J Fluoresc (2010) 20:9–17
DOI 10.1007/s10895-009-0515-6

ORIGINAL PAPER

Fluorescent Probe ABM and Estimation of Immune State in Patients with Different Pathologies (Review Article)

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Received: 4 February 2009 / Accepted: 29 June 2009 / Published online: 1 August 2009

Abstract The fluorescent probe ABM (3-aminobenzanthrone derivative) one of the fluorescent probes synthesized in Riga Technical University proved to be an excellent, independent model for studying cell membranes. In our work we have investigated the possibility of using the fluorescent probe ABM for detection of immune state in patients with different pathologies. There is a strong correlation among all studied ABM spectral parameters, immunological characteristics, clinical and laboratory investigations of the all observed patients groups. The obtained results suggest that ABM spectral parameters in cell suspension reflect the alterations of the cellular mechanisms of immunity. Therefore fluorescent method could be used as preliminary screening test in immune diagnostics instead of more expensive, time consuming methods (subset detection, radioisotope method etc.) used as routine in clinics. Spectral parameters of ABM reflect a wide range of interrelated (interdependent) characteristics of cells (physico-chemical state and microviscosity of membrane, proliferating and lipid metabolic activity of cells, distribution of cells among subsets). The observed change of the studied parameters reflects 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.

Keywords Fluorescent probe · Peripheral blood mononuclear cells · Immune state · Different pathologies · Radiation effects

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Introduction

New methods for synthesis of heteroaromatic compounds with marked luminescent properties were worked out as research on them started since the middle of the 1980s at the Department of Organic Chemistry, Technical University of Riga. Many of these compounds have been successively approved as fluorescent probes [1–4]. 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 composition and molecular organization are the principal determinants of the

alterations of membrane fluidity observed in many human diseases. Many immunological functions may be heavily dependent on cell membrane structure [5, 6].

It is very important for clinics to receive information on the properties of immune competent cells—peripheral blood mononuclear cells (PBMC) by an express method. The fluorescent probe ABM (3-aminobenzanthrone derivative) one of the fluorescent probes synthesized in Riga Technical University, proved to be an excellent, independent model for studying cell membranes. In our work we have investigated the possibility of using the fluorescent probe ABM for detection of immune state in patients with different pathologies.

ABM: synthesis and properties

Synthesis

ABM (conditional name) was synthesized at the Riga Technical University, of Riga, Department of Organic Chemistry (Riga, Latvia) (Fig. 1).

Synthesis was performed by means of substituting the bromide atom in 3-bromobenzanthrone with an appropriate amine [2].

Spectral characteristics of ABM in organic solvents

Spectral characteristics of compound ABM dissolved in various organic solvents are registered. Solvents (from cyclohexane to methanol) are used in order of orientation polarity delta f increase defined by the dielectric constant of the solvent and refraction coefficient [7]. Obtained results indicate that the fluorescence of this compound is sensitive to polarity changes in the microenvironment. The maximum wavelength of fluorescence for ABM increases in correspondence with Δf. Maximum λmax change on passing from cyclohexane to methanol for a compound is 123 nm [2, 8].

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

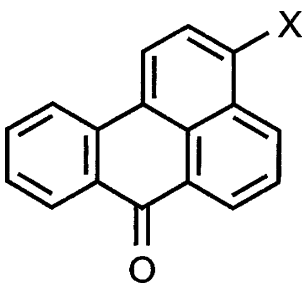

Fig. 1 Chemical structure of the probe ABM [2]

Binding of ABM with liposomes

Liposomes made of phosphatidyl choline alone (egg lecithin) and enriched with cholesterol, human PBMC, each showed different emission spectra maxima and fluorescence intensities. The ABM emission spectra in both liposomes enriched with cholesterol (phosphatidyl choline: cholesterol molar ratio, 1: 2) and PBMC exhibited 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 came along with decrease in fluorescence intensity. In the cell suspension the ABM fluorescence intensity inversely correlated with membrane anisotropy.

