Fluorescent Methods in the Study of UV-Induced Changes in Structural and Functional State of Human Blood Lymphocytes

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Structural and functional state of human blood lymphocytes after exposure to UV light (240-390 nm) in doses of 151-1359 J/m^2 was studied by methods of laser flow cytofluorometry, indirect immunofluorescence, and fluorescent probes. Using a combination of these methods, we have showed that UV light in the specified doses induced changes in the surface phenotype of T cells: stimulation or suppression of the expression of antigen-recognizing receptor complex molecules (CD3, CD4, and CD8 markers) and their redistribution on the surface of immunocompetent cells (capping effect) with the formation of receptor clusters of various types.

Key Words: *fluorescent methods; lumphocytes; UV radiation; antigen-recognizing complex molecules (CD3, CD4, and CD8 markers)*

Fluorescent methods are widely used in biomedical and immunological studies for quantitative and qualitative analysis of cell membranes in native state and after modification with various physicochemical agents.

8-Anilinonaphthalene-1-sulfonate (1,8-ANS) is a universal probe sensing various structural rearrangement in membranes. ANS is a classic probe redistributing between the aqueous and membrane phases in measurable proportion. The intensity of ANS fluorescence in lymphocytes depends on their transmembrane potential [1,3].

Flow cytofluorometry and fluorescent microscopy are now actively used in laboratory diagnostics for immunocompetent cell phenotyping. For visualization of certain membrane antigens, the studied cells are treated with specific monoclonal antibodies labeled with a dye, fluorochrome (fluorophore), fluorescing upon light exposure with appropriate wavelength. To this end, FITC (492 nm/518 nm excitation/emission wavelengths), rhodamine isothiocyanate (RITC; 550 nm/585nm excitation/emission wavelengths), *etc*. are used [6,8].

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Expression of surface receptors mediating perception of external signals is the key stage of immune system functioning. Molecules of the receptor complex involved in antigen-recognizing functions of T cells (CD3-TCR, CD4 and CD8 co-receptors) are abundantly expressed on the membrane of T cells [10,11]. Thus, the absence or low expression of functionally important molecules on immunocytes in various pathologies weakens signal transmission into the cell and reduces the intensity of the immune response to foreign antigens [4].

The method of autotransfusion of UV-irradiated blood (AUVIB-therapy) is used in clinical practice for the correction of immune system disturbances. Bactericidal, immunocorrective, complex immunostimulating, and other therapeutic effects of this procedure were demonstrated [2,5,9]. However, the mechanisms of the modulating effects of UV light related to changes in the structure and functional state of human lymphocytes require further investigation; this will help to develop the methods for targeted immunocorrection of various pathological states by using AUVIB.

Here we studied the effects of UV light (240-390 nm) in doses of 151-1359 J/m^2 on structural changes in lymphocyte membrane and expression and localiza-

tion of antigen-recognizing receptor complex (CD3, CD4, and CD markers) on the membrane of human blood T cells.

MATERIALS AND METHODS

Lymphocytes were isolated from donor peripheral blood by centrifugation in Ficoll-urografin density gradient (ρ=1.077 g/cm^3). The T cell-rich suspension (prepared on columns packed with Nylon wool [7]) and T/B lymphocyte mixture were used in the experiments. The cell suspensions were irradiated with a DRT-400 quartz-mercury lamp with a UFS-1 light filter (240-390 nm transmission band) in a thermo-controlled (37°C) glass cuvette with constant stirring. The intensity of irradiation was 151 J/m^2/min at a distance of 0.23 cm from the object. The exposure durations were 1, 3, 6, and 9 min, which corresponded to irradiation doses of 151, 453, 906, and 1359 J/m^2.

UV-irradiated lymphocytes were incubated in RPMI-1640 medium supplemented with 1% glutamine, 5×10^{-5} M β-mercaptoethanol (Sigma), HEPES (Serva), and 10% ECS for 24 h at 37°C and 5% CO_2.

