric Wilcoxon rank test for two related samples, Kruskal-Wallis one-way analysis, and χ^2 test were used.

Experimental Results

CMI response to the specific and non specific antigen expressed as a relative number of non adherent T lymphocytes was examined in the groups of healthy women (H), of patients with cervical carcinoma (Ca), and with precancerous lesions (P).

Adherence of T lymphocytes taken from healthy women and from cervical cancer patients without antigens is shown in Figure 2. The relative frequency p/N of cases is plotted as a function of the number M of non adherent T lymphocytes for $\Delta M = 5\%$. In both cases without antigen the M values are in the range of healthy humans (i.e., below the significant normalized level $IP < 1.3$). In individual cases, the M_i values of healthy humans and cervical cancer patients cannot be distinguished.

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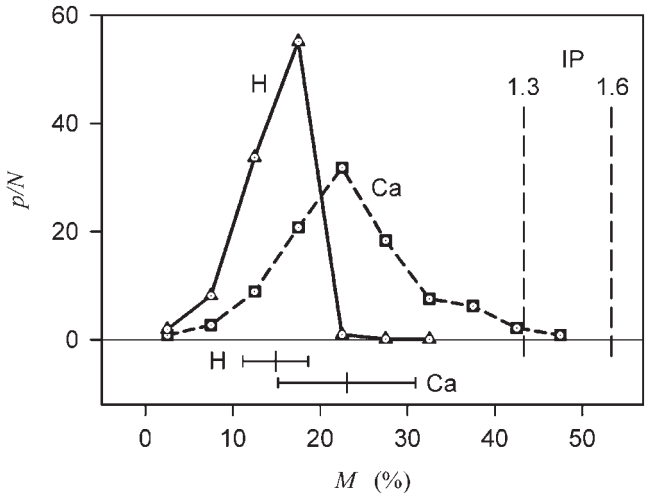


Figure 2. Adherence of T lymphocytes prepared from peripheral blood of healthy women (H, $N_H = 1{,}721$) and patients with cervical carcinoma (Ca, $N_C = 371$) without antigens. Relative frequency p/N of cases is plotted as a function of the number M of non adherent lymphocytes (p is the absolute value of the frequency of cases in a range $\Delta M = 5\%$). The dashed vertical lines denote the significant normalized values of M (i.e., IP = 1.3 and IP = 1.6—the upper and lower boundaries of the regions with negative and positive indications for cancer, respectively). The mean values and standard deviations are plotted under the p/N curves.

Effects of specific and non specific antigens on adherence of T lymphocytes of healthy women, of patients with cervical precancerous lesions, and of patients with cervical carcinoma are in Figures 3a and b (p/N vs. M). The significant IP levels (IP = 1.3 and IP = 1.6) separate regions with negative, positive, and indifferent indications for cancer (Jandová et al., 2001). The majority of cases in the group of healthy humans and cervical carcinoma patients have IP $<$ 1.3 and IP $>$ 1.6, respectively, that corresponds to the experimental analysis in Jandová et al. (2001). The p/N values of patients with precancerous lesions are distributed over the whole region of M, but the greater part of cases has the carcinoma M values (i.e., IP $>$ 1.6, Table 1).

The "precancerous" CMI response curves in Figures 3a and b include data of patients with all the CIN stages of lesions. It was assumed that CMI response might depend on the precancerous evolution stage. Therefore, we evaluated data for each CIN group. Figure 4 shows number of non adherent T lymhocytes of CIN1, CIN2, and CIN3 groups and of groups of healthy humans and cervical cancer patients (number of cases is given in Table 1). M values of healthy humans and cancer patients are about 30% and above 80%, respectively. M values of all three CIN groups are between 60–70% (i.e., the average IP $>$ 1.6) but significantly smaller than M values of the cancer patient group (Table 2a, with exception of Ca $\times$ CIN1 for specific antigen). The CIN groups do not display significant differences in M values when the specific and non specific antigens are used, i.e., as if evolution of precancerous lesions along the pathway to carcinoma had no effect on CMI.

Standard deviation values in all the precancerous groups shown in Figure 4 are greater than those in the healthy and in the carcinoma group. To analyze the CMI response each CIN group of patients was divided into two subgroups: one corresponding to healthy women (IP $<$ 1.3) and the other to cervical carcinoma patients (IP $>$ 1.6); see

