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Review

IMMUNE STATE OF PATIENTS WITH DIFFERENT PATHOLOGIES MONITORED BY FLUORESCENT PROBE 3-AMINOBENZANTHRONE DERIVATIVE

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Research results of the 3-aminobenzanthrone derivate ABM (developed at the Rīga Technical University, Rīga, Latvia) as a potential fluorescent probe for determination of the immune state have been summarised. Spectral characteristics of the compound in different solvents as well as binding to model lipid membranes (liposomes), albumin and human peripheral blood mononuclear cells (PBMC) were determined. The fluorescence of the compound was found to be sensitive to polarity changes in the environment. Distinctions were observed in the spectral characteristics of ABM when bound to PBMC of healthy donors and patients with several diseases (advanced lung tuberculosis, multiple sclerosis, rheumatoid arthritis, oncological diseases etc.) and those who have been subjected to ionising radiation during the clean-up work in Chernobyl. It was established that spectral parameters of ABM reflect a wide range of interrelated (interdependent) characteristics of PBMC: physico-chemical state and microviscosity of membrane; proliferating and lipid metabolic activity of cells; distribution of lymphoid subsets. The spectral characteristics of ABM were correlated with the clinical view of investigated diseases, their phase and type, clinical and laboratory indices and several immunological parameters. The observed changes of the studied parameters reflect alterations of the cellular mechanisms of immunity and therefore can be applied as a preliminary screening test of the immune state.

Key words: *fluorescent probe, lymphocytes, immune state.*

INTRODUCTION

New methods for synthesis of heteroaromatic compounds with marked luminescent properties have been elaborated since the middle of the 1980's at the Department of Organic Chemistry of the Faculty of Chemistry Technology, Rīga Technical University. Many of these compounds have been successively approved as fluorescent probes (Kalnina *et al.*, *1996*; Kalnina and Meirovics, 1999; Kalnina *et al.*, 1999; 2005). As known, pathological cellular function can be caused by or result from changes in cellular membrane structures. The molecular structure and mechanism of action of membranes pose challenging problems. Changes in the composition and molecular organisation are the principal determinants of the alterations of membrane fluidity observed in many human diseases. Recent studies of structure and function relationships in biological membranes have shown that membrane lipids play an important role in the regulation of cellular function. Many immunological functions may be largely dependent on cell membrane structure (Shinitzky, 1984; Qulinn and Chapmann, 1980; Извекова, 1991).

It is very important for clinics to obtain information on the properties of immune competent cells – peripheral blood mononuclear cells (PBMC) using an express method. The fluorescent probes proved to be an excellent, independent model for studying cell membranes (Lakowicz, 1994; Slavik, 1994). In our work we have investigated the possibility of using the fluorescent probe ABM (conditional name) for detection of ~~the immune~~ state in patients with different pathologies.

ABM: SYNTHESIS AND PROPERTIES

Synthesis. ABM (derivative of 3-aminobenzanthrone) was synthesised at the Department of Organic Chemistry, Rīga Technical University (Rīga, Latvia). Synthesis was performed by means of substituting the bromine atom in 3-bromobenzanthrone with an appropriate amine. Figure 1 gives the chemical structure of the probe ABM (conditional name).

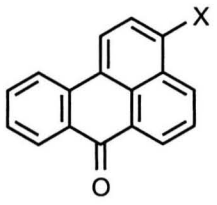

Fig. 1. Chemical structure of the probe ABM.

Spectral characteristics of compound ABM dissolved in various organic solvents. Solvents are listed in order of increasing orientation polarity defined by the dielectric constant of the solvent and refraction coefficient (Lakowicz, 1994). These results indicate that the fluorescence of this compound is sensitive to polarity in the microenvironment. The maximum wavelength of fluorescence for ABM increases in correspondence with *f*. The maximum λ_{max} change on passing from cyclohexane to methanol for a compound is 123 nm (Kalnina *et al.*, 1996; Kalnina and Meirovics 1999; Kalnina *et al.*, 2004).

Structural and functional alterations of cell membranes resulting from unfavourable conditions outside the cell or disturbed intracellular metabolism have been reported in many pathologies. Therefore, it is important that the fluorescent probe when bound to the membrane is sensitive to the above-mentioned changes. Compound ABM is sensitive to polarity deviations in the microenvironment.