The results obtained are in agreement with previous theoretical indications that a high cholesterol level may cause a blue shift of emission spectra [5].

Binding of ABM with albumin

Fluorescent probe ABM binds with human serum albumin [1, 8]. Changes of pH in the range from 3 to 12 strongly affected the fluorescence intensity and spectrum of albumin-bound ABM. The most prominent changes in fluorescence characteristics occurred at pH values known to cause conformational transitions of proteins (Fig. 2) [9].

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; fluorescent intensity decreases. According to the literature [19], 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, fluorescent intensity increases. That is so-called N-F transition;
3 - pH 7.4 (control spectrum);
4 - pH 9.0 So called x-B transition. The fluorescence zone is shifted to the short wavelength region by 19 nm accompanied by fluorescent intensity decrease. Significant alterations in albumin structure are not characteristic;
5 - pH>11.5 The fluorescence zone is shifted to long wavelength region 4 nm, fluorescence intensity decreases: significant alteration in albumin structure.

These data suggest that ABM can be used as a probe sensitive to conformation changes of proteins.

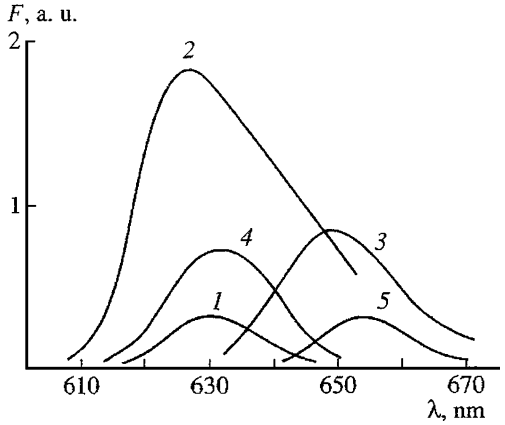

Fig. 2 Spectral characteristics of ABM in human serum albumin (exc. wavelength 485 nm) [8]. 1-pH 1–2; 2-pH 3,6; 3-pH 7,4; 4-pH 9,0; 5-pH>11,5, Albumin concentration 2 mg /ml. Concentration of probe in sample 10 μM

Distribution of ABM in cells

Fluorescent microscopy revealed the distribution of ABM in such membranes of ly as plasma, mitochondrial, and nuclear, but there were no signs of localization of ABM inside the nucleus [2, 3].

Flow cytometric experiments revealed a strong bimodal distribution, with high and low ABM fluorescence intensity (F), respectively. Nearly 90% of PBMC showed high F, and the other 10% low F (Fig. 3) [2, 3].

The results might be explained by certain differences in properties of the membranes of T and B cells. There are data that lymphocytes can be divided into two groups according to the fluorescence intensity (F) of another related probe, 3-methoxybenzanthrone (MBA), "bright" and "dim." The proportion of bright PBMC corresponded to the B cell content, but the proportion of dim corresponded to the content of T cells. The differences which distinguish T and B cells revealed by fluorescent probe are based on certain differences in properties of the membranes [10]. The chemical structure of the synthesized compound ABM bears a resemblance to the structure of the fluorescent probe MBA. But despite is extensive spectrum of possibilities, the use of MBA is limited by two properties of this probe: first, it destroys cells after a short period of time; and fades rapidly in fluorescent light (approx, 80% in 6–8 min). Our data witness 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 37C resulted in increased ABM F in comparison with data obtained at 20 C. The above facts suggest localization 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 [2]. Thus the environment of ABM in the phosphatidyl choline bilayer is quite polar, similar to that of methanol [2, 8].

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 [5, 6]. Above mentioned changes in cell membrane have an influence on the incorporation of fluorescent probe ABM. It was established that spectral parameters of ABM in cell suspension reflects 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 [2, 8, 11–17].

