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A Monoclonal Antibody to the Human Epidermal Growth Factor Receptor

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A monoclonal antibody of the IgG class, EGFR1, has been isolated using cells of the epidermoid carcinoma line A431 as immunogen. The A431 antigen recognized by EGFR1 has an apparent molecular weight of approximately 175,000, is a cell-surface molecule which can be specifically cross-linked to EGF, exhibits an EGF-stimulated protein kinase activity, binds to EGFR1 in a number of human cell lines to a degree which parallels EGF binding, and shows EGF-dependent internalization in A431 cells and human fibroblasts. We therefore conclude that EGFR1 is directed against an antigenic site on the human EGF receptor. EGFR1 is not mitogenic for human fibroblasts and does not inhibit EGF binding under a variety of assay conditions. The characterization of EGFR1 has allowed the unambiguous assignment of the structural gene for the human EGF receptor to chromosome 7. Preliminary results suggest that a convenient method for isolating a range of anti-EGF receptor monoclonal antibodies can be developed, based on a hybridoma supernatant screening assay in which positive supernatants bind selectively to a human-mouse cell hybrid containing human chromosome 7.

Key words: monoclonal antibody, A431, EGF receptor, chromosomal location, internalization

The regulation of the growth and division of cells in vivo and in vitro can be influenced by a wide variety of compounds and experimental conditions. Some of the best-characterized mitogens are the small polypeptide growth factors, and among these, epidermal growth factor (EGF) has been the subject of the most intensive experimental study. Through the work of Cohen, Carpenter, and their colleagues, and subsequently of other groups, mouse EGF has been purified, its primary structure

Abbreviations used: BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; EDTA, [Ethylenedinitrilo]tetraacetic acid; EGF, epidermal growth factor; FCS, fetal calf serum; HEPES, (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid); PBS, phosphate-buffered saline; SDS, sodium dodecyl sulphate; wash buffer, 50 mM Tris HCl, pH 7.4, 0.5% NP40, 1 mM EDTA, 0.15 M NaCl.

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established, and the biological and biochemical effects of EGF studied in vivo and in vitro (reviewed [1]). Since highly purified EGF has become readily obtainable, with the characterization of the human epidermoid carcinoma cell line A431 which has an unusually large number of EGF receptors ($2–3 \times 10^6$/cell) [2–4], and through studies on a variety of cells, notably human and mouse fibroblasts (reviewed in [1]), it has been possible to start to dissect the biochemical events that follow ligand binding to the receptor. Many diverse responses are initiated by EGF, including receptor clustering and internalization followed by EGF degradation [5–12], receptor phosphorylation [13 and more recently in 14–19], alterations in membrane transport [20–25], and changes in cell morphology and in the cytoskeleton [26–28]. After about 6–8 hr of receptor occupancy a mitogenic effect occurs in some cell types [29–32]. However, it is presently unclear which, if any, of the responses known to be initiated by EGF represents or is directly related to a key mitogenic signal, and it is also uncertain whether the generation of such a primary signal normally requires the internalization of EGF together with its receptor.

Recently, an important aspect of the mechanism of EGF-induced mitogenesis has emerged from experiments using a nonmitogenic derivative of EGF which was obtained by cyanogen bromide cleavage of EGF [33–36], and antibodies against EGF and its receptor [31,34,36–38]. These studies suggest that the primary function of EGF may be to induce cross-linking or conformational changes of the EGF receptors, and that following such an activation step, all the information necessary for triggering the mitogenic response may reside in the receptor molecule itself. Irrespective of whether a continued association of EGF with its receptor might be required to maintain the activated state, it seems clear that a detailed knowledge of the structure, transmembrane disposition, and internalization pathway of the receptor is likely to be a prerequisite for understanding the mechanism of EGF-triggered mitogenesis.

