A Modified Immunofluorescence Technique for Detection of Surface Ig[1] (37561)

VITHAL K. GHANTA, JERRY R. MCGHEE, AND RAYMOND N. HIRAMOTO

The Department of Microbiology, University of Alabama Medical Center, Birmingham, Alabama 35294

It is conceivable that plasma cells secreting immmunoglobulins can have the same immunoglobulin on their cell surface which serves as receptor. Undoubtedly the surface receptor density is influenced by the cell cycle or stage of development (1, 2). The direct staining of receptors of plasma cells has been relatively unsuccessful. New and sensitive procedures have been developed for the detection of the surface receptors of lymphoid cells, indicating the presence of Ig receptors on the surface of such cells by immunofluorescence (3–5), and immunocyto-adherence of sensitized erythrocytes (6), or with radiolabeled anti-immunoglobulin antibodies (7). Recently, Pernis *et al.* (8) showed the presence of IgM receptors on the plasma cells of bone marrow of a case of Waldenstrom's macroglobulinemia. However, Paraskevas *et al.* (9) and Pernis *et al.* did not demonstrate the surface immunoglobulins in murine myeloma cells of the IgG class.

In this paper we report the experiments of staining of surface immunoglobulins of the plasma cell lines of the ascites tumors. We have utilized MOPC 104E which secretes an IgM antibody to bacterial dextran B-1355, MOPC 315 which secretes an IgA antibody to DNP, Adj Pc5 which secretes an IgG_2 and Ehrlich carcinoma. We also present the immunofluorescence evidence for the presence of IgG_2-like globulins on the surface of tumor cells of MOPC 104E.

Materials and Methods. Preparation of tumor cell suspension. Ascites tumors were collected from mice bearing MOPC 104E, MOPC 315, Adj Pc5, and Ehrlich carcinoma, centrifuged and the supernatant removed. The cells were washed four times with saline and kept at 0°.

Preparation of monospecific antisera. Preparation of mouse IgM. Purification of mouse IgM was accomplished since the IgM reacts with bacterial dextran B-1355. Sepharose 2B was washed with distilled water to remove sodium azide preservative and then with 0.1 *M* $NaHCO_3$, pH 11.0 and placed in an ice bath. CNBr (2.5 g) was dissolved in 1.0 ml of *N,N*-dimethylformamide and added to the cold gel slurry (15.0 ml packed volume) with stirring. The pH was maintained about 11.0 with 0.5 *N* NaOH for 1 hr. After the reaction, the gel was washed with cold distilled water and then with cold 0.1 *M* borate–saline buffer, pH 8.6 and reacted with BSA (600 mg/50 ml gel) in the same buffer for 2 days at 4° with minimal stirring (10). Prior to conjugation with dextran the Sepharose 2B-BSA was poured into a column and nonconjugated BSA was removed. A dextran, fraction S from *Leuconostoc mesenteroides* NRRL B-1355 was oxidized with $NaIO_4$ in 0.01 *M* acetate buffer at pH 6.0 (11). The oxidized dextran 50 mg (10 mg/ml) was allowed to react with 20 ml packed volume Sepharose 2B-BSA in borate–saline buffer, pH 8.6 for 4 hr at 37° and reduced with 2–5 mg of $NaBH_4$ for 15-30 min. The gel was washed with the same buffer and used as an immunoadsorbent column for isolation of tumor IgM from MOPC 104E ascites fluid. Fifty ml of MOPC 104E ascites fluid was passed through a 50-ml Sepharose 2B-BSA-dextran column and washed free of any unbound protein with borate–saline buffer. The IgM was eluted

[1] This investigation was supported by Grant GB 13804 from the National Science Foundation and the American Cancer In-66J Grant.

with 0.1 *M* glycine–HCl buffer, pH 2.8 followed 0.1 *N* HCl, with simultaneous neutralization of the eluate with borate–saline buffer. The IgM samples were concentrated using Collodion bags and was pure by IEP (12).

Monospecific goat anti-μ. An immunoadsorbent column of purified IgM (100 mg/20 ml gel) was prepared by coupling to CNBr activated Sepharose 2B as described above. A λ light chain column (140 mg protein/35 ml gel) was made using the final peak of MOPC 104E ascites fluid from a Sephadex G-200 column which contained both albumin and λ light chains. Goat antimouse IgM serum was passed through the IgM column and washed free of unbound proteins with 0.1 *M* borate–saline buffer pH 8.6. Both adsorbed anti-μ and anti-λ antibodies were eluted with 0.1 *M* glycine–HCl pH 2.8. The eluates were neutralized and passed through the λ-light chain column to separate anti-μ from anti-λ antibodies.