Lymphocyte membrane structure was studied using a fluorescent probe 1,8-ANS. The intensity of 1,8-ANS fluorescence (rel. units) in the test samples was performed on a Shimadzu RF-1501 spectrophotometer (excitation maximum at 360-361 nm and emission at 470-490 nm).

The expression of the studied markers on the membranes of native and UV-modified T cells was evaluated by flow cytofluorometry on a CyFlow space flow cytofluorometer (Partec) using FITC-labeled monoclonal antibodies LT3, LT4, and LT8 (Sorbent). IgG-FITC was used as the antiidiotypic control. The intensity of fluorescence of the analyzed cells correlates with antigen density on the cell surface. The level of the expression of the studied molecules after UV exposure was assessed by the mean fluorescence intensity (MFI; arb. units) of cells after interaction with specific antibodies labeled with the fluorophore; the results were calculated (in %) by the formula:

$$\frac{\text{MFI sample - MIF isotype - control}}{\text{MFI control}}\times100\%,$$

where MFI sample is MFI of photomodified sample, MFI isotype-control is MFI of isotypic control, and MFI control is MFI of not irradiated cells.

The distribution of CD3 complexes and CD4 and CD8 receptors on the surface of native and UV-modified T cells was assessed by the method of indirect immunofluorescence with the use of monoclonal antibodies LT3, LT4, and LT8 to CD3, CD4, and CD8 receptors, respectively. The squashed drop preparation was examined under a Mikmed-2 microscope (LOMO) in visible light (blue-violet band; OSRAM ShBO lamp) under immersion. The preparations prepared similarly, but treated with Hanks saline instead of monoclonal antibodies served as the negative control. The number of capping immunocytes in lymphocyte suspension was assessed visually by counting cells with different distribution patterns of fluorescing complexes.

The data were processed statistically using Statistica 6.0 software. Significance of differences between the control and experimental samples was evaluated using Student *t* test.

RESULTS

The study of structural state of native and UV-modified lymphocyte membranes showed that the intensity of 1,8-ANS fluorescence in membranes of intact lymphocytes was 15.0±0.9 rel. units and the spectrum of probe fluorescence was characterized by a maximum at 480±2 nm.

UV irradiation of lymphocyte suspension in a dose of 151 J/m^2 significantly reduced the intensity of 1,8-ANS fluorescence by 19% (to 12.1±1.3 rel. units) from the control (Fig. 1). Increasing the irradiation dose to 453, 906, and 1359 J/m^2 led to a decrease in fluorescence intensity by 23% (11.5±1.1 rel. units), 35% (9.7±0.7 rel. units), and 42% (8.6±0.7 rel. units), respectively in comparison with that in native cells (Fig. 1). Moreover, a short-wave shift of fluorescence maximum to 473±1 and 471±1 nm was observed after exposure to 151 and 453 J/m^2, respectively, which attested to changes in probe microenvironment from polar to hydrophobic and increased microviscosity of the lipid bilayer [3].

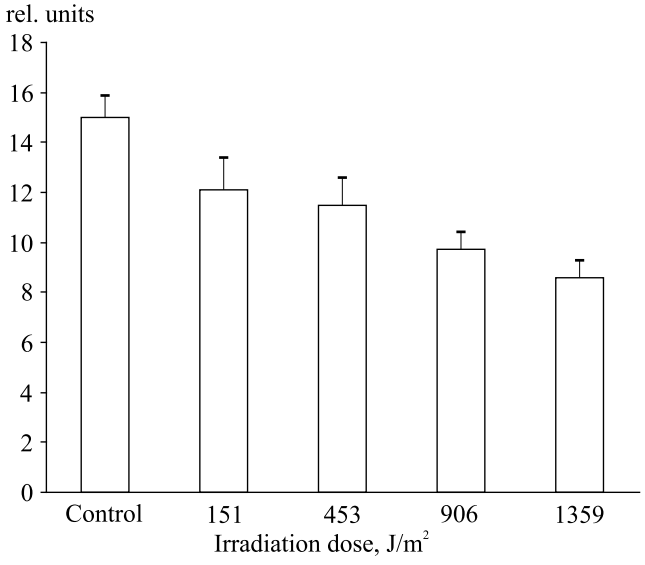

Fig. 1. Changes in 1,8-ANS fluorescence intensity in lymphocyte membranes after UV irradiation.

