Blood albumin alterations and lymphocyte populations in advanced cancer patients

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Summary

Objective: Patients with cancer (namely advanced cancer) exhibit poorly functioning immune system. It is now widely accepted that dynamics of changes, along the certain types of alterations in structures of lipid/protein themselves of immune system cells and blood plasma, plays a critical role in the maintenance of the immune status of organism. Biomarkers for prediction of disease outcome are of great interest in human medicine.

Methods: The fluorescent probe ABM was used to characterize lymphocyte membranes and blood plasma albumin of cancer patients suffering from advanced cancer with wide metastasis and intoxication. The aim of these studies was to evaluate the potential applicability of measures of ABM fluorescence intensity as a standard tool in the analyses of host immune status and for a clinical interpretation of alterations in albumin per se and in lymphocyte functional activity in cancer patients receiving palliative care. We registered probe ABM spectral parameters in patients’ lymphocytes and blood plasma, and ABM auto-fluorescence in plasma.

Results: The fluorescence intensity of ABM in blood plasma and lymphocyte suspension differed from control values and showed specific differences in patient groups in accordance (correlation) with survival rate. A significant decrease in ABM fluorescence in plasma could be explained, in part, by a diminished binding capacity of the albumin of these patients. The lymphocyte distribution among the subsets of patients also differed. Interestingly, the ABM fluorescence in the cell suspension and blood plasma was also found to correlate with selected immunological parameters (CD4⁺:CD8⁺ ratios, lymphocyte counts, etc.).

Conclusion: Results strongly correlated with changes in ABM spectral characteristics and both clinical and pathological estimates of patient immune state. ABM spectroscopy appears to be useful for clinicians to monitor the course of certain diseases (e.g., gastrointestinal cancers).

Key words: Advanced cancer; Fluorescent probe; Immune status; Lymphocyte; Plasma albumin

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Introduction

Patients with cancer (namely, advanced cancer) exhibit a poorly functioning immune system that is characterized, in part, by decreases in T-lymphocyte proliferation [1, 2] and reduced CD4⁺:CD8⁺ ratios [3, 4]. In different pathologies (including these and other types of cancers), membrane damage in immune cells (and other cell types) often evolves as a consequence of alterations that are induced in cell-associated lipids and proteins in the affected patients [5, 6]. It is now widely accepted that the dynamics (i.e., actual rate of occurrence; not only incidence) of these changes, along with the certain types of alteration in structure(s) of lipids/proteins themselves of the immune system cells, play a critical role in the maintenance of the immune status of any given organism [7].

It is known that the prognosis of cancer depends on tumor aggressiveness and host immune response. Although the mechanisms that underlie specific immunological alterations are not completely understood, it is clear that both functional and quantitative defects in immunity are observed alongside with cancer, especially in its advanced stages [8]. Biomarkers for prediction of disease outcome are of great interest in human medicine. Based on the dynamics, we investigate relationship between clinic-pathological characteristics, immunological parameters (abs. lymphocytes count, CD4⁺:CD8⁺ ratio, etc.) and ABM parameters to identify potential prognostic factors affecting survival in patients with advanced cancer, receiving palliative and supporting care. This work was performed using a novel fluorescent probe ABM.
(derivative of benzanthrone) as a biomarker. Synthesis and properties of this probe are described in our previous works [9-12]. Our aim was to elaborate a new fluorescent-based method for use in immunological-based diagnostics of gastrointestinal cancer (e.g., colorectal) patients.

Materials and methods

Study subjects

The research outline used in this study had been approved by the Central Ethics Commission (Riga, Latvia) and all patients enrolled in this investigation had provided their informed consent prior to the research. Blood samples from 49 patients (33 males, 16 females; 68 years of age on the average) with advanced cancer (UICC stages II-IV), undergoing palliative resection of the colorectal (primary) tumor, were included in this study. As for the enrollment in these studies, it was a prerequisite that the cancer patients did not undergo any chemotherapy or radiation treatment(s) prior to their scheduled surgical procedures. Individuals who had been under treatment for acute infections or another autoimmune disease were excluded. To serve as a control group, 24 healthy age-matched volunteers of both sexes were involved in order to ascertain normal (i.e., baseline) levels of lymphocyte and plasma albumin fluorescence intensity, and total and sub-population lymphocyte characteristics. As noted above, the aim of this study was to characterize the immune status of these cancer patients (here; using the ABM probe) before they underwent surgical treatment, as this is often a very important prognostic indicator of their long-term survival. On the basis of the obtained results, the patients were divided into two groups in accordance with their survival rate (0-6 months and >24 months).

