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THE MICROFLUORIMETRIC AND FLUORESCENCE MICROSCOPIC ESTIMATION OF THE ANTIGENICITY OF CELL MEMBRANES IN TWO HUMAN LYMPHOBLASTOID CELL LINES

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Abstract

By the microfluorimetry an attempt was made to estimate the results of staining with FITC-labelled antibody, according to the fluorescence strength emitted from isolated cells stained with indirect immunofluorescent method. Two human lymphoblastoid cell lines established from peripheral blood of different donors were used, and it could be known that the antigenicity of the cell membrane of both cells is somewhat different, owing partly to the alloantigen and partly to some other factors, e.g., the modification of cell membrane with viral infection, and that the usage of microscopic observation of samples under fluorescence microscope and microfluorimetry at the same time is very useful to estimate the results objectively.

INTRODUCTION

Recently, the development of microfluorimetric instruments have made it possible to measure a faint fluorescence emitted from single cells, and such instruments have been used for the studies with immunofluorescent method[1,2].

In the present study we have tried to distinguish the fluorescence strength of two groups of cells, stained with indirect membrane immunofluorescent method using the antiserum against to one cell line MM-1. With fluorescence microscopy the different antigenicity of cells was clarified, i.e., only MM-1 cells were stained with antiserum against

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MM-1 cells, and the usage of microfluorimetry at the same time was very helpful to understand the data and to draw the conclusion about them.

MATERIALS AND METHODS

Cells: Two human lymphoblastoid cell lines were used, i.e. one cell line LO-1 (lymphoid cell type) established from the peripheral blood of a male patient with thymoma combined with myasthenia gravis, and the other cell line MM-1 (reticulum cell type)[3], which was established from the peripheral blood of a patient 33-year old male with myelomonocytic leukemia. The both cell lines were maintained with the medium RPMI 1640 supplemented with 30% fetal calf serum in Petri dishes at 37°C. The concentration of CO_2 in the air was kept at 5%.

Antiserum to MM-1: 2.5×10^9 cells of cell line MM-1 were emulsified with complete Freund's adjuvant and injected to rabbit subcutaneously. The immunization was repeated twice in a week. Thirty days after the 10th injection the booster was performed with 2.5×10^9 cells. The whole blood was collected 7 days after the 11th injection. The anti-MM-1 rabbit serum was stored at -70°C. Before the staining with immunofluorescent method, 0.5 ml of the serum was absorbed twice with 1×10^9 of LO-1 cells for 15 min at 37°C. After the centrifugation the serum was filtered with the millipore filter (pore size; 0.45μ), and prepared for the use.

Immunofluorescent studies: Cells of both cell lines were washed three times and suspended at the concentration of 3×10^6 cell/ml in PBS (pH 7.2). For the indirect membrane immunofluorescent test, 0.1 ml of cell suspension was treated with 0.1 ml of anti-MM-1 serum (diluted 1:2) for 30 min at 37°C. After washing twice in PBS the cells were resuspended in 0.1 ml of PBS and incubated with the fluorescein isothiocyanate-conjugated anti-rabbit IgG (F/P: 1.8) for 30 min at 37°C. The cells were washed twice in PBS, and mounted with buffered glycerol. FITC labelled antiserum was absorbed twice with aceton powder of hamster liver before use. The specimens were studied under a Nikon fluorescence microscope (Type PFM) with UV illumination.

Microfluorimetry: The microfluorimetry was performed using an Olympus single beam microfluorimeter (Type MMSP-FS). The fluorescent magnified image was formed on the plane of the image diaphragma and the light flux was measured by the photomultiplier, using the conditions of pinhole; 10, diaphragm; 10, sensitivity; 10^3, with the high voltage of

700V. The diameter of the exciting spot on the specimen was 20μ. Details about the instrument were described by Ikeda elsewhere[4].