The data obtained using 1,8-ANS fluorescent probe attest to modification of the lymphocyte membrane structure after exposure to UV light, which, in turn, modulates the expression of surface receptors, their localization and rearrangement in the membrane, and functional activity of immunocompetent cells.

Analysis of CD3, CD4, and CD8 markers on the surface of photomodified T cells showed that MFI of $CD3^+$ cells after exposure to UV light in doses of 151, 453, and 906 J/m^2 increased by 12, 11, and 15%, respectively, while after UV exposure in a dose of 1359 J/m^2 it decreased by 10% in comparison with that in native cells (Fig. 2). UV irradiation in all doses increased MFI of $CD4^+$T lymphocytes by 20-30% and $CD8^+$ cells by 16-34%, respectively, in comparison with not modified samples (Fig. 2).

Thus, the flow cytometry data showed the dynamics of CD3, CD4, and CD8 expression on human blood T cells after exposure to UV light (151-1359 J/m^2).

Changes in the expression of the studied cell markers on T-lymphocyte surface after UV irradiation can be related to conformation changes and subsequent rearrangement of these molecules on the membrane. It is known that UV-induced structural modifications of the cell membranes can lead to antigenic drift modulating the localization of antigenic determinants and receptors on the cell surface [2]. These rearrangements lead to unmasking of latent receptors and their capping resulting in the formation of receptor clusters.

For detection of capping on the T-cell membrane after UV irradiation (151-1359 J/m^2), immunofluorescent analysis was performed. The types of distribution of fluorescing complexes are presented in Figure 3.

Evaluation of the distribution of CD3 complexes on the surface of native human blood T cells showed that 56% lymphocytes were characterized by uniform (peripheral) fluorescence, while 44% cells was in a state of capping (32 and 12% of cap $^1/_2$ and cap $^1/_4$ cells respectively). UV exposure in a dose of 151 J/m^2 did not increase the count of capping cells, but led to the appearance of lymphocytes with ameboid type of fluorescence (4%). After exposure to UV light in doses of 453, 906, and 1359 J/m^2, the relative content of capping cells significantly increased to 62, 64, and 64%, respectively. UV exposure in a dose of 1359 J/m^2 increased the count of cap $^1/_4$ cells to 26% in comparison