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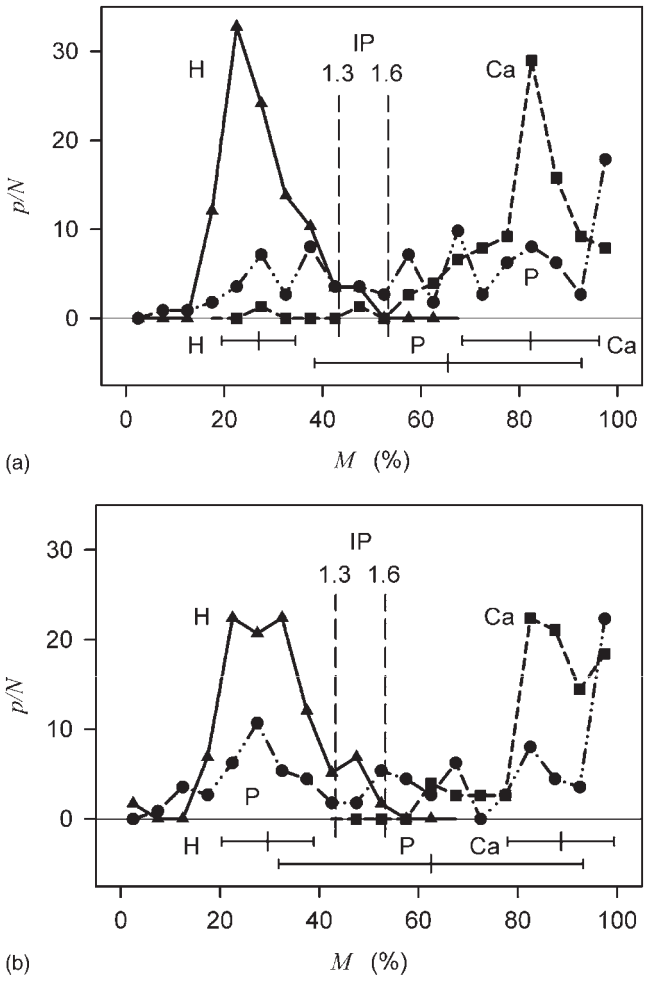


Figure 3. Relative frequency p/N of cases as a function of the number M of non adherent lymphocytes. T lymphocytes were prepared from peripheral blood of healthy humans (H, $N_H = 58$), patients with cervical precancerous lesions (P, $N_P = 112$), and patients with cervical carcinoma (Ca, $N_C = 76$). Significance of the dashed vertical lines is the same as in Figure 2. Specific (a) and non specific (b) antigens were used.

Figure 5. Table 2b shows that there are no significant differences between M values of CIN1, CIN2, and CIN3 individual subgroups with IP > 1.6 and M values of cancer groups using both the antigens. Similar assessment holds for M values of CIN groups with IP < 1.3 and healthy groups, except for CIN2 group and the specific antigen.

Table 2c shows that statistically significant difference in M emerges among CIN1, CIN2, and CIN3 subgroups for IP > 1.6 using non specific (LDH virus) antigen.

Discussion

Precancerous lesions enable us to follow and analyze a certain period of cancer transformation before final pathological product is formed—malignant cell. In the cervical precancerous evolution period the immune system is able to suppress the

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Table 1
Number N of cases in the groups of healthy humans (H), patients with cervical precancerous lesions (P) divided into groups according to CIN grades and to IP values, and patients with cervical carcinoma (Ca). SA (NA) denotes specific (non specific) antigen

		IP < 1.3		1.3 ≤ IP ≤ 1.6		IP > 1.6	
Group	Total number	SA	NA	SA	NA	SA	NA
H	58	55	52	3	6	—	—
P							
CIN1	28	6	8	1	2	21	18
CIN2	40	14	14	3	1	23	25
CIN3	44	11	17	2	3	31	24
Ca	76	1	—	1	—	74	76

cancer development and the probability of successful cancer eradication is not negligible. Nevertheless, behavior of precancerous events is unpredictable and invasive carcinoma may develop from any level of abnormalities (Koss, 1997). Cervical carcinoma cells fail to induce an effective specific cytotoxic T lymphocyte response and tumor cells escape host immune surveillance (Kaufmann et al., 1996).

Cell-mediated immunity response of the healthy humans and of cancer patients examined by LAI assay displays large statistically significant differences for both antigens. CMI response to the specific and non specific antigen seems to be similar for healthy humans as well as for cervical carcinoma patients (as might be assessed from Figures 4 and 5) but the differences are statistically significant. Therefore, the specific and the non specific antigen may monitor the same pathological process but elicit responses of different magnitude, but we have also to admit that the antigens monitor different processes and trace different parts or branches of the carcinogenesis pathway.