Blood collection

Blood was drawn at a fixed time point, usually at 8-10 AM. When blood was drawn from healthy volunteers, this was also performed at 8-10 AM. In all cases, the peripheral venous blood samples were collected into vacutainer tubes containing preservative-free heparin (30 IU per tube); a total volume of ≈7 ml was the routine target collection volume.

Preparation of peripheral blood lymphocyte suspension and determinations of subsets

Lymphocytes were separated from the freshly drawn heparinized venous blood on a Ficol-Verographine gradient, using standard methodologies [13]. After removal of some of the isolated cells for use in the ABM binding experiments (see below), tagging of the remaining cells with appropriate specific monoclonal antibodies (Becton Dickenson, Stockholm, Sweden) was performed using the company’s protocols. After this labeling, both absolute numbers and relative percentage of various lymphocytic cell types (i.e., CD4+, CD8+ and CD4+:CD8+) in the samples were evaluated using an Ortho Spectrum III flow cytometer (Ortho Diagnostics Systems Inc., Westwood, MA, USA). For each blood sample, a total of 25,000 events were ascertained to permit statistical analyses.

Effective and total albumin content in blood plasma

Effective albumin concentration (EA) is a signal of “healthy” albumin in blood plasma, measured by the fluorescent method (in this case using probe ABM). Fluorescence intensity is in proportion to the number of free, unoccupied binding sites of plasma albumin (equivalent of “healthy” albumin in blood plasma). The total albumin concentration (TA) is more conservative. The total albumin concentration was measured by using bromocresol purple [14]. The content of total albumin in the samples was calculated by the calibration curve of the dependence of fluorescence intensity on the concentration of albumin in the blood plasma sample of healthy donors. Reserve of albumin binding capacity was determined as EA/TA.

Sample preparation and fluorescence measurements

Investigations here, as in earlier studies, were performed using the ABM fluorescent probe [9-12, 15, 16], developed at Daugavpils University, Daugavpils, Latvia. In the current study, blood plasma (200-fold diluted) or cells (5 x 10^6 per assay) incubated without probe were used as each patient’s personal “blank” for each experiment. ABM (resulting concentration in sample = 19.6 µmol/l) was added to 1 ml aliquot of each patient’s blood plasma (or 1 ml solution containing cells) at the temperature of 18-20°C and the mixture was then allowed to set for ≈5 min. The time interval between cell/plasma isolation and fluorescence measurement was held constant for all samples (i.e., ±3 hr). Resulting fluorescence parameters were then registered by the Spectrofluor JY3 spectrofluorometer (ISA Jobin Yvon Instruments S.A., Longjumeau, France) at the excitation wavelength of 470 nm and an emission wavelength of 520-700 nm. To register luminescence, every sample was placed in a cuvette (1 x 10 x 40 mm³) fixed at the angle of 30° to the excitation light beam. Fluorescence intensity was
then recorded and reported in terms of arbitrary units (AU). The final intensity value for each patient’s sample was then calculated taking into account a corresponding personal “blank”; this approach thereby eliminated any potential contributions from any autofluorescing constituents in the plasma sample.

Statistical analysis
Statistical differences among groups having different spectral characteristics were determined using the Student’s t-test and Mann-Whitney’s U-test. Correlative relationships between spectral characteristics of the ABM and the measured lymphocyte parameters were determined as outlined by Duncan [17]. The correlations were determined according to Pearson Correlation Coefficients (PCC) using the following PCC values: r = 0.1-0.4, weak; r = 0.4-0.7, mid-strength; and r > 0.7, strong.

Results
ABM binding with plasma albumin
In advanced cancer patients, the emission spectra maximum (650 nm) after combination with the patients blood plasma was not altered in comparison to that seen with the plasma from healthy control volunteers (Fig.1).

The average fluorescence intensity of ABM in blood plasma of group 1 and group 2 patients was lower by 37.4% and 20.2%, respectively, as compared to that seen in healthy donors (Table 1). In group 2, average intensity is significantly higher (p < 0.05); it differs from both group 1 and control group values by 10.6% and 31.7%, respectively. Total albumin concentration in group 1 and group 2 patients is by 23.5% and 15.1% lower, respectively. Effective albumin concentration was lower by 30.4% and 16.1%, respectively. The lowest value of EA/TA in plasma of these patients reached 0.61 (group 1) and 0.66 (group 2). In the donor group, it is 0.79-0.81 (Table 1).

