Vol. 149, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1987 Pages 309-312

BIOLOGICAL SCREENING OF MONOCLONAL ANTIBODIES

Ralph A. Jacobson[1], J. Denry Sato[2] and Gordon H. Sato

W. Alton Jones Cell Science Center, Inc.
10 Old Barn Road
Lake Placid, NY 12946

Received October 22, 1987

SUMMARY: A method was developed to screen hybridomas secreting immunoglobulin to cell surface receptors by observing the ability of antibodies to inhibit cell attachment and survival. The model used to develop the screening procedure involved mouse hybridomas secreting monoclonal IgG to human epidermal growth factor (EGF) receptors. Conditioned medium from these hybridomas inhibited the attachment and subsequent growth of human foreskin fibroblasts unless excess EGF was added to the cultures. This procedure allows for the selection of hybridomas producing increased levels of immunoglobulin.

Cellular events induced by hormones and growth factors are mediated by interactions between these ligands and specific cell surface receptor molecules (1). Such interactions take on added significance since they have been implicated in diseases as diverse as diabetes, cancer, and atherosclerosis (2-4). Monoclonal antibodies directed against hormones and growth factors or their receptors have potential applications in controlling such diseases. For example, it has been demonstrated that monoclonal antibodies to surface immunoglobulin (5), transferrin receptors (6), and EGF receptors (7) can reduce or inhibit the growth of tumor cells bearing these receptors.

Traditional methods of selecting hybridomas of interest usually involve immunoglobulin binding assays based on differential screening, inhibition of ligand binding or binding to purified antigen. These approaches do not ensure that antibodies selected in early rounds of screening will have potentially useful intrinsic biological activities. This limitation would be most acute in

[1]Current address: Chemistry Department, California Polytechnic State University, San Luis Obispo, CA 93401
[2]To whom correspondence should be addressed.

0006-291X/87 $1.50

cases where neither a growth factor nor its receptor were available in purified form. We describe a method of screening immunoglobulins based on their ability to inhibit cell attachment and survival. The method was developed using a monoclonal antibody to human EGF receptors (8,9), but it should be flexible enough for use with antibodies to any growth factor or cell surface growth factor receptor. This method can also be used to identify subclones that exhibit increased antibody production.

MATERIALS AND METHODS

Cell culture. Hybridoma 528 (8,9) and A431 human epidermoid carcinoma cells (10) were maintained as described previously (9). Human foreskin fibroblasts were maintained in a 1:1 mixture (by volume) of Dulbecco's modified Eagle's medium (DME, Gibco) and Ham's F-12 medium (Gibco) supplemented with 15 mM Hepes buffer (Sigma), and either 5% fetal calf serum (Scott Laboratories) or 2 µg/ml human transferrin (Sigma) and bovine insulin (Sigma), 4 µg/ml fibronectin (Collaborative Research), 1 µg/ml hydrocortisone (Sigma) and 16 ng/ml EGF (Collaborative Research).

Screening. Hybridoma cells were plated in microtiter wells at 1-2 cells per well in defined medium (9) supplemented with 5 mg/ml bovine serum albumin (BSA fraction V, Sigma) to increase cloning efficiency. When clones had developed, three replicas were made of each plate. After colonies were macroscopic in size, the replica plates were centrifuged, and 100 µl of medium from each well were transferred to an additional microtiter plate, which was incubated overnight at -20°C to kill any transferred hybridoma cells. The following day the plates were warmed to 37°C and 40-80 foreskin fibroblast cells were added to each well. EGF was added to replicate wells to final concentrations of 0, 1, and 8 µg/ml. Control plates contained no conditioned medium or medium conditioned with a non-inhibitory mouse monoclonal antibody. The plates were inspected daily for 3 or 4 days for fibroblast attachment and growth.

Quantitation of antibodies. Conditioned medium was collected after 2-4 days from hybridoma subclones plated at 1 x 10^6 cells per well in serum-free medium (9), and the medium was clarified by filtration. Secreted antibody was measured by two methods: peroxidase-linked immunoassay (Kirkegaard and Perry) using A431 target cells and purification on protein A-Sepharose (Sigma) (9) and protein determination by the method of Lowry, et al. (11) using commercial mouse IgG as a standard.