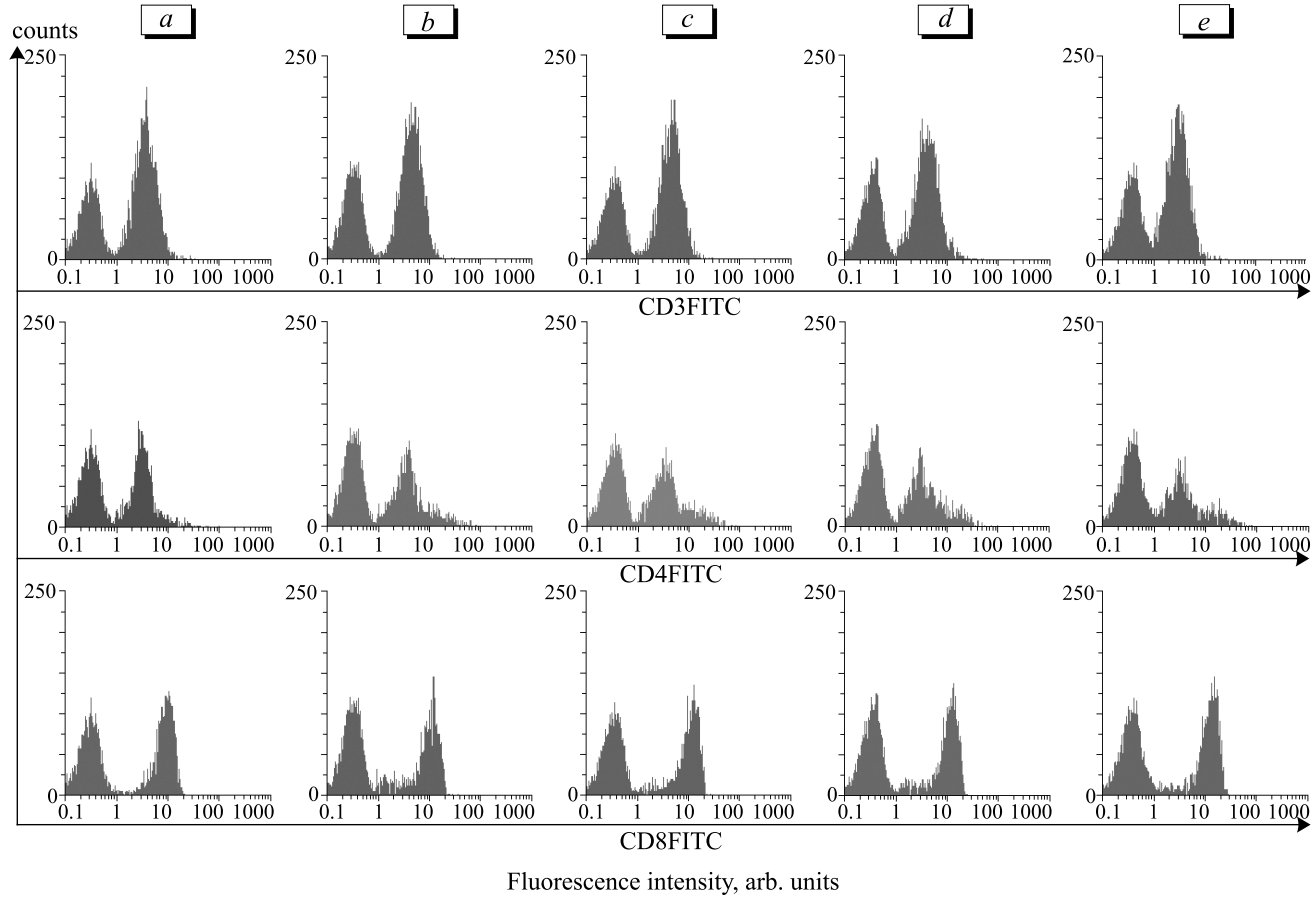

Fig. 2. Changes in the expression of CD3, CD4, and CD8 markers on the surface of UV-modified human lymphocytes (histograms; left: isotype-control, right: specific staining, *i.e.* cells binding considerable amount of FITC-labeled antibodies to CD3/CD4/CD8 markers). *a*: native cells; *b, c, d, e*: lymphocytes exposed to UV light in doses of 151, 453, 906, and 1359 J/m^2, respectively.