ABM binding with lymphocytes
In patients, the ABM emission spectra maximum (630 nm) after combination with the patients lymphocytes (as with their plasma) was not altered in comparison to that seen with the cells from the healthy control volunteers (Fig.2).

The ABM fluorescence intensity in the samples from advanced cancer patients in group 1 was not different from the average control value (0.25 vs. 0.25 fluorescence units, respectively; as shown in Table 2). In contrast, there was a significant increase in this parameter in the cells from cancer patients in group 2, by 80.8%, as compared with group 1, and by 112% comparing with healthy donors (0.25 vs. 0.52 units) (Table 2).

Lymphocyte counts and sub-populations
The levels of lymphocytes, both total and the CD4⁺ and CD8⁺ sub-populations, in the blood samples of cancer patients and healthy volunteers were also assessed in the research. The results show that among the patients in both advanced cancer groups, the relative number of lymphocytes was

Table 1. Spectral characteristics of ABM in blood plasma of advanced cancer patients (values are shown as mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Survival rate</th>
<th>F (Pl)</th>
<th>EA</th>
<th>TA</th>
<th>EA/TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-6 month</td>
<td>1.17 ± 0.14</td>
<td>39 ± 1.1</td>
<td>63.8 ± 1.02</td>
<td>0.61</td>
</tr>
<tr>
<td>2</td>
<td>&gt;24 month</td>
<td>1.42 ± 0.09</td>
<td>47 ± 1.2</td>
<td>70.8 ± 1.14</td>
<td>0.66</td>
</tr>
<tr>
<td>3</td>
<td>Healthy controls</td>
<td>1.87 ± 0.13</td>
<td>65 ± 1.3</td>
<td>83.4 ± 1.16</td>
<td>0.78</td>
</tr>
</tbody>
</table>

p < 0.05 between Groups 1-2, 1-3, 2-3

F (Pl), fluorescence intensity in blood plasma; EA, “effective” albumin concentration; TA, total albumin concentration; EA/TA, reserve of albumin binding capacity.
Table 2. ABM fluorescence intensity in lymphocytes. Peripheral blood lymphocytes subpopulation counts in advanced cancer patients (values are shown as mean ± SEM)

<table>
<thead>
<tr>
<th>Group</th>
<th>Survival rate</th>
<th>F(Ly), a.u.</th>
<th>Ly (%)</th>
<th>CD4⁺ (%)</th>
<th>CD8⁺ (%)</th>
<th>CD4⁺:CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-6 month</td>
<td>0.25 ± 0.03</td>
<td>13.31 ± 1.16</td>
<td>20.93 ± 1.13</td>
<td>27 ± 1.39</td>
<td>0.78 ± 0.09</td>
</tr>
<tr>
<td>2</td>
<td>&gt;24 month</td>
<td>0.53 ± 0.11</td>
<td>16.95 ± 1.18</td>
<td>26.14 ± 1.32</td>
<td>26.7 ± 1.31</td>
<td>0.98 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>Healthy controls</td>
<td>0.25 ± 0.03</td>
<td>28 ± 1.3</td>
<td>38.4 ± 2.1</td>
<td>19.5 ± 1.2</td>
<td>1.88 ± 0.16</td>
</tr>
</tbody>
</table>

F(Ly), fluorescence intensity in lymphocytes.

significantly lower, i.e., by 52.6% and 39.6%, respectively, as compared to corresponding control values. It is necessary to note, though, that relative number of lymphocytes in group 2 is significantly higher than in group 1 patients, but lower than the control value (see Table 2).

In patients with advanced cancer and metastases, there is a reduction both in numbers of lymphocytes and proportions of CD4⁺:CD8⁺ T-lymphocytes, which are thought to play an important role in cell-mediated immunity. Because shifts in CD4⁺:CD8⁺ ratios were often used as indices of altered host immune status, these values were also calculated from the patient blood samples. The results indicate that in group 1 and group 2 patients, the ratio CD4⁺:CD8⁺ is significantly lower by 58.5% and 47.9%, respectively, relative to the corresponding control level. The actual values of group 1 and group 2 are 0.78 and 0.98, respectively, with the control value being 1.88. It is of interest to note that in group 2 this parameter remains significantly higher as compared with the results in group 1, but does not reach the control value.