Physical and chemical alterations of membrane structure may contribute to the increased membrane microviscosity. The fluorescence of ABM was found to be sensitive to changes in fatty acid composition of the lymphoid cell membrane [2, 8, 11, 13].

Changes in the fatty acid composition of human PBMC membranes during the period of blastic transformation. Studies on lymphoid cells have demonstrated an enrichment of polyunsaturated acids in the early steps of activation, and that is associated decrease of membrane microviscosity [18, 19]. There are various pathological states in which the lipid composition and content of fatty

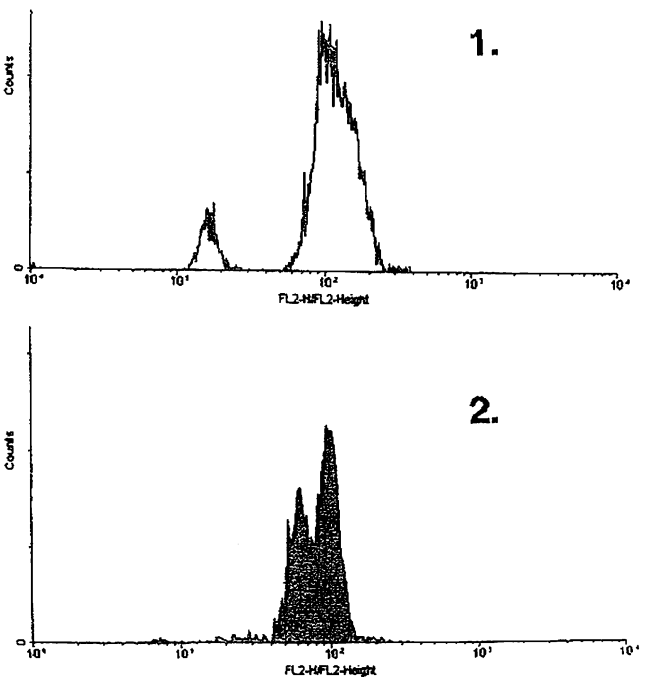

Fig. 3 ABM fluorescence in lymphocytes recorded by FACScan [2] Healthy donors (1); tuberculosis patients (2)

acids in the PBMC plasma membrane are disturbed. It is known that various human cancers result in changes in the membrane composition of host tissues. For example, colorectal cancer patients have abnormal plasma and erythrocyte fatty acid profiles characterized by decreased levels of most saturated, monounsaturated, and essential fatty acids, as well as their polyunsaturated metabolites [20, 21]. In cases of active lung tuberculosis, the degree of fatty acid unsaturation decreases resulting in raised membrane microviscosity [2, 22]. ABM F in PBMC 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) [23]. 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 was examined. We measured 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) (Fig. 4). At the same time, 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 is λ 630 nm—no different from 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 the ABM. Results testify that both ABM and LBTR characteristics are increased in PBMC suspensions activated by T mitogenes and B mitogenes in comparison with control cells. The above-mentioned characteristics are higher with PHA or Con A than in the case of PWM. It must be mentioned that the averaged values of these characteristics for the observed donor groups are different. It was established that 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 [2].

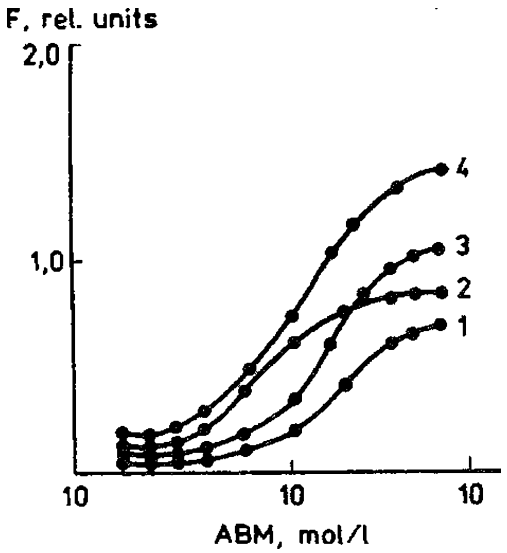

Fig. 4 ABM fluorescence intensity in activated lymphocyte suspension in dependence of its concentration [2]. Intact cells; (2) activated by PWM (0.1 mg/ ml); (3) activated by Con A (0.5 mg/ml); (4) activated by PHA (0.5 mg/ml) ABM concentration in sample: 19.6 μM (1,3,4) and 9.9 μM (2)