Binding of ABM with liposomes. Liposomes made of phosphatidyl choline alone (egg lecithin) and those enriched with cholesterol or human PBMC, each showed different emission spectra maxima and fluorescence intensities (Kalnina *et al.*, 1996; Kalnina *et al.*, 2004).

The ABM emission spectra in both liposomes enriched with cholesterol (phosphatidyl choline: cholesterol molar ratio, 1 : 2) or PBMC exhibit a shift towards shorter wavelengths (630 nm) as compared to the ABM spectrum in phosphatidyl-choline liposomes (650 nm). The blue shift of emission spectra is associated with a decrease in fluorescence intensity. In cell suspension the ABM fluorescence intensity was inversely correlated with membrane anisotropy (Kalnina and Meirovics, 1999).

The results obtained are in agreement with previous theoretical indications that a high cholesterol level may cause a blue shift of emission spectra (Shinitzky, 1984).

Binding of ABM with albumin. Fluorescent probe ABM binds with human serum albumin. Changes of pH in the range from 3 to 12 strongly affect the fluorescence intensity and spectrum of albumin-bound ABM. The most prominent changes in fluorescence characteristics occur at pH values known to cause conformational transitions of proteins, as follows:

(1) pH 1–2. The fluorescence zone is shifted by 20 nm to the short wavelength region, as compared to the spectrum at pH 7.4; fluorescence intensity decreases. According to the literature (Грызунов и Добрецов, 1994) the acidic expansion of albumin globule takes place;

(2) pH 3.6. The fluorescence zone is shifted to the short wavelength region by 23 nm, fluorescence intensity increases. This corresponds to the so-called N–F transition;

(3) pH 7.4 (control spectrum);

(4) pH 9.0. The so called x–B transition. The fluorescence zone is shifted to the short wavelength region by 19 nm accompanied by decrease of fluorescence intensity. Significant alterations in albumin structure are not characteristic;

(5) pH 11.5. The fluorescence zone is shifted to the long wavelength region by 4 nm and fluorescence intensity decreases, accompanied with significant alteration in albumin structure.

The observed changes in relation to pH suggest that ABM can be used as a probe sensitive to conformation changes of proteins (Kalnina *et al.*, 2004).

DISTRIBUTION OF ABM IN CELLS

Fluorescence microscopy has revealed the distribution of ABM in membranes of PBMC such as plasma, mitochondrial, and nuclear, but there is no evidence of localisation of ABM inside the nucleus (Kalnina and Meirovics, 1999).

Flow cytometry experiments show a strong bimodal distribution, with high and low ABM fluorescence intensity (F), respectively. Nearly 90% of PBMC showed high F, and the others low F (Kalnina and Meirovics, 1999). This might be explained by specific differences in the properties of the membranes of T and B cells. Lymphocytes can be divided into two groups, "bright" and "dim," according to the fluorescence intensity (F) of another related probe, 3-methoxybenzanthrone (MBA). The bright lymphocytes showed correspondence to the B cell content, and the dim group to the content of T cells. The differences which distinguish T and B cells revealed by fluorescent probe are based on properties of the membranes (Korkina *et al.*, 1981). The chemical structure of the synthesised compound ABM bears a resemblance to the structure of the fluorescent probe MBA. However, despite its extensive spectrum of possible applications, the use of MBA is limited by two properties of this probe: (1) it destroys cells after a short period of time; and (2) it fades rapidly in fluorescent light (ap-

prox. 80% in 6–8 min). Our results indicate that ABM is photostable and nontoxic for cells.

The addition of Triton X-100 did not lead to any changes in ABM fluorescence intensity, while incubation of PBMC at 37 °C resulted in increased ABM F in comparison with that obtained at 20 °C. The above suggests localisation of ABM deep in the phospholipid bilayer. The emission maximum of ABM in phosphatidyl choline liposomes is at 650 nm, which corresponds closely to that of the compound in methanol (Kalnina and Meirovics, 1999). Comparison of the maxima of the fluorescence spectrum of membrane bound probe with that of the probe in different organic solvents (Waggoner and Stryer, 1970) indicated that the environment of ABM in the phosphatidyl choline bilayer is quite polar, similar to that of methanol.

ABM SPECTRAL CHARACTERISTICS AND PROPERTIES OF PBMC

Significant structural, metabolic and functional deviations of mononuclear cells play a leading role in the pathogenesis of different human pathologies (Shinitzky, 1984; Bail and Koopmen, 1986; Извекова, 1991; Hohfeld *et al.*, 1995; Skapenko *et al.*, 1999). The changes in the cell membrane influence the incorporation of fluorescent probe ABM to the cell.