**Determination of relationship between ABM fluorescence and investigated parameters**

Correlation between ABM fluorescence intensity in lymphocyte suspension and blood plasma, and other immunological parameters for advanced cancer are determined. The ABM fluorescence intensity in blood plasma and lymphocytes was found to correlate with CD4⁺:CD8⁺ ratios in both groups of advanced cancer and the absolute number of lymphocyte subtypes in their blood. In advanced cancer groups, in contrast to other groups, there is direct correlation between a relative lymphocyte count and ABM fluorescence intensity. There seems to be a good relationship between total lymphocyte (and subpopulation) levels and ABM fluorescence in both groups of patients. There is also a good association between the plasma albumin fluorescence measurements and each of the individual lymphocyte/albumin-associated total and “effective” albumin concentration and reserve of albumin binding capacity.

**Discussion**

It is known that the prognosis of cancer depends on cancer aggressiveness and host immune response. Although the mechanisms that underlie the specific immunological alterations are not completely understood, it is clear that both functional and quantitative defects of immunity develop alongside cancer, especially in its advanced stages [8]. This work was performed using a novel fluorescent probe ABM. The probe ABM synthesis and its properties were described in our previous works [9-12, 15]. We must reproduce results of previous works devoted to examination of gastrointestinal cancer patients. The noted changes in ABM fluorescence could reflect modifications in one or more independent properties of lymphocytes: 1) outer membrane physicochemical state; 2) membrane microviscosity; 3) proliferative
activity; 4) lipid metabolism; and/or 5) phenotypical profile [11, 12]. The above mentioned changes seem to be useful in reflecting alterations within the lymphocytes of cancer patients in each sub-group before and after operation; they change as a function of cancer staging and therefore may ultimately be of use as potential indices of altered cellular immunity in these hosts [9-12].

The present study showed that spectral characteristics (fluorescence intensity) differed among the advanced cancer patient sub-groups. These findings suggest similar physical (structural) and functional alterations in the patients’ cells. Table 1 and Table 2 show statistically significant differences in ABM fluorescence intensity in cell suspension, blood plasma, and other detected parameters among observed groups of patients. The main research findings are based on analysis of overall survival of cancer patients, emphasizing the role of absolute counts of T-lymphocyte subsets.

Literature data show that cells have an important role in the functioning of immune system, but qualitative indicators of lymphocyte subsets are also important. The higher the absolute number of lymphocytes, their functional activity might be associated with the longer survival rate in patients with advanced cancer. On the other hand, cell activation is associated with membrane microviscosity and lipid metabolism [18]. It is known that ABM fluorescence intensity can change in accordance with environment polarity and, consequently, in relation to plasma membrane microviscosity that, in turn, correlates with cell lipid metabolism. There are various pathological states (i.e., cancer) in which the lipid composition and specific fatty acid content in lymphocyte membranes and blood plasma are disturbed [19]. For example, colorectal cancer patients have abnormal plasma and erythrocyte fatty acid levels, as well as of their polyunsaturated metabolites [20]. Ultimately, in lymphocytes, because membrane physicochemical status and cell lipid metabolism play pivotal roles in signal transduction pathway(s) activities important in maintaining cell function [21], it would not be unexpected that disturbances in these parameters could result in altered immunocompetence in hosts with these affected cells.

Fluorescence intensity of ABM in lymphocyte suspension is significantly higher in group 2 patients (longer survival rate) as compared with group 1 patients. Shifts in magnitude of ABM fluorescence could reflect modifications in one/more interdependent properties of cells [9]. As seen in the studies mentioned here, some cells’ properties are responsible for this phenomenon of proliferative activity and phenotypical character and physicochemical status of the membrane. In comparison with patients with stage II local cancer, the functional activity of lymphocytes, estimated as fluorescence intensity, tended to increase with progress of cancer. The study of Milasiene et al [2] also suggests that immunosuppression covers many aspects of the complex immune system, and therefore, we have many unexpected findings.

In patients with advanced cancer, in contrast to other previously observed gastrointestinal cancer groups [9-12], the relative number of lymphocytes had direct (not inverse) correlation with fluorescence intensity. Patients who lived more than 24 months had high levels of lymphocyte functional activity and also higher (p < 0.05) relative numbers of lymphocytes in comparison with patients who lived only 0-6 months (Table 2).