Monospecific anti-IgG_1 and anti-IgG_2. The two monospecific antibodies were acquired in collaboration with Dr. Alexander Lawton using IgG_1 and IgG_2 immunoadsorbent columns. Goats were immunized with purified mouse IgG_1 or IgG_2 globulins. Goat Anti-IgG_2 serum was passed through an IgG_1 column first to remove anti-κ-light chain activity and the effluent was passed through an IgG_2 column. Monospecific anti-IgG_2 was obtained by eluting the antibody off the immunoadsorbent column. To obtain anti-IgG_1 antibodies, the goat anti-IgG_1 serum was first passed through an IgG_2 column and the effluent passed through an IgG_1 column. Monospecific anti-IgG_1 was eluted from this column.

Fluorescein staining of tumor cells. All tumor cells were incubated at 37° for 30 min in Eagle's medium. To 0.1 ml of tumor cells (2×10^5 cells), 0.05 ml monospecific goat anti-mouse IgG_2 antibodies (2.0 mg/ml) was added, incubated 30 min in an ice bath. To the mixture 0.025 ml fluorescein-conjugated mouse 7S globulin (~ 2 mg/ml) was added to make microprecipitate. The mixture was allowed to react for 1 hr at 0° with occasional mixing. After incubation the background fluorescence was removed by centrifugation with 0.5 ml Eagle's medium in a serofuge for 30 sec and supernatant discarded. This was repeated until the background fluorescence was reduced. Fluorescence was observed with a uv microscope. Staining with other monospecific antibodies and fluorescein-conjugated antigens was carried out as described above. The optimum conditions for each antigen–antibody complex was arrived at empirically before the staining procedure.

Results. In an attempt to develop an immunofluorescence method for the detection of surface Ig, a sensitive microprecipitate method is employed. The method utilizes the formation of microprecipitates of fluorescein-conjugated antigen and monospecific antibody in the presence of the cell receptor to be detected. In this study, distinctions between lymphocytes, phagocytic granulated cells and plasma cells or tumor cells were made. The staining of some viable lymphocytes and macrophages was seen.

Evidence for the presence of IgM receptors to dextran on the surface of neoplastic plasma cells of MOPC 104E has been demonstrated with fluorescein-conjugated dextran (13). MOPC ascites fluid or pure IgM isolated from the ascites fluid forms microprecipitate with dextran and reacts with the IgM receptors of tumor cells (Fig. 1). Under these conditions tumor cells were seen to attach microprecipitate to its surface. Macrophages and lymphocytes did not show staining. Our experiments show essentially all viable MOPC 104E tumor cells have IgM receptors on their surface. The specificity of the IgM–dextran complex is verified using MOPC 315 plasmacytoma and Ehrlich carcinoma cells, Table I. These cells did not pick up the complex of IgM–dextran.

MOPC 104E, MOPC 315, and Ehrlich carcinoma were also treated with fluorescein-conjugated λ light chains and pure monospecific anti-λ light chain antibodies. MOPC 104E and MOPC 315 showed a positive reaction, but Ehrlich carcinoma did not stain. This indicates that both MOPC 104E and MOPC 315 have λ light chains as part of the surface Ig.

When these cells were similarly treated with fluorescein-conjugated mouse 7S globulin

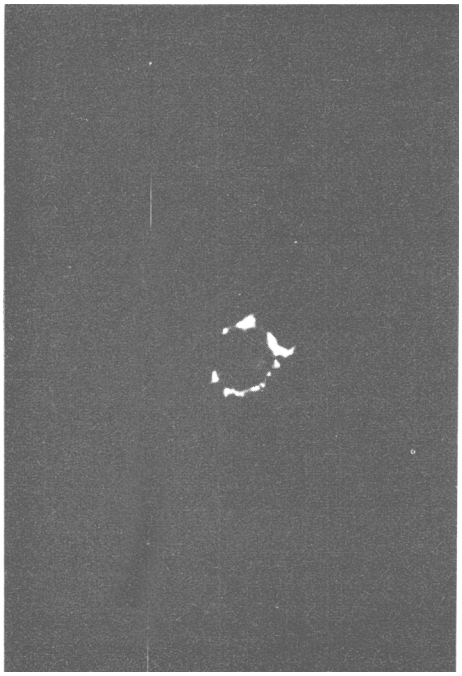

FIG. 1. Viable MOPC 104E tumor cell showing specific surface attachment of microprecipitates of fluorescein-conjugated dextran and IgM, 460×.

fraction and monospecific anti-IgG_2 the presence of IgG_2 was detected on MOPC 104E cells. Adj Pc5 tumor, which secretes IgG_2 was employed as a positive control. Table I summarizes the different microprecipitate combinations used in the staining of plasma cells. The reaction is indicated by positive (+) when tumor cell surface was stained or negative (—) when tumor cells were not stained.