The spectral parameters of ABM in cell suspension indicate several properties of PBMC: (1) physico-chemical state of membrane, (2) membrane microviscosity, (3) proliferating activity of PBMC, (4) metabolic activity of lipids, (5) phenotypical characteristics of PBMC.

Physical and chemical alterations of membrane structure may contribute to the changed membrane microviscosity. The fluorescence of ABM was found to be sensitive to fatty acid composition of the lymphoid cell membrane (Kalnina *et al.*, 1998; Klimkane *et al.*, 1998; Kalnina and Meirovics, 1999; Kalnina *et al.*, 2001; Kalnina *et al.*, 2004; Bruvere *et al.*, 2003; Klimkane *et al.*, 2003).

Changes in the fatty acid composition of human PBMC membranes during the period of blastic transformation and different pathologies have been reported (Anel *et al.*, 1990; Huber *et al.*, 1994). Studies on lymphoid cells have demonstrated an enrichment of polyunsaturated acids in the early steps of activation, which is associated with a decrease of membrane microviscosity (Huber *et al.*, 1994).

There are various pathological states in which the lipid composition and content of fatty acids in the PBMC plasma membrane are disturbed (Qulinn and Chapmen, 1980; Извекова, 1991; Huber *et al.*, 1994; Kalofoutis *et al.*, 1996; Lekaz, 1997; Robinson *et al.*, 2001). It is known that various human cancers result in changes in the membrane composition of host tissues. For example, colorectal cancer patients have an abnormal plasma membrane and erythrocyte fatty acid profiles characterised by decreased levels of most saturated, monounsaturated, and essential fatty acids, as well as their polyunsaturated metabolites (Robinson *et al.*, 2001).

In cases of active lung tuberculosis, the degree of fatty acid unsaturation decreases resulting in raised membrane microviscosity (Jack *et al.*, 1994). ABM F in cell suspension was observed to be inversely correlated with membrane microviscosity.

Changes in membrane microviscosity of cells have been shown to correlate not only with physical and chemical changes of the membranes, but also with a decline of the cell responsiveness to mitogens (functional activity) (Huber *et al.*, 1994).

Mitogenic assay plays a very important role in clinical immunology. The proliferation potential of a cellular population is determined not only by the number of proliferating cells, but also by their respective proliferation rates.

The possibility of using ABM to determine functional properties of PBMC has been examined (Kalnina *et al.*, 1998; Kalnina and Meirovics, 1999). We have measured the spectral characteristics of native and mitogene-stimulated cells taken from healthy test donors. Isolated cells were stimulated *in vitro* by mitogens, such as phytohemagglutinin (PHA-P) and concanavalin A (Con A) (mostly T cells) and poke weed mitogen (PWM, mostly T-dependent B cells). At the same time, the characteristics of the PBMC blast transformation reaction (LBTR) (DNA synthesis activity) in these cells were measured. It was ascertained that the fluorescence maximum of ABM in the stimulated cell suspension was ė 630 nm, similar to that of the intact cells.

The method is more sensitive when using PWM at a lower concentration of ABM: 9.9 μM not 19.6 μM as with PHA and Con A. The subpopulation stimulated by PWM (mostly T-dependent B cells) is more sensitive to ABM.

Both ABM and LBTR characteristics are increased in PBMC suspensions activated by T mitogens and B mitogens in comparison with control cells. The above-mentioned characteristics are higher with PHA or Con A than in the case of PWM. ABM fluorescence intensity and LBTR characteristics were observed to be correlated when cells were stimulated by the tested mitogens.

The spectral parameters of ABM in the cell suspensions depend on the mitogen used (PHA, Con A, PWM), its dose, and on the sub-population of PBMC stimulated by the mitogen. ABM is therefore prospective for the determination of the proliferative activity of PBMC (Kalnina *et al.*, 1998; Kalnina and Meirovics, 1999).

Membrane-associated events, especially lipid metabolism, play a pivotal role in the regulation of cellular function in PBMC, such as signal transduction, expression of surface markers and cellular activation, all of which are important in immune cell function (Kim *et al.*, 1999).

Changes in the plasma membrane can influence cell-associated signal transduction molecules of T cells (Kim *et al.*, 1999).