Although the fluorescence associated with CD4+ or CD8+ lymphocyte subtypes in the patients’ samples was not measured directly, we hoped to find out if the observed changes in ABM intensity associated with the parent lymphocyte pool might be useful to reflect current CD4+ and/or CD8+ status in the hosts. In this regard, the same as above disease-related differences in the relationship were apparent between changes in ABM fluorescence and those in CD4+ levels in the patients. It was of great surprise that even with this lack of concordance between the changes in ABM fluorescence among the parent lymphocyte populations and the CD8+ cell levels in the advanced cancer patients, the calculated CD4+CD8+ ratios for these subjects still showed a good trend relationship with the fluorescence outcomes.

The noted shifts in CD4+ levels were expected; the cancer-related CD4+ cell deficiency is a frequent finding in gastric cancer patients [4]. In our previous investigations, CD4+:CD8+ ratios tended to parallel ABM fluorescence levels, i.e., it was the lowest among the patients whose lymphocyte suspensions manifested lower fluorescence [9-12]. In those earlier studies, CD4+:CD8+ ratios gradually decreased as CD8+ levels increased with the progression of cancer stage [9, 23]. In the present study, the shifts seem to depend more on decreases in CD4+ levels as the disease becomes metastatic. These outcomes are in keeping with the studies by Tancini et al [23] and McMillan et al [24] to some extent. They indicate
that decreases in CD4+:CD8+ ratios in gastric cancer patients mainly depend on increases in CD8+ T-cytotoxic cells in patients with an early stage disease; at the same time, the CD4+:CD8+ ratios are due to decreases in CD4+ T-helper cells in patients with metastases (later stage disease). Reduction of CD4+ lymphocytes is more expressed in group 1 (survival rate 0-6 months) as compared with group 2 data and stages IIA-IIIB local colorectal cancer [8, 12]. Among colorectal patients, percentage of CD4+ cells is lower by 25.5% and 38.3% comparing to the control levels as stages progressed [12]. In advanced cancer Groups 1 and 2, this parameter is lower by 45.5% and 32%, respectively.

The reason for examining albumin is that this protein is practically the only source of ABM binding in blood plasma with a very high selectivity and subsequent fluorescence. Fluorescent method reveals the “effective” concentration of albumin (equivalent to “healthy” albumin in blood plasma). The total concentration of albumin is conservative [5, 6]. The present study revealed significant changes in ABM fluorescence associated with plasma (re: albumin) of the cancer patients. In general, serious alterations in plasma albumin levels often suggest poor outcomes in cancer patients [25]. This observation is based on the fact that presence of liver metastasis and low serum albumin levels are most powerful adverse prognostic factors. In the present study, the effective and total albumin concentrations (and the EA/TA ratio) in patients of the observed groups seem to correlate with the changes in ABM fluorescence relative to corresponding values in the healthy control group. We suggest that the albumin in the studied patients had undergone modifications that affected its ability to bind ABM. As well known, molecules of albumin display heterogeneity (polydispersity). Fatty acids and other metabolites increasingly occupy the binding sites; it results in conformational alterations of the albumin molecule (changes in binding sites counts, rigidity of molecule, especially in its tryptophanyl region, etc.). Rigidity (dehydration) of the tryptophanyl region is more expressed in group 1 as compared to the control group and group 2. The fluorescence intensity of ABM in this is halved more significant as compared to the control group and group 2. Tryptophanyl residue is located in a conformationally labile hydrophobic fold of the structure which is accessible for water. The hydrophobic fold is closed due to albumin transformation and under this process the environment of tryptophanyl becomes more rigid (dehydrated) [5, 6]. The additional ‘binding shifts’ seen in plasma samples of cancer patients could be partly due to decreased binding of albumin because of conformational changes in it. There are several ways in which tumor- and/or treatment-associated agents can bind to albumin and cause allosteric modifications that lead to structure and function changes: 1) tumor cells release a variety of bioactive proteins/peptide fragments; sequestration by carrier proteins (such as albumin) protects these materials from clearance and amplify their circulating levels [26]; 2) plasma content of select key unsaturated fatty acids (i.e., oleic and arachidonic acids) is increased; these then increasingly occupy binding sites of albumin [5, 6, 27]; and 3) an array of drugs, e.g., ibuprofen, indomethacin, etc., as well as their metabolites, commonly ingested by cancer patients readily bind to albumin [27]. As was the case with lymphocytes, the shifts in cancer patient plasma ABM fluorescence intensity were related to a disease stage [12]. While moderate alterations in albumin-ABM signals were already noted at early phases of both forms of cancer (Stage II), the effects were amplified as each cancer evolved to Stages III-IV. It is likely that as each cancer progressed, the levels of pathological/pharmacological metabolites in the patient’s blood increased and their albumin could not ultimately bind them all. One consequent structural-functional alteration induced in albumin could be a shift in ABM binding away from normal primary high affinity sites to others with lower affinities/specificities. Such shifts would be in agreement with the observations of Togashi and Ryder [28] and Rolinski et al [29] who noted that albumin molecules contained different binding sites (i.e., classes) that differed in affinity, quantum yield, and degrees of polarization, i.e., higher mobility of a bound probe and increased accessibility by water, for ABM and various other probes.