RESULTS

Figs. 1 and 2 demonstrate the characteristic pattern of fluorescence observed in the MM-1 cells stained with indirect membrane immunofluorescent method. MM-1 cells demonstrated areas of intense fluorescence, sometimes linear and most often cap formed. On the contrary, the cells of LO-1 cell line showed no obvious fluorescence after the similar staining with indirect membrane immunofluorescent method. As shown in Figs. 1 and 2 the cap formation of fluorescent cell membrane of MM-1 cells after the treatment of anti-MM-1 cell antiserum was observed. From this phenomenon of cap formation we know that the membrane fluorescence after the staining is specific reaction between the cell membrane and the antiserum to cell components. This phenomenon was not observed in the control group LO-1 cells. It means that the antibodies directed to the components of MM-1 cells do not react with those of LO-1 cells, after the absorption with LO-1 cells, and that there can be observed the differences in the antigenicity of cell components between MM-1 cells and LO-1 cells. Sometimes cells of both cell lines showed autofluorescence with orange colour derived from the lysosomal granules. From these results we can tell that the cell membrane of MM-1 cells can be specifically stained with the indiret membrane immunofluorescent method using the rabbit antiserum directed to MM-1 cells, and that the cells of MM-1 and LO-1 cell lines possess somewhat different antigenic determinant on the cell membranes. By the observations under fluorescence microscope, we have tried to measure the strength of the fluorescence with the microfluorimeter, for the purpose of estimating the observations above more objectively. Fig. 3 illustrates the results of the microfluorimetry using the cell lines MM-1 and LO-1 stained and unstained with the indirect membrane immunofluorescent method. Unstained cell group in both cell lines show less fluorescence strength, which represents the autofluorescence and derived from lysosomal substance. The fluorescence strength of the MM-1 cells stained with the indirect membrane immunofluorescent method using the anti-MM-1 serum presented so high values. On the contrary, that of LO-1 cells is as low as those of control groups unstained. From the fluorescence strength of cells in each group we can tell that MM-1 cells