While studies using chemically cross-linked EGF-receptor complexes [39–46], polyclonal antibodies [31,34,36,37], and an anti-EGF receptor IgM monoclonal antibody [38] have been invaluable for establishing the identity and some properties of the EGF receptor, a series of characterized monoclonal antibodies against different antigenic sites on the receptor should allow a more detailed analysis of its biosynthesis, structure, and function. This report introduces a convenient method for isolating a range of monoclonal antibodies against the EGF receptor, and presents the characterization of an IgG monoclonal antibody suitable for use in biosynthetic and structural studies of the receptor molecule.

MATERIALS AND METHODS

Cells and Materials

A431 cells and 3T3K cells were kindly provided by Drs G. J. Todaro and E. Rozengurt, respectively. Fluorescent rabbit antimouse antibody was purchased from Miles Laboratories Limited.

Growth of Cells

Cells were grown at 37°C in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) in an atmosphere of 5% CO_2-95% air, except for A431 cells, which were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS in an atmosphere of 10% CO_2-90% air.

Monoclonal Antibody Production

A male Balb/c mouse was injected intraperitoneally with 10^7 trypsinized A431 cells in 0.1 ml of complete Freund's adjuvant. Four weeks later the mouse was injected intraperitoneally with 10^7 cells in 0.1 ml of phosphate-buffered saline (PBS). After a further 7 days the mouse was boosted with 2×10^6 cells injected intravenously. On the fourth day following the last injection, the spleen was removed and the cells used for fusions to produce monoclonal antibodies. Antibody-producing hybrids were isolated according to the method of Kohler and Milstein [47] except that polyethylene glycol 4,000 was used in the fusion [48], and the nonsecretory line P3-NSI/1-Ag4-1 [49] was used as the mouse myeloma parent. Fusion products were distributed in 4 × 96-well plates (Flow) in RPMI 1640 medium supplemented with 20% FCS and HMT (10^{-4} M hypoxanthine, 10^{-5} M methotrexate, 1.6×10^{-6} M thymidine) selection component. Hybrids whose supernatants inhibited binding of EGF to A431 cells were cloned twice by limiting dilution and then maintained in the above medium with the FCS level lowered to 10%. EGFR1 was purified from ascitic fluid using a Protein A-Sepharose column as described [50]. Antibody class was established using immunodiffusion as described [51].

Immunofluorescence

After the treatments described in Figures 2 and 3 and in the text, the cells were washed five times with 1 ml PBS/0.1% bovine serum albumin (BSA), fixed in 2 ml methanol:acetone (1:1, v/v) for 15 min at 21°C, and washed five times with PBS/0.1% BSA. The cells were then incubated with 50 μl PBS with or without EGFR1 (0.75 mg/ml) at 37°C for 30 min, washed five times with PBS/0.1% BSA, 20 μl fluorescein-conjugated or rhodamine-conjugated rabbit antimouse IgG (diluted 1 in 20 with PBS) added, and the cells incubated at 37°C for 30 min. Finally the cells were washed five times with PBS/0.1% BSA, and mounted with Gelvatol 20–30. Photographs were taken with a Zeiss III RS Photomicroscope using Kodak Ektachrome film for Figure 2, and with an apparatus for video intensification microscopy as described [52] using Ilford FP4 film for Figure 3.

Preparation and Iodination of EGF

EGF prepared as described [53] was iodinated using the soluble lactoperoxidase procedure [54] as outlined in [55], or using the chloramine-T procedure as previously described [56].

Hybridoma Supernatant Screening for EGF-Binding Inhibition

A431 cells were grown in 24-well tissue culture plates (Linbro) until confluent. Hybridoma supernatants (200 μl) were added to test wells and incubated for 1 hr at 37°C. Binding of ^{125}I-EGF (2.25 pmol/well, 13.6 Ci/mol) labeled using the lactoperoxidase procedure was then carried out for 1 hr at 37°C as described [55].

Protein Assay

Protein was assayed by the method of Lowry et al [57].

Polyacrylamide Gel Electrophoresis and Autoradiography

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis was carried out according to Laemmli [58]. After staining in Coomassie blue the gels were dried and autoradiographs prepared using Fuji RX x-ray film.