While increased binding of tumor- or/and treatment-associated agents (leading to the sequela outlined above) could be a means by which changes in albumin-ABM fluorescence evolved here, there are other means by which the albumin ability to bind the probe may have been altered. In advanced cancer patients, variations in total lymphocyte levels clearly and consistently paralleled the corresponding changes in ABM fluorescence and also in patients’ blood plasma (compare Table 1 vs. Table 2) for the advanced cancer subjects. It is likely that “polydispersity” of albumin molecule is related to physiological status of the organism.
The investigated lymphocyte count (and sub-populations) and plasma albumin level correlate with the prognosis of patients with advanced tumor. Thus, our results suggest that measures of ABM fluorescence intensity values for lymphocytes and plasma albumin could potentially be used in clinical immunological screenings instead of the routinely used tests to provide a snapshot of immune status in these cancer patients. The use of these measures could be further extended onto determination of human disease states.

Fluorescence behaviour of ABM could be useful in observing alterations in lymphocytes in each subgroup, and they may ultimately be of use as potential indicators of alterations in cellular immunity in individuals. We also sought to ascertain whether shifts in ABM binding with plasma albumin could be potentially used as part of an overall preliminary immunodiagnostic screening test in cancer patients. Taken together, all the results show that measures of ABM spectral characteristics could potentially be a useful tool to estimate the immune status of gastrointestinal patients. Compared to many commonly used diagnostic protocols, this fluorescence-based method is less expensive and not very time consuming, technically simple and 100-times more sensitive than standard absorbance-based methods.

Taken together, it appears that progression of cancer is associated with changes of immune function and, more specifically, a reduction in absolute number of CD4+ T-lymphocytes and increase or no change in the absolute count of CD8+ T-lymphocytes. The present study suggests that a higher number of absolute lymphocyte count and the value of CD4+:CD8+ ratio have a beneficial effect on overall survival of patients with advanced tumor. The latter also depends on quantitative parameters of cellular immunity of cancer patients. Thus, the status of the immune system of patients with advanced tumor before treatment is important for their survival. The immunosuppression and metastatic spread are interconnected. A low level of plasma albumin was also identified as a bad independent marker of prognosis. The fluorescent-based method is pertinent to pathway profiling, target validation, clinical diagnosis, prediction of therapeutic efficacy, and monitoring of treatment outcomes. ABM fluorescence intensity values for plasma albumin and lymphocytes (as reflection of their functional activity) might be a useful tool for studying evolution of the immune status of patients.

In our previous works probe ABM was also used to characterize peripheral mononuclear cells of healthy donors, patients with several nonmalignant diseases (advanced lung tuberculosis, multiple sclerosis, rheumatoid arthritis) [11], and those who have been subjected to ionizing radiation during the clean-up work in Chernobyl [30]. Obtained patterns of spectra suggest that specific and qualitatively different changes of membrane properties are evident in Chernobyl clean-up workers cells, similar to that of lymphoid leukemia patients. In all observed groups of patients with nonmalignant and malignant diseases and Chernobyl clean-up workers, correlation was obtained between probe fluorescence intensity, anisotropy, and the ability of cells to produce interferons when induced in vitro by Newcastle disease virus phytohemagglutinin [11]. The spectral characteristics of probe and functional activity of cells, correlate with albumin “effective” concentration and binding sites characteristics in blood plasma; with clinical and laboratory characteristics of the groups. Likewise, the observed changes in the ABM spectral parameters in blood plasma are probably coupled with alterations in cellular mechanisms of immune regulation in the organism. Results showed that measures of ABM fluorescence intensity values for plasma albumin and/or lymphocytes could potentially be a useful tool in clinical immunological screenings to estimate the immune status of patients. Compared to many other commonly-used diagnostic protocols, this fluorescence-based method is less expensive and not very time consuming, technically simple, and 100-times more sensitive than standard absorbance-based methods.

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