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 being important in immune cell function [20]. Changes in plasma membrane can influence cell associated signal transduction molecules of T-cell [24]. 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 [24–26].

Rheumatoid arthritis is classified as a deficiency of the T-suppresors. It is considered that a decrease T-lymphocytes 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 [26, 27]. It is suggested that the pathogenesis of multiple sclerosis involves a disregulation of myelin-specific T-cells [25].

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 ionizing radiation [28, 29].

Application of ABM in clinical diagnostics

ABM was also used to characterize PBMC of healthy donors, patients with several nonmalignant diseases (advanced lung tuberculosis, multiple sclerosis , and rheumatoid arthritis, malignant diseases (gastrointestinal cancer, gynaecological cancer, patients with advanced tumor, receiving palliative and supportive care), and of those who have been subjected to ionizing radiation during the clean-up work in Chernobyl.

Nonmalignant diseases

The spectra and their wavelength maxima from healthy patients did not differ from those of patients with myeloleukemia or with nonmalignant diseases. So, the absence of blue-shift emission of spectra indicates no significant cholesterol increase in PBMC of observed groups of patients. These groups showed only differences in the fluorescence intensity [12].

Advanced lung tuberculosis

ABM F and stimulation of the DNA synthesis in the PBMC of tuberculosis patients decrease, but membrane fluidity increased, in comparison with those parameters in healthy donors. Lowered functional activity of cells could bcing consequence of their structural changes.

Changes in membrane microviscosity of patients with advanced lung tuberculosis was 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 is disseminated in pulmonary tissue. We have revealed significantly decreased ABM F among highly fluorescent PBMC. Moreover, the strong bimodal distribution disappeared due to cells, with intermediate F occurrence. Results revealed testify that changes of spectral parameters are in positive correlation with the severity of the disease [2, 14].

Multiple sclerosis (MS)

The patients were divided according to the phase- exacerbation (EX) or remission (RE) and type (remitting or chronic progressive) of the disease. Based on results of ABM fluorescence intensity in PBMC suspension three groups have been distinguished in each of multiple sclerosis phase: decreased F , normal F, and increased F .

Lymphocytes distribution among the detected subsets CD3+, CD4+, CD8+, CD16+, HLA.DR+ showed differences in each group corresponding to the certain level of F and according to the phase and type of multiple sclerosis [15].

We suppose that F depends on different activation of each PBMC subset depending on the phase of disease. In the EX phase a direct correlation between F and the increased number of CD16+ cells exists (r = +0.76), 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 a diminished ratio of CD4+/CDI6+ (r = −0.835) was obtained. In the RE phase an inverse correlation between F and the decreased ratio of CD4+/CD8+ (r = −0.755) was observed.

These results changes in the immune state of patients and correlates with the clinical view of the disease which is confirmed by a 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 [24]. 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 and is persistently low in patients with chronic progressive MS. [12, 15]

Rheumatoid arthritis (RA)

Patients with RA were divided in two groups in selection 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 rheumatoid arthritis patients in relation to the titre of RF in blow. Patients with 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 In the better, the PBMC deficiency is compensated to some extent by increased functional activity of these cells. The ABM F correlated not only with membrane anisotropy (r = +0.97), but also with the proliferative activity of PBMC (r = −0.98). Among these groups a direct relationship between the F and the ratio CD4+/CD8+ was observed.

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 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 disfugaration and deformation of articulations with pronounced destructive alterations [12, 16].

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 were investigated by using the fluorescent probe ABM [11, 13, 17, 30].