When the tumor cells were stained with monospecific anti-IgG_1 and fluorescein-conjugated mouse 7S globulin fraction none of the tumor cells stained. But granulated cells and small lymphoid cells were stained to a small extent in all these cases. The possibility that MOPC 104E might bind mouse IgG_2 immune complexes by way of the Fc piece was considered. The inability of the MOPC 104E tumor cells to bind the fluorescein-conjugated BSA and mouse anti-BSA microprecipitate excludes the possibility of the binding of the immune complex through the Fc determinant of mouse IgG. The results indicate that MOPC 104E tumor cells bear IgM and IgG on their surface. Thus far only few cells containing both γM and γG have been demonstrated (8) and it appears significant that in this instance both immunoglobulins are detected in a differentiated plasma cell line.

The results described here have been obtained using MOPC 104E, MOPC 315, Adj Pc5 and Ehrlich carcinoma cells. Pernis *et al.* showed the presence of IgM in the cytoplasm and also on the surface of plasma cells from bone marrow of a case of Waldenstrom macroglobulinemia. However, Paraskevas *et al.* were unable to detect surface immunoglobulins on murine myeloma cells of IgG class.

Discussion. The discovery of surface receptors of lymphoid cells (14) has answered many aspects of immune reactions and

TABLE I. Summary of the Staining Reactions of Tumor Cells with Anti-Ig and Fluorescein-Conjugated Antigens.

Anti-Ig	Antigen-Fl	Ehrlich	MOPC 104E	MOPC 315	Adj Pc5
(IgM) anti-Dextran	Dextran	—	4+	—	—
Anti-light chain (λ)[a]	Light chain fraction	—	2+	+	—
Anti-IgG_1[a]	Mouse 7S globulin	—	—	—	—
Anti-IgG_2[a]	Mouse 7S globulin	—	+	—	+
Anti-BSA (mouse serum)	BSA	—	—	—	—
Anti-BSA (rabbit)[a]	BSA	—	—	—	—
Anti-IgG_1[b] serum	Mouse 7S globulin	—	—	—	—
Anti-IgG_2[b] serum	Mouse 7S globulin	—	+	—	+

[a] Anti-Ig were purified with immunoadsorbent columns.

[b] Antisera was obtained from Meloy Laboratories.

recognition. Recently, the data is being accumulated on the identification of receptors on plasma cells with fluorescein staining (8) and radiolabeling (7). The method described here gives a sensitive method for the detection of receptors on plasma cells. This will enable us to estimate the number of plasma cells secreting immunoglobulins in a population, but care should be taken to identify small lymphocytes and macrophages if they stain. The procedure to be effective required the use of monospecific antiserums obtained from immunoadsorbent columns. Under these circumstances the fluorescein-conjugated antigens used need not be purified, since specificity is determined by the antiserum. No nonspecific fluorescence of the viable tumor cells were seen under the conditions used. The antigen could be highly conjugated, and very small amounts are required for microprecipitate formation. The level of sensitivity is brought to that of rosette formation. For example MOPC 104E cells form rosettes with dextran-coated erythrocytes (15). However, when such cells are incubated with soluble fluorescein-conjugated dextran for various times (30 min to 2 hr) no staining could be detected, indicating that while the cell surface receptors are present (as demonstrated by RFC) the amount of fluorescein-labeled antigen localized on the surface was not sufficient to be visible. The use of microprecipitate, however, showed essentially all tumor cells to possess surface IgM receptors.

Three mouse tumors known to be secreting immunoglobulins were tested for the presence of immunoglobulin on their surface. MOPC 104E, an IgM and λ chain secreting tumor, reacted positively to monospecific anti-μ, anti-λ, and anti-γG_2. MOPC 315, an IgA (λ chain) secreting tumor, was positive with anti-λ. Adj Pc5, γG_2 (κ chain) producing tumor, was positive with anti-γG_2 serum. Ehrlich ascites tumor served as control and was negative. Rabbit and mouse anti-BSA serums were also used to make nonspecific microprecipitate and these controls were negative.

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Received May 3, 1973. P.S.E.B.M., 1973, Vol. 144.