RESULTS

Isolation of Anti-EGF Receptor Monoclonal Antibodies

Monoclonal antibodies were raised in mice against A431 cells. Following fusion of spleen cells with NS1 cells, 600 clones were screened for production of antibodies inhibiting binding of ^{125}I-EGF to A431 cells. Six clones inhibited binding by 20–30%, and one clone was obtained as a stable line which produced large amounts of IgG2b (EGFR1) in ascitic fluid. Surprisingly, purified EGFR1 did not inhibit EGF binding to A431 cells in preliminary experiments. However, since tenfold reciprocal changes in the number and affinity of EGF receptors of A431 cells at different cell densities have been reported [59], the observed inhibition of EGF binding by EGFR1 seen during screening may relate to particular growth conditions which we have been unable as yet to reproduce in several larger-scale tissue culture experiments. Further experience in isolating anti-EGF receptor monoclonals has superseded the use of EGF binding as a primary selection procedure. Recent studies have shown that after prescreening hybridoma supernatants for binding to A431 cells, about 30% of the positive supernatants bound to a human-mouse cell hybrid containing human chromosome 7, on which the gene for the EGF receptor is located (see below). Of these positive supernatants, all gave immunoprecipitates showing on SDS polyacrylamide gels a major component with a molecular weight indistinguishable from that of the EGF receptor (see below). These studies suggest that anti-EGF receptor monoclonals can be readily isolated using one simple cell binding assay.

Evidence That EGFR1 Recognizes Determinants on the EGF Receptor

The potential use of EGFR1 as a reagent depends on its specificity for the EGF receptor, thus properties of the antigenic A431 molecule recognized by EGFR1 were compared with the currently known properties of the EGF receptor.

Immunoprecipitation of extracts of A431 cells and membranes. A431 cells and membranes, labeled in four different ways, were immunoprecipitated with EGFR1 after solubilization. Autoradiographs of SDS polyacrylamide gels of these immunoprecipitates (Fig. 1) showed in each case a major component with an apparent molecular weight (MW) of 175,000.

Figure 1A shows that this major component was observed when cells were metabolically labeled with ^{35}S-methionine for 15 hr. Shorter labeling times gave reduced quantities of the 175,000 MW component, but other labeled bands, which may be biosynthetic intermediates or degradation products, were observed with apparent molecular weights of 95,000 and 160,000 (data not shown). Figure 1B shows that when whole A431 cells were externally labeled with ^{125}I, the 175,000 MW component was again labeled, consistent with it being a cell-surface molecule. The first direct evidence for the identity of the 175,000 MW molecule was obtained after treatment of A431 cells with ^{125}I-EGF under conditions known to cross-link EGF to its receptor. Figure 1C shows that treatment with ^{125}I-EGF prepared using chloramine-T gave a labeled band of approximately 175,000 MW. This band was not visible if the treatment was carried out with ^{125}I-EGF prepared using lactoperoxidase (data not shown), in agreement with observations using labeled EGF to study cross-linking to its receptor [46]. These results, which strongly suggest that the 175,000 MW molecule is the EGF receptor, were confirmed after the detection of the activity of an autophosphorylating EGF-stimulated cyclic nucleotide-independent protein ki-

nase, now thought to be an integral part of the EGF receptor [14,17,19]. Figure 1D shows a phosphorylated band at 175,000 MW from an EGFR1 immunoprecipitate of an extract of A431 membranes incubated with gamma-^{32}P-ATP. This band was visible after longer exposures of the gel track of the reaction mixture without EGF, and is clearly stimulated by EGF. Thus EGFR1 immunoprecipitated a 175,000 MW molecule showing two characteristic properties of the EGF receptor-kinase.