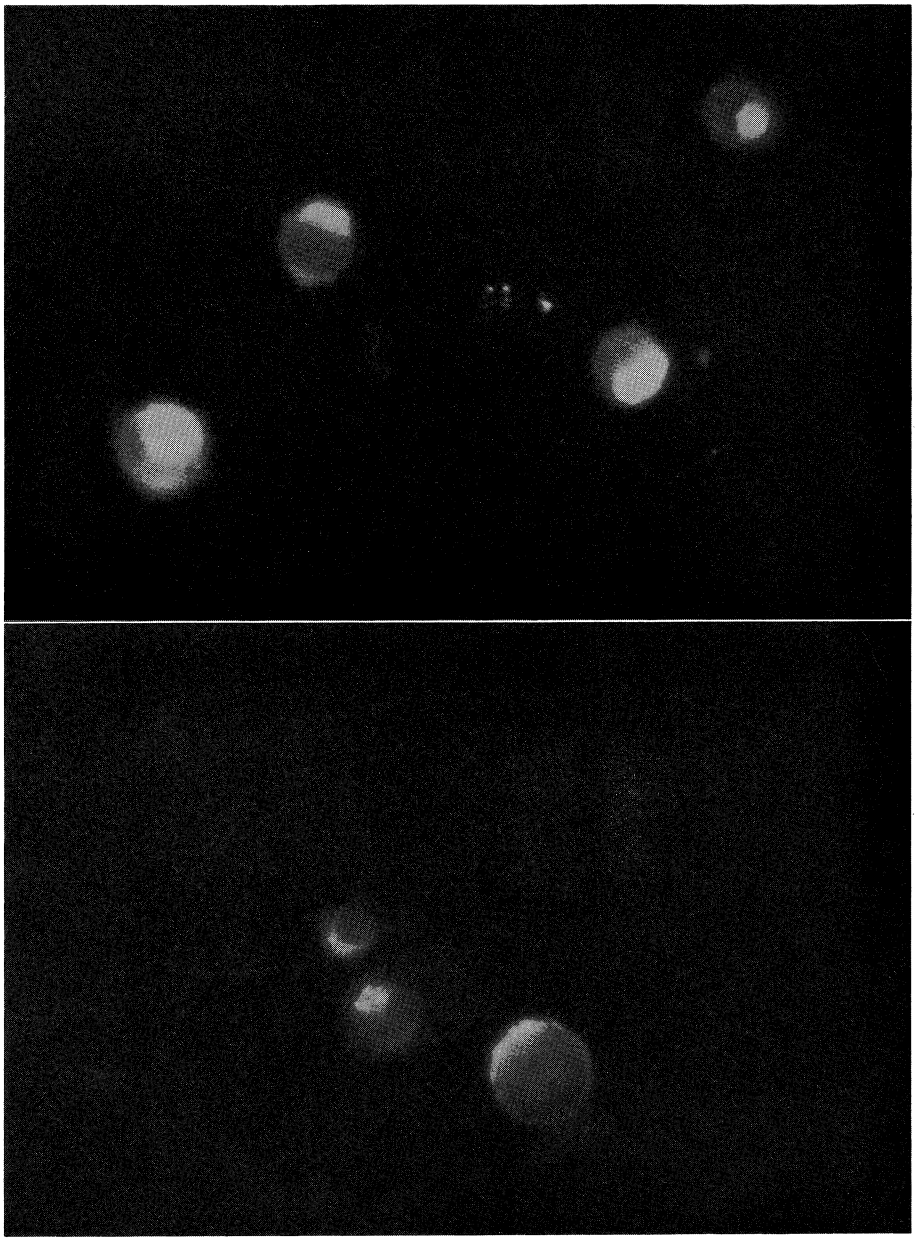

Fig. 1. Cap formation of the cell membrane coupled with the antibody; one hour after the staining of indirect membrane immunofluorescent antibody method, using anti-MM-1 serum. MM-1 cell line. ×1000

Fig. 2. The mode of fluorescence of the cell membrane was sometimes linear, one hour after the staining. MM-1 cell line. ×10000

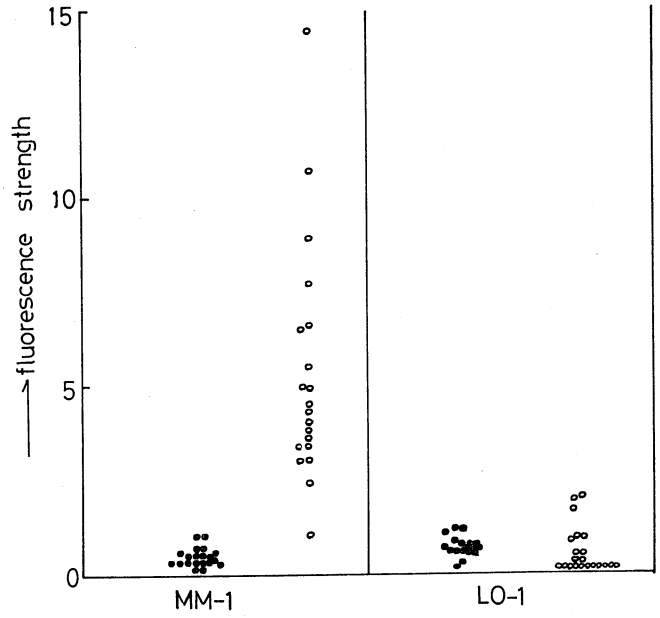

Fig. 3. The fluorescence strength of cells stained with indirect membrane immunofluorescent antibody method, using anti-MM-1 serum. The antiserum was absorbed twice with the cell suspension of LO-1 cells before use.
• control, unstained
∘ stained with indirect immunofluorescent antibody method

have been stained specifically with indirect membrane immunofluorescent method using anti-MM-1 cells.

According to the observations from fluorescence microscopy and microfluorimetry we conclude that anti-MM-1 rabbit antiserum can demonstrate the membrane antigenicity to differ from that on the LO-1 cell membranes.

DISCUSSION

When we stain single cell membrane with membrane immunofluorescent technique, the estimation of the results sometimes needs the personal scientific experience of high level, e. g., autofluorescence must be distinguished from the specific fluorescence derived from fluorescent substance conjugated with antibody or antigen. In such a case the simultaneous use of two or three techniques might be useful to estimate the results objectively.

In this study we have tried to utilize the microfluorimetry as well

as the immunofluorescence microscopic observations, for the purpose of estimating the results obtained from the membrane immunofluorescent staining method. As mentioned above, it is known that the microfluorimetry is a helpful method to estimate the results obtained from immunofluorescent techniques. Since our material was suspended cells, it was very convenient to neglect the background or other complicated factors.

It is known that the antigenicity of the cell membrane of both cell lines used here is somewhat different. Some speculation could be made, e. g., the difference of the antigenicity is due to the alloantigen as HL-A, or on the contrary, due to the modification of the cell membrane with viral infection and transformation.[5,6]

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