Gastrointestinal cancer patients

ABM spectral characteristics (fluorescence intensity) were different in gastric and colorectal groups of patients. It is closely related with physical (structured) and functional properties of cells membrane. PBMC distribution among the subsets differed in correspondence to the level F (decreased, increased, normal). The 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 the F of the whole PBMC suspension.

We revealed an inverse relationship between numbers of PBMC (relative and absolute) and F. The most important such as correlation was between F and CD38+ cells (activated T-lymphocytes). Our observation that the relative number of CD4+ cells was the lowest, and that the relative and absolute numbers of CD8+ cells was the highest, in the group with decreased F, was expected because CD4+ helper cells stimulate and CD8+ (cytotoxic) cells inhibit the immunological response, thereby affecting the functional activity of the whole ly 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 increase along with the increasing of F level in the cancer patients.

Surgical treatment affected the main immunological parameters and the number of active PBMC was elevated. Before surgery in gastric and colorectal cancer patients groups F correlated with the relative number of CD38+ cells (r=+ 0.96, r=+ 86, respectively), supporting the view that F depends on the level of activated cells. After surgical treatment a reverse 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) were revealed in patients with cervical cancer. We also found inverse correlation in the group of patients with cancer of corpus uteri between F and absolute (r = −0.86) and relative numbers (r = −0.93) of PBMC.

Patients receiving palliative and supportive care

Relative and absolute numbers of PBMC showed direct (not inverse) correlations with F in patients with advanced tumors. 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 correlated with the survival rate in patients with advanced tumors. Results suggest that F of ABM in cells suspension reflects the functional activity of PBMC and could be used as additional test to characterize immune activities. It is possible that high functional activity of PBMC have certain roles in prolongation of survival rates of patients with disseminated cancer, but low level of F in these patients are a bad sign. It is possible, that special stimulation of PBMC functional activity can exert on patients survival and life quality in late stages of the disease.

Radiation effects

ABM spectral characteristics in PBMC suspension (Dynamical Changes at Ten Years Period-1997–2007)

The first examination of these patients was performed in 1997 (11 years after they participated in the cleaning-up of the Chernobyl accident aftereffects). Screening of individuals showed 5 patterns of ABM fluorescence spectra [8, 12]. The emission maximum of ABM in PBMC suspension for healthy donors is 630 nm.

Screening of the individuals in April 2006–June 2007 (20 years after the work in Chernobyl) showed the following two patterns of fluorescence spectra [31].

1. Fluorescence zone is shifted to the short-wave region of spectrum by 10–50 nm (max 580–640 nm). The obtained shift is more remarkable than in 1997 (3–13 nm) and characterize 83% of observed patients (n=45; in 1997 n=12, 12%). Max shift on passing from 620 nm to 580 nm is accompanied by increasing of ABM fluorescence intensity: 1,3–4,0 times higher than that observed in healthy donors ; in 1997 it was 2.1 times higher.
2. A wide fluorescence maximum (plateau) in the region of 625–650 nm was obtained only for 8 (15%) patients (in 1997 n=54, 56%). Fluorescence intensity is 2.3 times higher than that in healthy donors group.
3. The fluorescence zone is shifted to the long-wave region of the spectrum by 25 nm (n=1) (in 1997 n=14, 14.4%).
4. There are no patients with ABM fluorescence maximum at 630 nm corresponding to the fluorescence observed in healthy persons (in 1997 n=17, 18%).

Such spectral characteristics of PBMC of Chernobyl clean-up workers (the 1st and the 2nd pattern of spectra) obtained 20 years after the work in Chernobyl are due to

ABM fluorescence originating from lipid-bound ABM and from protein-bound ABM.

