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A new approach to studies of cell-antibody interactions using fluorescent lipid probes

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The interaction of poly- and monoclonal antibodies against the L-chain of human Ig with Burkitt lymphoma EB-3 cells was studied using a fluorescent lipid probe, anthrylvinyl-labelled sphingomyelin, incorporated into the cell plasma membrane. Binding of the antibodies to Ig receptors on the surface was shown to induce changes in the fluorescence polarization of the probe. The high sensitivity of the method allows one to detect less than 100 antibody molecules per cell. The possibility of using cells or liposomes carrying antigens and fluorescent lipids for the determination of antibodies in solution is discussed.

Lipid fluorescence; Fluorescent probe; Immunoglobulin receptor; (Burkitt lymphoma cell)

1. INTRODUCTION

In a previous paper [1] we proposed a new approach for ligand-receptor interaction studies. The method is based on the following considerations. When a ligand binds to a membrane receptor, this is accompanied by conformational transformation of the receptor protein which in turn must be sensed by a large number of lipid molecules in the receptor's environment, i.e. changes are induced in their packing characteristics. Such changes can be followed by incorporating a fluorescent lipid probe (anthrylvinyl-labelled sphingomyelin, ASM) into the target membrane and measuring the difference in fluorescence anisotropy or fluorescence polarization before and after addition of the ligand. Conditions under which the ligand-induced fluorescence anisotropy changes are proportional to the number of occupied receptor sites were formulated [2]. Using as an example the binding of the B-subunit of the plant toxin ricin to Burkitt lymphoma cells the fluorescence method was demonstrated to have the advantages of higher sensitivity and specificity in comparison with radioligand measurements [2].

Here, we examine the possibility of using the same fluorescence method for the detection of antibodies in solution by studying the influence of poly- and monoclonal antibodies against the light chains of human Ig on the fluorescence polarization of Burkitt lymphoma EB-3 cells prelabelled with ASM.

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Abbreviations: ASM, anthrylvinyl-labelled sphingomyelin, *N*-(12-(9-anthryl)-11-*trans*-dodecenoyl)sphingosyl-1-phosphocholine; Ig, immunoglobin; *P*, fluorescence polarization

2. MATERIALS AND METHODS

2.1. *Fluorescence labelling and fluorescence polarization*

The synthesis of ASM was described in [3]. Burkitt lymphoma EB-3 cells were routinely cultivated in RPMI medium, supplemented with 10% heat-inactivated fetal calf serum, 2×10^{-3} M glutamine, 50 IU/ml penicillin and 50 μg/ml streptomycin. Cells were grown at 37°C in a 5% CO_2 incubator.

Prior to fluorescence labelling, cells were washed 3 times with PBS. The cells were resuspended in the same solution to a concentration of 10^7 cells/ml. An ethanolic solution of ASM was

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added with stirring to a probe/phospholipid molar ratio of 1:100. Probe incorporation into cells was controlled by measuring the enhancement of fluorescence intensity. Fluorescence spectra were recorded with a Hitachi 650-60 spectrofluorimeter with thermostatted cells and quartz cuvettes (5 × 5 mm); slit width was 2 nm for excitation and 10 nm for emission. Fluorescence polarization was calculated by the processor of the spectrofluorimeter (cf. [4]).

2.2. *Antibodies*

Polyclonal antibodies were obtained by immunizing rabbits with human Ig light chains and were isolated from serum by affinity chromatography as in [5]. Normal rabbit Ig was isolated from nonimmune serum by Na_2SO_4 precipitation with subsequent chromatography on DEAE-cellulose. Hybridomas, producing monoclonal antibodies to light chains of human Ig, were obtained in the Laboratory of Immunology of the USSR Cardiology Research Center. Antibodies were isolated from ascites fluid by Na_2SO_4 precipitation and subsequent DEAE-cellulose chromatography [6]. Antibodies were analysed by SDS-PAGE and isoelectrofocusing with LKB 2117 Multiphor.

2.3. *Binding of ^{125}I-antibodies to cells*

Labelling of antibodies was performed according to Bale et al. [7]. ^{125}I-labelled proteins had a specific activity of 0.3–0.6 × 10^6 cpm/mg. Binding of ^{125}I-labelled antibodies was studied as in [6] with minor modification. Briefly, cells were washed with Hanks' solution (Flow Labs) containing 1 mg bovine serum albumin (Calbiochem) per ml. The cells were resuspended in this solution to 10^7 cells/ml and various amounts of ^{125}I-labelled antibodies were added. After 1 h incubation at 4°C the cells were washed and the amount of bound ^{125}I-antibodies was determined. As shown in [6], the method employed measures the specific binding of our antibodies to EB-3 cells.

3. RESULTS AND DISCUSSION

The binding to lymphoma cells of ^{125}I-labelled antibodies against the L-chain of human Ig was studied in detail previously [6]. The results of monoclonal antibody binding obtained here are presented as a Scatchard plot in fig.1. The calculated apparent association constant amounted to 1.2 × 10^8 M^{-1}, while the number of binding sites per one lymphoma cell was equal to 5.4 × 10^5. For polyclonal antibodies, the association constant was 1.5-fold lower and only about 10% of the antibodies interacted with L-chains of Ig on the lymphoma cell surface.