Growth inhibition assay. Fibroblasts were plated in duplicate at 4 x 10^3 cells/well in serum-free medium containing 4 ng/ml EGF and 10% hybridoma conditioned medium. Control wells received no conditioned medium. After one week the cells were trypsinized and counted with a model Z_{B1} Counter counter.

RESULTS AND DISCUSSION

Antibody screening procedures must be rapid, specific and unequivocal. We have used a well-characterized mouse monoclonal antibody to human EGF receptors, 528 IgG (8,9), to demonstrate that these criteria can be met in a biological assay for receptor antibodies based on antibody-mediated growth inhibition of target cells. Medium conditioned by 528 subclones was tested for the ability to inhibit the attachment of human foreskin fibroblasts. In the

Table 1. Antibody Production and Fibroblast Growth Inhibition

Clone	IgG From EIA (Units/ml)	Protein A Purified IgG (μg/ml/day/10^6 cells)	Inhibition of Fibroblast Growth (%)
528	1.0	6.0	60
D3	1.2	7.0	69
2F7	4.2	24.0	78
1G8	3.8	19.0	67
2C12	3.4	26.0	88
1F9	3.1	18.0	73
2B1	2.2	12.0	73
1D8	2.0	11.0	60
2C11	1.3	10.0	73
1A9	0.9	9.0	Not determined
2G12	0.7	4.0	60
2B11	0.6	2.5	42

presence of EGF receptor antibody the fibroblasts either did not divide and died, or they rounded up and detached from the culture surface. This effect was inhibited by the addition 1 or 8 μg/ml EGF, and it was not seen with initial fibroblast densities above 2,000 cells/ml. 528 hybridoma cells and eleven subclones were expanded, and conditioned media were assayed for antibody concentration and for the ability to inhibit fibroblast growth (Table 1). For each clone antibody levels measured by enzyme-linked immunoassay corresponded well with antibody production measured by Lowry determinations of purified IgG. Antibody production varied approximately 10-fold between clones with the highest producers secreting 4-5 times as much antibody as the original 528 culture. In the fibroblast growth inhibition assay the degree of inhibition correlated with the amount of antibody produced by each clone.

This procedure can thus be used to assess directly the intrinsic effect on cell growth of receptor monoclonal antibodies. In principle this assay could be used to detect growth factor-specific antibodies (eg., 12) as well as receptor-specific antibodies. The only requirement is that target cell growth be dependent upon or responsive to the growth factor-receptor interaction under study. This method may be particularly useful in instances where a growth factor and its receptor are not available in pure form or where they have not been previously characterized.

ACKNOWLEDGMENTS

This research was supported by NIH grants CA 40294 and CA 37589 to G.H. Sato

REFERENCES

1. Kahn, C.R. (1981) J. Cell Biol. 70, 265-275.
2. Kasuga, M., Akanuma, Y., Tsushima, T., Suzuki, K., Kosaka, K. and Kibata, M. (1978) J. Clin. Endocrinol. Metab. 47, 66-77.
3. Bowen-Pope, D.F., Vogel, A. and Ross, R. (1984) Proc. Natl. Acad. Sci. USA 81, 2396-2400.

4. Ross, R., Glomset, J., Kariya, B. and Harker, L. (1974) Proc. Natl. Acad. Sci. USA 71, 1207-1210.
5. Miller, R., Maloney, D., Warnke, R. and Levy, R. (1982) N. Engl. J. Med. 306, 517-522.
6. Trowbridge, I.S. and Domingo, D.L. (1981) Nature 294, 171-173.
7. Masui, H., Kawamoto, T., Sato, J.D., Wolf, B., Sato, G.H. and Mendelsohn, J. (1984) Cancer Res. 44, 1002-1007.
8. Kawamoto, T., Sato, J.D., Le, A., Polikoff, J., Sato, G.H. and Mendelsohn, J. (1983) Proc. Natl. Acad. Sci. USA 80, 1337-1341.
9. Sato, J.D., Kawamoto, T., Le, A., Mendelsohn, J., Polikoff, J. and Sato, G.H. (1983) Mol. Biol. Med. 1, 511-529.
10. Fabricant, R.N., DeLarco, J.E. and Todaro, G. (1977) Proc. Natl. Acad. Sci. USA 74, 565-569.
11. Lowry, O.H., Rosebrough, A.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
12. Hsu, Y.-M., Barry, J.M. and Wang, J.L. (1984) Proc. Natl. Acad. Sci. USA 81, 2107-2111.