The results for groups tested suggest that various qualitative changes of membrane properties are evident in Chernobyl clean-up workers examined in 2006–2007 as compared with membrane characteristics joining examination in 1997. The results 1997 showed ABM fluorescence anisotropy values in Group 1 (A=0.261) and Group 2 (A= 0.245) higher than in 2007 (Group 1-A = 0.233, Group 2 A = 0.219). In dynamics the membrane lipophilic apartment fluidity increases in both groups of patients.

Authors [1, 2] showed that changes in the fluidity of irradiated membranes were primarily due to conformational and biochemical changes of membrane proteins and/or lipids. The two obtained fluorescence patterns (Groups 1, 2) have never been seen in healthy individuals or in patients with lung diseases, multiple sclerosis, rheumatoid arthritis etc., which we tested previously using ABM. The emission spectra of PBMC-ABM of the patients represented zone of fluorescence without sign of fluctuating structure. The emission spectra fluorescence intensity and their wavelength maxima of these patients did not differ from those of healthy donors. The obtained two patterns of Chernobyl clean-up workers spectra suggest that various qualitative changes of membrane properties are evident in their PBMC as compared with previously examined healthy donors or patients having no professional contact with radioactivity [1–4, 8, 12, 15–17]. Spectral characteristics of patients PBMC are due to ABM fluorescence originating from lipid-bound and from protein-bound ABM. We looked at whether the modification of the oxidative status and the resulting structural change after radiation exposure might have affected the overall biophysical properties of the membrane, most specifically, its fluidity. Lipophilic phase of the membrane appeared more fluid whereas the lipid–protein interface of the membrane was show more rigid in observed patients as compared with those in healthy donors. Patterns of spectra are associated with membrane anisotropy and conformational changes of blood plasma albumin.

Characterization of albumin in patients blood plasma

Albumin is a single source of ABM fluorescence in human blood plasma (max 650 nm). In the Group 1 the fluorescence zone is shifted to the short-wave region of spectrum by 20–50 nm. The 2 Group had a fluorescence maximum at 650 nm corresponding to the fluorescence observed in healthy donors. Fluorescence intensity in observed clean-up workers decreases (in Group 1 more remarkable as compared by healthy donors and Group 2). These observations may be consistent with the ABM decreased binding and/or conformational changes of albumin molecule, significantly differing in patients groups. In previous experiments with human serum albumin are obtained that changes of pH in the range from 3 to 12 strongly affect the fluorescence intensity and spectrum of albumin-bound ABM [8].

The most prominent changes in spectral characteristics occurred at pH values known to cause conformational transitions of proteins [13–15]. Data in Group 1 (630 nm–620 nm) was similar to that obtained at pH 1–2: the fluorescence zone is shifted to the short wavelength region as compared to the spectrum at pH 7.4; fluorescence intensity decreases. According to the literature, the acidic expansion of albumin globule takes place [9, 32]. Results in Group 1 600–615 nm) to resemble so called N-F transition albumin. The shift is accompanied by a blue shift of ABM fluorescence spectra maximum from 630 nm to 600 nm and significant increasing of fluorescence intensity. It may be ex plained by an increase of albumin binding sites capacity [9, 32, 33]. No so significant albumin structure alterations are obtained in Group 2 as compared with Group 1. Conformational changes of membrane proteins by ionizing radiation are described by Verma and Sonwalkar [34] using Raman spectroscopy. Their data demonstrate that the state of lipid-protein phase , the secondary structure of proteins and the environment of aromatic amino acids were altered in the plasma membrane.

In human serum albumin for ANS exists 2-3 binding sites differed in affinity for this probe [9, 32]. The fluorescence from these binding sites has a different quantum yield and relatively large degree of polarization (i.e., higher mobility of bound ANS, higher accessibility of water to these binding sites was obtained). On excitation the human blood serum with 286 nm (emission wavelength 330 nm) practically single source of fluorescence are tryptophanyl of albumin molecule. The shift of fluorescence zone to short wave region (318–309 nm, Group I) is evidence of hydration of tryptophanyl region of albumin molecule [9]. Obtained results may be explained by conformational changes of proteins. Results is in agreement to ANS lower degree of polarization Group 1 patients (i..e. higher mobility of bound ANS, higher accessibility of water to these binding sites as compared with Group 2. Tryptophanyl of plasma albumin are located in a conformation labile hydrophobic fold of structure which is accessible for water. This hydrophobic fold is closed due to N-F transition of the molecule. Under this process the environment of tryptophanyl becomes non-polar.