Autoimmune T cells play a key role as regulators and effectors of autoimmune diseases (e.g., multiple sclerosis, rheumatoid arthritis, cancer, etc.). They are responsible for the regulation of immune response and maintenance of immune tolerance (Bail and Koopmen, 1986; Hohfeld *et al.*, 1995; Skapenko *et al.*, 1999).

Rheumatoid arthritis is classified as a deficiency of the T-suppresors. It is considered that a decrease in T-lymphocyte number is not the only alteration of the immune system in rheumatoid arthritis, as the functional activity of these cells, and regulation in the T-suppressor subpopulation and their interaction with other regulatory cells are also affected (Bail and Koopmen, 1986; Skapenko *et al.*, 1999). It has been suggested that the pathogenesis of multiple sclerosis involves a disregulation of myelin-specific T cells (Hohfeld *et al.*, 1995).

Membrane damage is also considered to play a key role in the killing of cells and loss of many different membrane functional properties induced by ionising radiation (Parasassi *et al.*, 1991; Tartara and Virsik, 1991).

APPLICATION OF ABM IN CLINICAL DIAGNOSTICS

ABM has also been used to characterise PBMC of healthy donors, patients with several nonmalignant diseases: advanced lung tuberculosis (Kalnina and Meirovics, 1999; Kruckova *et al.*, 1996), multiple sclerosis (Kalnina *et al.*, 1999), rheumatoid arthritis (Kalnina *et al.*, 2005); malignant diseases (gastrointestinal cancer, gynaecological cancer, patients with advanced tumours, receiving palliative and supportive care) (Klimkane *et al.*, 1998; 2003), and those who were subjected to ionising radiation during the clean-up work in Chernobyl (Bruvere *et al.*, 2003; Kalnina *et al.*, 2001; 2004).

Nonmalignant diseases. The spectra and wavelength maxima of PBMC from healthy patients did not differ from those of patients with myeloleukaemia or with nonmalignant diseases. The absence of blue-shift emission of spectra indicates no significant cholesterol increase in PBMC in the observed groups of patients. These groups showed only differences in the fluorescence intensity (Bruvere *et al.*, 2003; Kalnina *et al.*, 1999; 2005).

Advanced lung tuberculosis. ABM F and stimulation of DNA synthesis in the PBMC of tuberculosis patients is lower, and membrane fluidity greater, in comparison with those indices in healthy donors. Lowered functional activity of cells can be explained by their structural differences.

Changes in membrane microviscosity of patients with advanced lung tuberculosis were found to depend on the nature and dynamics of the tuberculosis process and the predominance of the oxidative or productive inflammation phase. A considerable reduction in functional activity of PBMC was found when the tuberculosis process was disseminated in pulmonary tissue. We have revealed a significantly decreased ABM F among highly fluorescent PBMC. Moreover, the strong bimodal distribution disappeared in cells with intermediate F occurrence. Changes of spectral parameters are correlated with the severity of the disease (Kalnina and Meirovics, 1999; Kruckova *et al.*, 1996).

Multiple sclerosis (MS). In a study, the patients were divided according to the phase (exacerbation or remission) and type (remitting or chronic progressive) of the disease. Based on results of ABM fluorescence intensity in lymphocyte suspension, three groups have been distinguished in each multiple sclerosis phase: (1) with decreased F, (2) with normal F, (3) with increased F (Kalnina *et al.*, 1999).

Lymphocyte distribution among the detected subsets CD3+, CD4+, CD8+, CD16+, and HLA.DR+ showed differences in each group corresponding to a specific level of F and according to the phase and type of multiple sclerosis (Kalnina *et al.*, 1999).

We suppose that F depends on different activation of each lymphocyte subset depending on the phase of the disease. In the exacerbation phase a direct correlation ($r = +0.76$) between F and the number of CD16+ cells was found, as well as for the ratio CD3+/CD8+ ($r = +0.747$) and CD4+/CD8+ ($r = +0.745$), while an inverse correlation was found between F and the numbers of CD3+ and CD4+ cells ($r = -0.665$, $r = -0.863$, respectively) and the ratio of CD4+/CDI6+ ($r = -0.835$). In the remission phase an inverse correlation between F and the ratio of CD4+/CD8+ ($r = -0.755$) was observed.

The immune state of patients was seen to be correlated with the clinical observation of the disease, which was confirmed by magnetic resonance examination of the patients.