Binding of EGFR1 and EGF to various cell lines. If the 175,000 MW molecule immunoprecipitated by EGFR1 is indeed the EGF receptor, EGFR1 binding should parallel EGF binding in different cell types. The binding of EGFR1 and EGF to various normal and transformed human cell lines, summarized in Table I, shows that a positive correlation exists between the two binding parameters for those cell lines which show high-affinity EGF binding. Those cells which show little or no high-affinity EGF binding did not bind EGFR1. In addition, EGFR1 did not bind to mouse 3T3K cells which are known to express the mouse EGF receptor (see Fig. 4). Taken together with the above immunoprecipitation data, these results suggest that not only is EGFR1 directed against determinants on the EGF receptor of A431 cells, but that it is also probably directed against the same determinants on EGF receptors of human cells in general.

Immunofluorescence studies with EGFR1. Although EGFR1 apparently recognizes a molecule having the major known physical and enzymatic properties of the EGF receptor in A431 cells, it was important to establish whether the putative receptor recognized by EGFR1 behaved as a functional receptor for EGF. Detailed studies have shown that following binding of EGF to A431 cells, human fibroblasts, or mouse 3T3 cells, the EGF-receptor complex is internalized and the EGF eventually degraded [3,5–7,10]. The receptor is thought to also be degraded and not recycled, although these conclusions are based on indirect experiments [60]. Since A431 cells have unusually large numbers of EGF receptors, and if the 175,000 MW molecule recognized by EGFR1 represents a significant proportion of the functional A431 EGF receptors, it should be possible to visualize EGFR1 binding and internalization with fluorescent-labeled second antibody using standard fluorescence microscopic techniques. Accordingly, A431 cells were treated first with EGFR1 at 37°C, followed by EGF, at 4°C to inhibit internalization. After fixation and permeabilization of the cells, fluorescent second antibody (rabbit antimouse) was added to visualize the 175,000 MW protein recognized by EGFR1. A comparison of the phase-contrast (Fig. 2A) and fluorescence (Fig. 2B) photographs of the same microscopic field indicates that EGFR1 binds fairly uniformly to the surface of all A431 cells. Figure 2C,D shows the appearance of cells treated similarly, but incubated for 1 hr at 37°C before fixation, permeabilization and second antibody addition. Although there is no major loss of surface label, many vesicular structures are present which are similar in appearance to those previously seen in A431 cells under comparable conditions using fluorescein-EGF [3]. By varying the plane of focus it was clear that these vesicular structures are intracellular, and that there are no visible aggregates on the cell surface. The formation of these intracellular vesicles requires both the presence of EGF and incubation at 37°C (data not shown). These results suggested that EGFR1 was being internalized together with the receptor molecule as a consequence of EGF-dependent receptor down-regulation, and that the 175,000 MW molecule is therefore a functional EGF receptor. Parallel experiments in which EGFR1 and fluorescent second antibody were both added to fixed and permeabilized cells after incubation for