When poly- or monoclonal antibodies were added to ASM-labelled lymphoma cells, the fluorescence polarization (P) increased, indicating reduction in the mobility of the probe (fig.2). The changes in P reached their maximal level within 5–7 min and seemed to be induced by specific interaction of the antibodies with the surface antigens because addition to the cell suspension of non-immune Ig from rabbit serum did not lead to measurable changes in P. The dose-response curves for poly- and monoclonal antibodies were similar (fig.2). In both cases, the antibody concentration causing maximal changes in P was 10^{-9} M. In order to evaluate these results we assumed that: (i) interaction of antibodies with Ig follows a bimolecular law; (ii) one antibody molecule binds to one receptor molecule; (iii) the changes in fluorescence polarization induced by antibody binding to Ig receptors are proportional to the number of binding sites occupied.

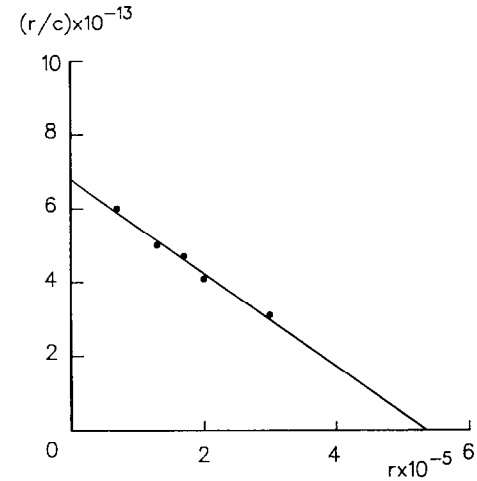

Fig.1. Binding of ^{125}I-labelled monoclonal antibodies against human Ig L-chain to Burkitt lymphoma EB-3 cells. Results are presented as a Scatchard plot: r, bound antibody molecules per cell; c, free antibody molecules, M.

With these conditions accepted, the changes in P can be used for determination of the number of bound antibody molecules causing maximal changes in P (see [2]). Since the saturation conditions for poly- and monoclonal antibodies are similar, the number of occupied Ig receptors must be almost equal. This amounts to 4.5 × 10^4 molecules/cell which is one order less than the value obtained from radioligand determinations. Apparently, occupation of a minor part of the antibody-binding sites on the cell surface prevents further increase in P. At least two explanations may be offered. The first is based on the assumption that the surface receptors are a heterogeneous population of proteins differing in either accessibility for or affinity to antibodies and that the

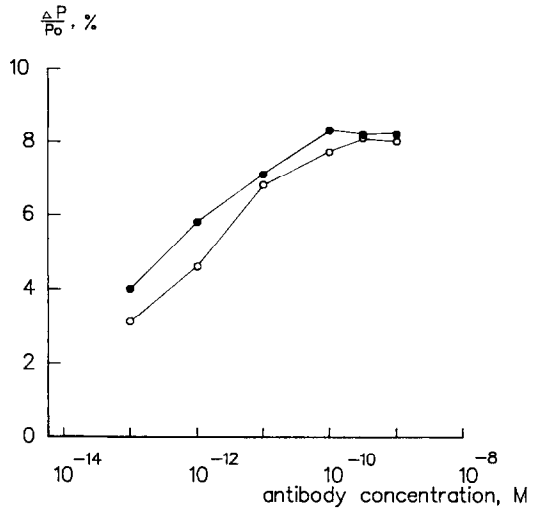

Fig.2. Dependence of the change in fluorescence polarization of Burkitt lymphoma cells labelled with the fluorescent analog of sphingomyelin ASM on antibody concentration. $\Delta P = P - P_0$, where P and P_0 are respective values of the fluorescence polarization after and before addition of the antibodies. (●) Monoclonal antibodies, (○) polyclonal antibodies.

fluorescence method detects only one receptor subpopulation (cf. [2]). Alternatively, the surface Ig molecules may comprise a homogeneous receptor population, but antibody binding to a minor part of the Ig molecules increases the rigidity of the lipid environment of the remaining Ig molecules to such extent that occupation of additional receptor sites would not have any effect on the fluidity of the surface domains sampled by ASM.

Although it is difficult at the moment to compare the results of radioligand and fluorescence anisotropy measurements, the fluorescence method offers a number of advantages. A significant feature of the fluorescent lipid probe method is its high sensitivity. Here, reliable changes in P were observed even at an antibody concentration of 10^{-13} M, which corresponds to approx. 100 antibody molecules or less per cell.

Thus, the present results open up the promising possibility of using liposomes or cells carrying a certain antigen and a fluorescent lipid probe for detection of antibodies in solution. The sensitivity of the method which uses unmodified antigens, the simplicity and speed of the procedure requiring just a few minutes for one determination could make it an attractive tool in antibody-antigen interaction studies.

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