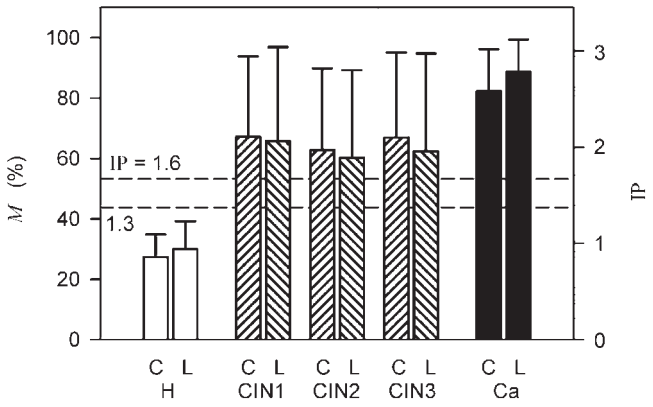


Figure 4. The relative number M (in %) of non adhering lymphocytes taken from healthy humans (H), patients with cervical precancerous lesions (CIN1, $N_1 = 28$; CIN2, $N_2 = 40$; and CIN3, $N_3 = 44$, Table 1), and patients with cervical carcinoma (Ca, $N_C = 76$). C—specific antigen, L—non specific antigen.

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Table 2

Statistical significance (s) of the differences between *M* values of the groups of healthy humans (H), patients with precancerous lesions (P), and patients with cervical carcinoma (Ca). SA (NA) denotes specific (non specific) antigen. X is a group given in the column "Group." NS means "not significant." We used: (a) the Mann-Whitney rank-sum test—(Table 2a); (b) the Mann-Whitney rank-sum test (MWRS), the paired samples statistics and the nonparametric Wilcoxon rank test for two related samples (PS + WR)—(Table 2b); (c) the Kruskal-Wallis one way analysis—(Table 2c)

	SA		NA	
Group	H × X	Ca × X	H × X	Ca × X
(a)				
H	X	0.01	X	0.01
P				
CIN1	0.01	N.S.	0.01	0.05
CIN2	0.01	0.01	0.01	0.01
CIN3	0.01	0.05	0.01	0.01
Ca	0.01	X	0.01	X

		H × X (IP < 1.3)		SA × NA
		Ca × X (IP > 1.6)		PS + WR
		MWRS		
Group		SA	NA	
(b)				
H		X	X	0.01
P				
CIN1	IP < 1.3	NS	NS	NS
	IP > 1.6	NS	NS	NS
CIN2	IP < 1.3	0.05	NS	0.01
	IP > 1.6	NS	NS	NS
CIN3	IP < 1.3	NS	NS	NS
	IP > 1.6	NS	NS	0.01
Ca		X	X	0.001

Group		
Antigen	IP value	CIN1 × CIN2 × CIN3
(c)		
SA	IP < 1.3	NS
	IP > 1.6	NS
NA	IP < 1.3	NS
	IP > 1.6	0.05

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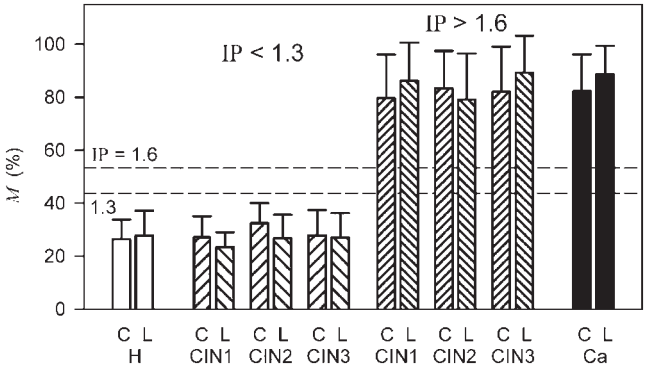


Figure 5. Relative number M of non adherent lymphocytes of healthy humans, of patients with cervical precancerous lesions, and patients with cervical carcinoma. With respect to the number of non adherent T lymphocytes, the patients with precancerous lesions are divided into subgroups with IP < 1.3 and IP > 1.6. The former and latter subgroup have the M values similar to those of T lymphocytes from healthy humans and from cancer patients, respectively. The number of cases in individual groups is given in Table 1.

As LDH virus is a parasite on the energy system of infected cells, the process monitored by the non specific antigen might be of the same nature—might affect oxidative energy production, i.e., mitochondrial activity.

Both the antigens elicit similar CMI response in the CIN groups: the smaller part of the patients in each group responses like healthy humans (IP < 1.3) and the greater part like cancer patients (IP > 1.6). But CMI response is distributed over the whole range of M (IP) values. The process of carcinoma evolution monitored during precancerous period may consist in accumulation of quantitative changes which—after reaching a threshold—trigger malignant behavior.