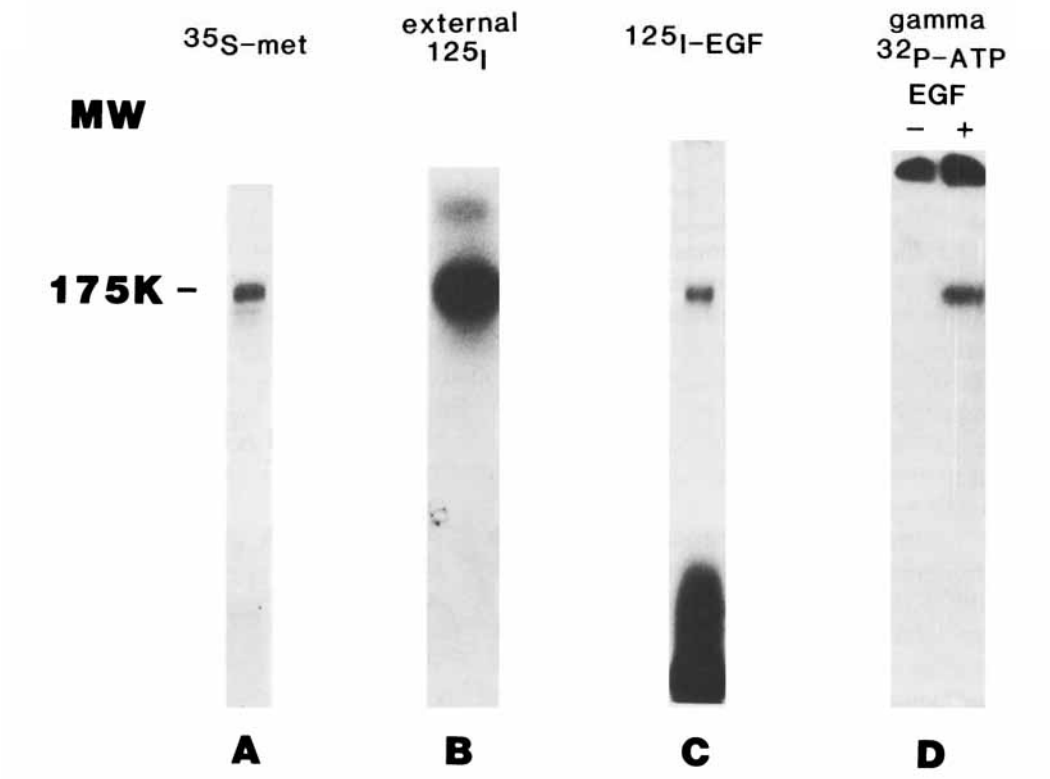

Fig. 1. Immunoprecipitation with EGFR1 of whole-cell and membrane extracts of A431: A) Subconfluent A431 cells (10^6 cells/35-mm dish) were incubated for 16 hr in 1 ml DMEM medium (10% normal methionine concentration) containing 5% dialyzed FCS and 50 μCi ^{35}S-methionine. The cells were lysed in 0.5 ml of 10 mM Tris HCl, pH 7.4, containing 1% NP40, 1 mM (ethylenedinitrilo)tetraacetic acid (EDTA), 0.15 M NaCl, 0.1% BSA, and 117 mM benzamidine. After centrifuging in a microfuge for 2 min, NaCl was added to the supernatant to a final concentration of 0.5 M. The mixture was incubated with 5 μg of EGFR1 for 15 min at room temperature, and then for 45 min with Protein A-Sepharose beads (40 μl of a 1:1 slurry in wash buffer (50 mM Tris HCl, pH 7.4, containing 0.5% NP40, 1 mM EDTA, and 0.15 M NaCl). The beads were washed with 1 ml of wash buffer containing a final concentration of 0.5 M NaCl, followed by 1 ml of wash buffer containing 0.1% SDS, and finally in 2 × 1-ml aliquots of 10 mM Tris HCl, pH 8.0, containing 0.1% NP40. They were then boiled in 40 μl Laemmli loading buffer for 5 min, run on a 10% SDS polyacrylamide gel, and treated as described in Materials and Methods. B) Confluent A431 cells (2 × 10^6/35-mm dish) were labeled with 250 μCi ^{125}I using the lactoperoxidase procedure. After washing with 5 × 1-ml aliquots of PBS containing NaI instead of NaCl, the cells were treated with 0.5 ml 10 mM Tris HCl, pH 7.4, containing 1% NP40, 1 mM EDTA, 0.15 M NaCl, 0.1% BSA, and 117 mM benzamidine, and an immunoprecipitate prepared and treated as described in A. C) A431 membranes (72 μg) prepared as described [67], EGFR1 (10 μg) and ^{125}I-EGF (100,000 cpm, 1.2 ng) prepared using the chloramine-T procedure, in a final volume of 10 μl, were incubated for 1 hr at 25°C. The incubation mixture was diluted to 1 ml with 10mM Tris HCl, pH 7.5, containing 1% NP40, 0.1 M NaCl and 1 mM EDTA, and incubated for 1 hr at 25°C with Protein A-Sepharose beads (50 μl in PBS). The beads were washed with 4 × 1-ml aliquots of 10 mM Tris HCl, pH 7.5, containing 1% NP40, 0.1 M NaCl and 1 mM EDTA, and treated as for A, except that a 12½% gel was used. D) A nonlabeled immunoprecipitate of A431 membranes (50 μg membrane protein in 1.0 ml lysis buffer) was prepared as described in A. After washing, the beads were resuspended in 20 mM (N-2-hydroxyethyl-piperazine-N′-2-ethanesulphonic acid) (HEPES), pH 7.4, and incubated at