The obtained results are in agreement with the concept that the pathogenesis of MS involves a dysregulation of myelin-specific T cells (Hohfeld *et al.*, 1995). The CD4+ cells mainly act as antigen-specific helpers of inflammatory cells and CD8+ cells act as antigen-specific killer cells. It has been established that CD8+ suppressor cell function is defective during disease attacks in MS patients and is persistently low in patients with chronic progressive MS (Hohfeld *et al.*, 1995).

Rheumatoid arthritis (RA). In a study, patients with RA were divided in two groups according to the titre of rheumatoid factor in blood. The ABM spectral parameters, the lymphocyte count, the T helper/ T suppressor ratio (CD4+/CD8+), functional activity of cells were studied in these groups of rheumatoid arthritis patients. Patients with a seropositive RA had a decreased functional activity and a lower number of PBMC in blood plasma, indicating greater alterations of the immunoregulating processes in these patients as compared to patients with seronegative RA. The PBMC deficiency was compensated to some extent by increased

functional activity of these cells. The ABM F was correlated not only with membrane anisotropy (r = +0.97), but also with the proliferative activity of cells (r = –0.98). Among these groups a direct relationship was found between F and the ratio CD4+/CD8+ (Kalnina et al., 2005).

The studied parameters are correlated with clinical manifestation of the disease. Medical examination revealed that in patients with the seropositive RA form, the clinical manifestations were much more pronounced as compared to the seronegative group. Seropositive patients suffered intensive pain in articulations, lengthy morning constraint, and rigidity coupled with pronounced proliferative phenomena. X-ray examination revealed significant disfiguraration and deformation of articulations with pronounced destructive alterations (Kalnina *et al.*, 2005).

ONCOLOGICAL DISEASES

PBMC of gastrointestinal cancer patients (gastric cancer, stage III and colorectal cancer patients, stages II–III), gynaecological cancer patients, and patients with other different cancer as well as cancers with massive cancer metastases and intoxication have been investigated by using the fluorescent probe ABM (Klimkane *et al.*, 1999; 2003).

Gastrointestinal cancer patients. ABM spectral characteristics (fluorescence intensity) differ in gastric and colorectal groups of patients, in relation to the physical (structured) and functional properties of cell membrane. PBMC distribution among the subsets differ in correspondence to F (decreased, increased, normal). An observed inverse relationship of the relative and absolute number of PBMC to ABM F, as well as the inverse correlations between PBMC numbers and F in gastric and colorectal cancer groups suggests that F of different PBMC subpopulations is distinct and that each subset provides a specific contribution to F of the whole PBMC suspension.

We found an inverse relationship between numbers of PBMC (relative and absolute) and F, particularly between F and CD38+ cells (activated T–lymphocytes). The relative number of CD4+ cells was the lowest and the relative and absolute numbers of CD8+ cells the highest in the group with decreased F, which were expected because CD4+ helper cells stimulate and CD8+ (cytotoxic) cells inhibited the immunological response, thereby affecting the functional activity of the whole lymphocyte suspension, estimated as ABM F. The highest ratio of CD4+/CD8+ was in the group with increased F and the lowest ratio in the group with decreased F. The relative number of CD38+ cells was also increased in association with an increased F level in cancer patients.

Surgical treatment was seen to affect the main immunological parameters and the number of active PBMC was elevated. Before surgery in gastric and colorectal cancer patients groups F was correlated with the relative number of CD38+ cells (r = + 0.96, r = + 86, respectively), supporting the point of view that F depended on the level of activated cells. After surgical treatment an inverse correlation of F was found with respect to the relative count of CD16+ cells (r = –0.79) and CD8+ cells (r = –0.81).

Gynaecological cancer patients. Inverse correlations between F and the absolute and relative numbers of PBMC (r = –0.89, r = –0.94, respectively) have been found in patients with cervical cancer, and between F and absolute (r = –0.86) and relative numbers (r = –0.93) of PBMC in the group of patients with cancer of corpus uteri.

Patients receiving palliative and supportive care. Relative and absolute numbers of PBMC show direct (not inverse) correlations with F in patients with advanced tumours. Patients who lived more than 24 months had high levels of PBMC functional activity and also higher numbers of cells in comparison with patients who lived only 0–6 months.

F is correlated with the survival rate in patients with advanced tumours. Our results suggest that F of ABM in cell suspension reflects the functional activity of PBMC and could be used as an additional test to characterise immune activities.