There are no significant differences in CMI response to specific antigen between CIN1, CIN2, and CIN3 subgroups either for IP < 1.3 or for IP > 1.6. Division of cervical precancerous lesions into the CIN groups is mainly based on assessment of morphological features of cells and of the structure of the tissue by cytological and histological examinations. Morphological changes of cells are connected with changes induced in the cell membrane, in the cytoskeleton, in the cytosol, and in the nucleus, which alters deformability of cells (Guck et al., 2005; Suresh, 2007; Suresh et al., 2005; Cross et al., 2007). Therefore, CMI response to the specific antigen does not monitor either morphological or structural tissue changes in cervical carcinoma evolution. A similar assessment holds for non specific antigen and CIN subgroups with IP < 1.3.

Variances of CMI response to non specific antigen among the CIN subgroups for IP > 1.6 are at the threshold of statistical significance. The variances might indicate reduction and restitution of the monitored process—very likely conditioned by or connected with changing activity of mitochondria which may cause a critical reversal in evolution of precancerous lesions.

In healthy cells, mitochondrial oxidative reactions produce much greater amount of energy transferred into ATP (adenosine triphosphate) than glycolysis. But mitochondrial activity is not limited to ATP production. In a healthy cell the wasted energy (nearly 60% of energy contained in pyruvates and fatty acids entering the mitochondrion) is liberated into the surroundings by heat efflux and also by emitted

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radiation (Batyanov, 1995) (the liberated energy has not only the heat form). The electric field associated with the mitochondrial membrane is measurable at a distance of several μm's from the mitochondrion (Tyner et al., 2007). The absolute value of the intensity is greater than 10^6 V/m at the membrane. Mitochondria tend to align along microtubules forming special conditions around them. Warburg disclosed that oxidative ATP (adenosine triphosphate) synthesis in cancer cells is suppressed and that vast amount of ATP production is switched to and replaced by glycolysis (Warburg, 1956; Warburg et al., 1924). He assumed that reduction of diminished oxidative activity is connected with structural changes in the cell. Cancer as a disease of the energy producing system in cells is described in Jandová et al. (1979). Contemporary research confirms a pathogenic role of suppression of oxidative processes caused by damage of mitochondria in cancer cells (Carew and Huang, 2002; Cuezva et al., 2002; Rossignol et al., 2004; Kroemer, 2006; Brandon et al., 2006; Chatterjee et al., 2006; King et al., 2006; Mathupala et al., 2006; Lill and Mühlenhoff, 2005). Dysfunction of mitochondrion in a cancer cell results not only in interruption of ATP production but also of the wasted energy efflux, and in extinction of the electric field in the cytosol. Mitochondrial energy production can be restored for instance by frataxin (Ristow et al., 2000; Schulz et al., 2006). DCA (dichloracetate) is used to boot the ability of mitochondria to generate energy (Bonnet et al., 2007). DCA restores the membrane potential, activates K^+ channels, and opens mitochondrial entry gate by activation of the mitochondrial enzyme PDH (pyruvate dehydrogenase) family converting pyruvate to acetyl CoA (acetyl coenzyme A). Restitution of mitochondrial activity in a cancer cell helps to restore the normal cellular functions or to switch on apoptosis of excessively aberrant cells.

It is well known that cancer evolution induces morphological changes and decrease of mechanical stiffness of the cells which signifies cytoskeleton damage (Guck et al., 2005; Suresh, 2007; Suresh et al., 2005; Cross et al., 2007). The stiffness changes are produced before the malignant properties are formed. Cytoskeleton of cervical epithelial cells is disintegrated by E4 HPV 16 protein (Roberts et al., 1993).

Conclusion

Cell-mediated immunity (CMI) in healthy humans, patients with precancerous lesions and with cervical carcinoma were investigated by LAI assay using cervical carcinoma and LDH virus antigens. The CMI response to antigens used is related to processes in cervical epithelial cells and transfers information about their cancer evolution. CMI response of healthy humans and cancer patients to the antigens are significantly different. CMI response of precancerous patients corresponds either to healthy humans or to cancer patients regardless of the CIN grade of the lesion. Therefore, cytology and histology examination (based on cellular and subcellular morphology and tissue structure) and LAI assay with two different antigens very likely trace different branches of carcinoma transformation pathway. Effect monitored by non specific antigen indicates that cervical cancer transformation may be connected with processes similar to those of LDH virus infection and suggests that mitochondrial dysfunction evolves in precancerous stages. The role of mitochondrial dysfunction in creation of invasive carcinoma should be further investigated. Any precancerous lesion must be carefully treated as the mitochondrial damage need not correspond to the CIN grades of precancerous lesions which is confirmed by experience of L. G. Koss that behavior of precancerous events is unpredictable.

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Acknowledgment

This work was supported under Grant OC 281.001 of the Ministry of Education, Youth, and Sports of the Czech Republic in the framework of COST 281. Dr. Ruth Tachezy is gratefully acknowledged for consultation and helpful comments. We thank Jitka Švihálková and Olga Pazderová from the National Institute of Public Health for expert assistance.

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