(continued next page)

TABLE I. Binding of EGFR1 and EGF to Human Cell Lines

Cell tested	Cell type	EGFR1 bound[a] (% A431)	^{125}I-EGF bound[b] (% A431 total bound) Total[c]	Nonspecific[d]
A431	Epidermoid carcinoma	100	100	4.0
LNSV	Transformed fibroblast	91	18.4	1.0
LS174T	Colorectal carcinoma	77	10.5	0.7
HT1080	Fibrosarcoma	68	14.7	0.8
BeWo	Choriocarcinoma	50	9.4	0.8
HEB7A	HeLa	23	6.5	0.2
HF2121	Fibroblast	18	11.1	0.6
K562	Promyelocytic leukemia	0	0.7	0.5
RAJI	Burkitt's lymphoma	0	2.3	0.8
MOLT4	T cell acute lymphocytic leukemia	0	3.8	3.4
KDK3B	Testicular teratocarcinoma	0	0.5	2.0

[a]Measured using the indirect radioimmunoassay as described [68]. Briefly, cells in PBS containing 5% FCS and 0.1% NaN_3 were incubated with antibody or control protein for 1 hr at room temperature and washed twice. PBS containing 200,000 cpm (approximately 20 μCi/μg) ^{125}I-labeled F(ab)$_2$' rabbit antimouse IgG was added to the cells, which were then incubated for 1 hr at room temperature, washed four times, and counted. EGFR1 and the negative control myeloma protein P3.X63.Ag8 [47] were used at a concentration of 20 μg/ml. The P3.X63.Ag8 value has been subtracted from each determination.
[b]Measured as described [55].
[c]Measured in the presence of 8 pmol/ml (195 Ci/mmole) ^{125}I-EGF for 1 hr at 37°C.
[d]Measured in the presence of 331 pmol/ml nonradioactive EGF for 1 hr at 37°C.

1 hr at 37°C showed that although similar limited numbers of receptors were internalized in the absence of bound EGFR1, they were visible inside the cells (as vesicular structures) only if 10 mM methylamine was present (data not shown). Methylamine is a lysosomotropic agent which is known to block EGF-receptor processing after internalization [61]. These results therefore suggested that limited numbers of A431 EGF receptors were internalized when EGF was present without EGFR1, and that these receptors were not visible under the conditions of fluorescence microscopy used unless they were localized in vesicular structures as a result of blocking the normal processing pathway with methylamine.

Despite the ready visualization of the EGF receptor in these experiments due to the large numbers of receptors/cell, A431 cells have major disadvantages for studying functional receptor down-regulation. First, as shown above and previously [3], many of the EGF receptors are not internalized after EGF addition; second, in contrast to other cell types, the low-density lipoprotein receptor is internalized in A431 cells with low efficiency [62]; and third, the growth of A431 cells is inhibited by EGF [59,63], suggesting that EGF receptor function may be abnormal in these cells. For these reasons additional experiments were carried out with human fetal fibroblasts and with the human SV40-transformed fibroblast LNSV [64], which would be expected to

4°C for 2 min. EGF (40 ng) was added to half the beads, and the two bead samples incubated at 4°C for 10 min. After adding BSA (7.5 μg), $MnCl_2$ (1 mM) and Zn $(CH_3COO)_2$ (10 μM), phosphorylation was carried out by adding 15 μM ATP containing 5 μCi gamma-^{32}P-ATP (> 5,000 Ci/mmol) and incubating at 4°C for 10 min. After adding 15 μl Laemmli loading buffer (4 times concentrated) the samples were boiled for 5 min, run on a 10% SDS polyacrylamide gel, and treated as described in Materials and Methods. The dark band at the top of the track represents material in the stacking gel.