It is possible that a high functional activity of PBMC has a role in the prolongation of survival rates in patients with disseminated cancer. We suggest that stimulation of PBMC functional activity can influence patient survival and life quality in the late stages of the disease.

RADIATION EFFECTS

In a study of Latvia's residents, who participated in the accident cleaning-up works in Chernobyl during 1986–1988, subjects were selected during May–June 1997.

Screening of the individuals showed five different patterns of fluorescence spectra. Four of the patterns had never been previously observed in healthy individuals or in patients with tuberculosis, multiple sclerosis, oncologic patients, etc., examined by us.

Screening of the ABM-labelled cell samples revealed five patterns of fluorescence spectra:

(1) the fluorescence zone shifted (compared to the spectrum for healthy donors) by 3–13 nm to the short wave region (617 ± 627 nm); (2) fluorescence zone is shifted to the long-wave region of spectrum by 19 ± 25 nm (max 649 ± 655 nm); (3) fluorescence with two maxima, at 619 ± 629 nm and 645 ± 655 nm; (4) fluorescence maximum observed in a wide region, 625 ± 655 nm; (5) fluorescence maximum at max 630 nm.

The spectral characteristics obtained in peripheral blood mononuclear cells (five patterns of spectra) are due to ABM fluorescence originating from lipid-bound probe and protein-bound probe. The five groups of the ABM-labelled

samples differed not only by the fluorescence spectra, but also by F and anisotropy index (A): F was significantly higher and A was lower in Group 2 in comparison with the others, and Group 1 differed from Group 5. The lowest F in clean-up workers was more than three times higher than that previously observed in healthy donors (Bruvere *et al.*, 2003; Kalnina *et al.*, 2004).

The obtained patterns of spectra suggest that various qualitative changes of membrane properties are evident in PBMC of Chernobyl clean-up workers, in comparison with previously examined healthy donors or patients having no professional contact with radioactivity.

Our results indicated that the probe ABM was localised in different transverse regions of cell membrane for the five groups of Chernobyl clean-up workers: from nonpolar (Group 1) to quite polar (Group 4).

A positive correlation between F and emission spectra max ($r = +0.77$) and a negative correlation between F and membrane microviscosity ($r = -0.97$) were observed.

Changes in membrane microviscosity of cells have been shown to correlate not only with physical and chemical modification of the membranes, but also with a decline of the cell responsiveness to mitogens (functional activity) with a decreased ability of leukocytes to produce interferons induced by ds RNA and with the levels of IgA, IgG, and IgM in peripheral blood.

We also found that PBMC of changed phenotype predominated in clean–up workers who suffered from seizures of unconsciousness and unspecified encephalopathies (Zvagule *et al.*, 2002).

There is a strong correlation among all studied ABM spectral parameters, immunological characteristics, and clinical and laboratory investigation results within all observed patient groups. Clinical and laboratory data were compared between the five above-mentioned groups differing in fluorescence patterns. Statistical analysis showed no significant differences among the groups with respect to smoking, alcohol consumption and occupational and medical exposure to ionising radiation. Significant differences of the incidence of several diseases in the groups were evident. The incidence of essential hypertension in the Group 1 was significantly greater than that in Group 5 [$F_{cmp}=4.7 > F_{0.95}=4.3$]. The incidence of unspecified encephalopathy in Group 2 was much greater than in the others [$F_{cmp2/1}=11.3 > F_{0.99}=7.9$], [$F_{cmp2/3}=7.9 > F_{0,99}=7.2$], [$F_{cmp2/4}=5.9 > F_{0,95}=4.2$], [$F_{cmp2/5}=14.5 > F_{0.999}=13.4$]. Also, in Group 2 there were more individuals with chronic inflammatory diseases of the upper respiratory tract (rhinitis, sinusitis, bronchitis etc.): [$F_{cmp2/1}=4.2 > F_{0.95}=4.0$], [$F_{cmp2/4}=5.9 > F_{0.95}=4.0$]. A characteristic symptom for Group 4 was seizures of unconsciousness, both in individuals with unspecified cerebrovascular diseases and in those with idiopathic epilepsy. 30% of individuals from Group 3 also had seizures of unconsciousness.