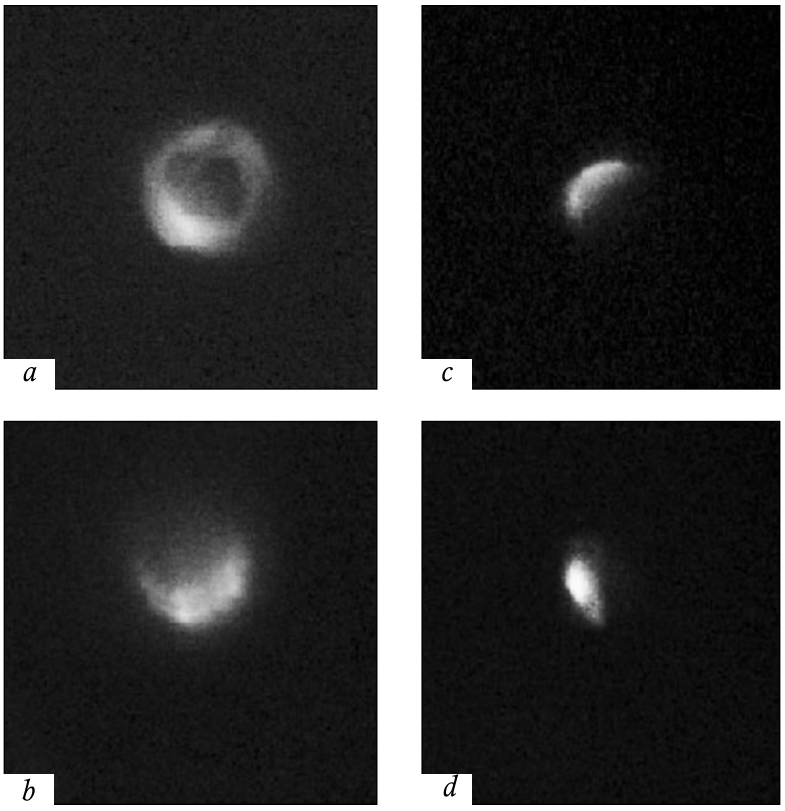

Fig. 3. Distribution patterns of fluorescing complexes (CD3 markers with FITC-labeled ligands) on the surface of native and UV-modified lymphocytes: *a*) peripheral; *b*) cap $^1/_2$; *c*) cap $^1/_4$; *d*) ameboid cap.

with native lymphocyte suspension. UV irradiation of $CD3^+$-cells in all studied doses led to the appearance of cells with ameboid pattern of fluorescence (2-4%).

Analysis of localization of CD4 markers on the surface of native T cells revealed peripheral fluorescence in 55% and capping in 45% cells (cap $^1/_2$). Cap $^1/_4$ and ameboid pattern of fluorescence of CD4 antigens was absent in native cells. UV exposure in a dose of 151 J/m^2 did not increase the count of capping cells, but led to the appearance of cap ¼ cells (12%). After UV irradiation in doses of 453, 906, and 1359 J/m^2, the count of capping cells increased by 63, 68, and 65%, respectively; it should be noted that the content of cap $^1/_2$ cells remained practically unchanged, but cap $^1/_4$ cells appeared. No cells with ameboid pattern of fluorescence were seen among UV-irradiated $CD4^+$ cells.

Peripheral fluorescence was noted in 49% $CD8^+$ cells, while 51% $CD8^+$ cells were in the state of capping (30 and 21% of cap $^1/_2$ and cap $^1/_4$ cells, respectively). Ameboid pattern of CD8 antigen fluorescence was absent in native cells. UV exposure in a dose of 151 J/m^2 did not increase the count of capping $CD8^+$ T cells, but led to the appearance of lymphocytes with ameboid type of fluorescence (5%). UV irradiation in doses of 453 and 906 J/m^2 increased the count of capping cells to 58 and 65%, respectively, and cells with ameboid fluorescence pattern appeared (4 and 6%, respectively). UV exposure in a dose of 906 J/m^2 increased the count of cap $^1/_4$ cells to 33% in comparison with native lymphocyte suspension. No significant differences in the distribution of fluorescing complexes in UV-modified $CD8^+$ lymphocytes (1359 J/m^2) and native cells were found.

Thus, immunofluorescence assay showed that UV light (151-1359 J/m^2) induced redistribution of CD3, CD4, and CD8 receptors on the surface of T cells and structural photomodification of their membranes manifesting in capping and formation of various types of clusters (cap $^1/_2$, cap $^1/_4$, ameboid cap), which suggested that immunocompetent cells can regulate the function of the antigen-recognizing receptor apparatus upon exposure to exogenous factors.

The combination of fluorescent probes, flow cytofluorometry, and indirect immunofluorescence showed that UV light (240-390 nm) in doses of 151-1359 J/m^2 modified the structure of lymphocyte membranes inducing changes in the surface phenotype of T cells, stimulation or suppression of the expression of antigen-recognizing receptor complex molecules (CD3, CD4, and CD8 markers), and their redistribution on the surface of immunpcompetent cells (capping).

The results of the study of UV-induced changes in structural and functional state of immunocytes are important for understanding of precise mechanisms of self-regulation and functioning of the immune system components under normal and pathological conditions. Our experimental findings can be useful for the devel-

opment of new approaches for immunocorerction of certain elements of the immune response in various pathological states using AUVIB-therapy.

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