Obtained patterns of fluorescence 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 [12].

Obtained modifications may be involved in the variation of the biophysical properties of the membranes that we noted after radiation exposure. Specifically, we observed that the lipid compartment of the membrane interface became more rigid. Taken together, these findings reinforce our understanding that the cell membrane is a significant biological target of radiation.

Thus the role of the biological membrane in the expression and course of cell damage after radiation exposure must be considered.

Results showed that radiation induced of membrane domain organization is also due to compositional changes and redistribution of membrane components. All these modifications results in patterns of ABM fluorescence spectra obtained in Chernobyl clean-up workers cell suspension.

Changes (alterations) in membrane proteins and lipids are accompanied by perturbations of binding site polarity. Comparison of the maximum of the fluorescence spectrum of protein-bound and lipid-bound probe with that of the probe in different organic solvents can provide information on binding sites. The fluorescence of ABM is sensitive to polarity changes in the microenvironment. A shift of fluorescence max on passing from benzene to ethanol for an ABM is 123 nm [8]. Our results indicated that the probe ABM was localized in different transverse regions of the PBMC membrane for the groups of Chernobyl clean-up workers: Group1: emission wavelength maximum of ABM in the cell membrane is intermediate between that of ABM in benzene (583 nm) and in chloroform (625 nm). The position of emission maximum indicates that the environment of ABM in membrane is distinctly non-polar.Group 2: emission wavelength maximum indicate that the environment of ABM in the membrane covers a wide region from non-polar to polar. Taken together, the groups of clean-up workers differed between themselves in all tested parameters.

According to the obtained results (ANS anisotropy values, ABM binding with blood plasma albumin, albumin auto-fluorescence characteristics) that alterations in proteins is more remarkable in Group 1 as compared with Group 2.

Whereas on the contrary ABM fluorescence anisotropy values and spectral characteristics demonstrate more pronounced changes of lipids in Group 2 as compared with Group 1. It can be concluded that the phospholipid compartment of cell membrane of Group 2 patients is more affected in comparison with the spectra obtained in healthy donors and Group 1. In our previous experiments (using probe 3-isopropyloxy-6-morpholino-2-phenilphenalen-1-ona) was obtained that in chronic B-cell lymphoid leukemia patients the fluorescence zone is also shifted to the long-wave region of spectrum and posses distinctively lower anisotropy values than those of healthy donors and chronic myeloleukemia patients [12].

The ABM and ANS spectral characteristics, fluorescence anisotropy values and albumin auto-fluorescence data demonstrate in Group 1 the most pronounced alterations of proteins as compared with Group 2. So 10 years dynamic of PBMC membrane characteristics by ABM (spectral shift and anisotropy indexes) in Chernobyl clean-up workers reveal progressive trend toward certain resemblance with those of chronic B-cell lymphoid leukemia [12].

The principal cause of the increase of membrane damage was probably the long-live radioisotopes incorporated in the clean-up workers bodies as a permanent radiation source.

There is a strong correlation among all studied ABM spectral parameters, immunological characteristics, clinical and laboratory investigations of the all observed patients groups. The obtained results suggest that ABM spectral parameters in cell suspension reflect the alterations of the cellular mechanisms of immunity. Therefore fluorescent method could be used as preliminary screening test in immune diagnostics instead of more expensive, time consuming methods. (subset detection, radioisotope method etc.) used as routine in clinics.

Conclusion

Spectral parameters of ABM reflects a wide range of interrelated (interdependent) characteristics of PBMS (physico chemical 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 parameters reflects 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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