Most individuals with unspecified encephalopathies and cerebrovascular diseases were concentrated in groups with λ_{max} shifted to the red-light region (Group 2, 3 and 4); and individuals with essential hypertension, in the group with λ_{max} shifted to the short-wave region (Group 1). It is interesting that in Group 5 (the fluorescence spectra correspond to those in healthy persons!) there were few individuals with encephalopathies, and no individuals with lumbosacral plexus disorders.

Lymphocytosis was found in 72.9–100% of individuals from groups 1–4. Group 5 significantly differed from groups 1–4 in having only 52.9% individuals with lymphocytosis. Elevated IgA levels were found in Groups 2, 3 and 4 (in 21%, 30%, and 59% of individuals, respectively) and normal levels in 80–82% of persons from Group 1 and 5. The IgA and IgG levels were correlated with F [$r = +0.354$, $r = -0.340$, accordingly]. IgM levels were normal also in 82.4% of persons of Group 5, whereas only in 50–60% of individuals of the other four groups.

Most of the individuals from groups 2, 3 and 4 showed a decreased ability of peripheral blood leukocytes to produce interferons when induced by Newcastle disease virus (NDV) and phytohaemagglutinin (PHA). When induced by double stranded ribonucleic acid (dsRNA), leukocytes of the individuals from all groups were poor interferon producers, but especially those from Group 1. Most individuals from Group 1 had leukocytes which produced normal amounts of interferons when induced by NDV and PHA but had very low activity when induced by dsRNA. The ability to produce interferons induced by dsRNA was correlated with the anisotropy index A (membrane viscosity indicator) ($r = +0.78$). Pb concentration in the peripheral blood was elevated (> 40 μg%) in 80% of individuals from Group 1. These data suggest that the decreased ability of peripheral blood leukocytes to produce interferons in Chernobyl clean-up workers may be associated with structural/viscosity changes of the cell membrane caused by Pb.

The changed phenotype in Chernobyl clean-up workers correlated also with the levels of disorders of electroencephalography and frequence of several diseases (Zvagule *et al.*, 2002).

CONCLUSION

The spectral parameters of ABM reflect a wide range of interrelated (interdependent) properties of PBMC (physicochemical state and microviscosity of membrane; proliferating and lipid metabolic activity of cells; phenotypical characteristics of lymphocytes (distribution of cells among subsets). The observed changes of the studied ABM spectral parameters reflect alterations of the cellular mechanisms of immunity which is a main focus for its application as preliminary screening test in immune diagnostics. The fluorescence-based method is sensitive, less expensive and time consuming, technically simple and convenient.

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JAUNA FLUORESCENTA ZONDE ABM: IMŪNĀ STĀVOKĻA NOTEIKŠANA PACIENTIEM AR DAŽĀDĀM PATOLOĢIJĀM

Apkopoti rezultāti par Rīgas Tehniskajā universitātē sintezētās zondes ABM (3-aminobenzantrona) atvasinājuma izmantošanas iespējām cilvēku imūnā stāvokļa izvērtēšanā. Noteikts ABM spektrālais raksturojums dažādos organiskos šķīdinātājos, kā arī saistība ar liposomām, albumīnu, cilvēku perifērajām asins mononukleārām šūnām (MNŠ). Konstatēts, ka ABM fluorescence ir jutīga pret vides polaritātes izmaiņām. Atšķirības ABM spektrālos parametros konstatētas, savienojumam saistoties ar MNŠ membrānām veseliem donoriem un pacientiem ar dažādām saslimšanām (tuberkuloze, multiplā skleroze, reimatoīdais artrīts, onkoloģiskās slimības u.c.), kā arī jonizējošai radiācijai pakļautajiem Černobiļas atomelektrostacijas avārijas seku likvidētājiem. Noskaidrots, ka ABM spektrālie parametri atspoguļo plaša diapazona savstarpēji atkarīgus (saistītus) MNŠ raksturlielumus: membrānu fizikāli ķīmisko stāvokli un mikroviskozitāti; šūnu proliferatīvo un lipīdu metabolisma aktivitāti, limfoīdo subpopulāciju sadalījumu. ABM spektrālie raksturlielumi korelē ar slimību klīnisko ainu, tās fāzi un tipu, klīnisko un laboratorijas izmeklējumu rezultātiem, kā arī ar virkni imunoloģisko parametru. ABM spektrālo raksturlielumu izmaiņas atspoguļo šūnu imunitātes mehānisma pārmaiņas, to var izmantot kā imūnā stāvokļa iepriekšējas pārbaudes testu.