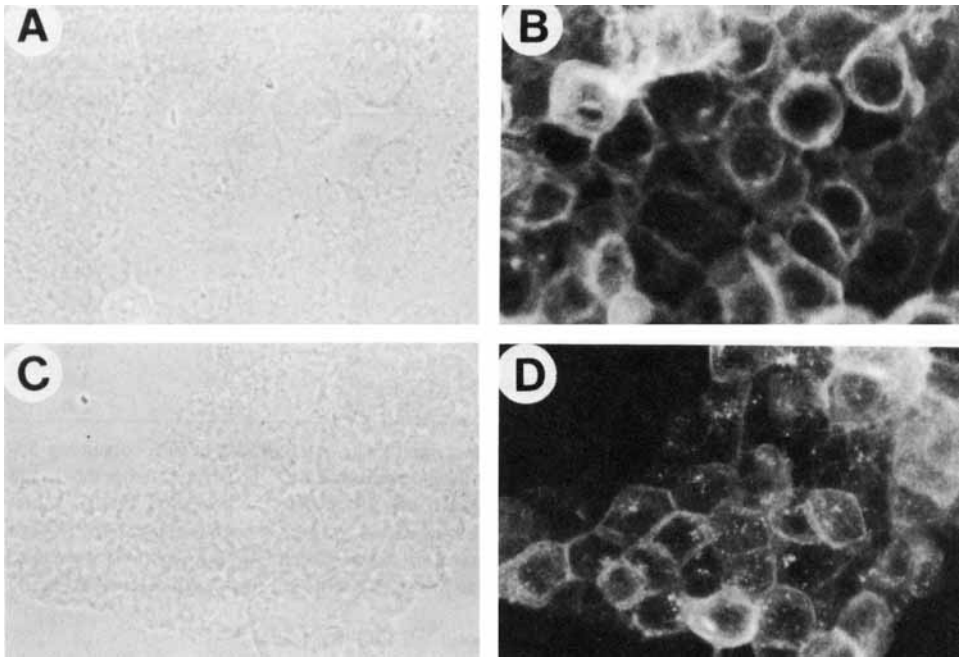

Fig. 2. Immunofluorescence visualization with EGFR1 of the EGF receptor in A431 cells: A431 cells grown on coverslips in 24-well tissue culture plates (Linbro) were incubated at 37°C for 30 min with 50 μl EGFR1 (0.76 mg/ml) added directly to the growth medium. They were then washed twice with 1 ml Hank's medium at 4°C, and incubated at 4°C for 40 min with 0.5 ml DMEM/0.1% BSA containing 300 ng EGF. The cells were washed five times with 1 ml Hank's medium/0.1% BSA at 4°C, twice with 1 ml Hank's medium at 4°C, and then incubated at 37°C with 0.5 ml DMEM/0.1% BSA for 0 min (A and B) or 60 min (C and D). After washing and fixing, the cells were treated with fluorescein-conjugated rabbit antimouse IgG, and the preparations mounted and photographed as described in Materials and Methods. A and C) phase-contrast. B and D) fluorescence. (×300).

exhibit less abnormal EGF receptor function. When LNSV cells were treated as described for Figure 2 with EGFR1 at 37°C, and EGF, at 4°C to inhibit internalization (Fig. 3A,B), the entire surfaces of the cells were covered with EGFR1. After incubation of similarly treated cells for 1 hr at 37°C (Fig. 3C,D), the antibody was completely cleared from the cell surface, although the intracellular vesicles present were visible only if 10 mM methylamine was included in the medium. Similar results were obtained using human fetal fibroblasts (data not shown). These data, which are consistent with observations of extensive EGF receptor down-regulation in human and mouse fibroblasts, confirmed that EGFR1 is directed against functional EGF receptors.

Chromosomal Location of the Gene Controlling Expression of the Human EGF Receptor

The properties of EGFR1 as an antihuman EGF receptor monoclonal antibody made it possible to resolve an important question concerning the chromosomal location of the gene for the human EGF receptor. Human chromosome 7 has been implicated in the control of the expression of the human EGF receptor as a result of

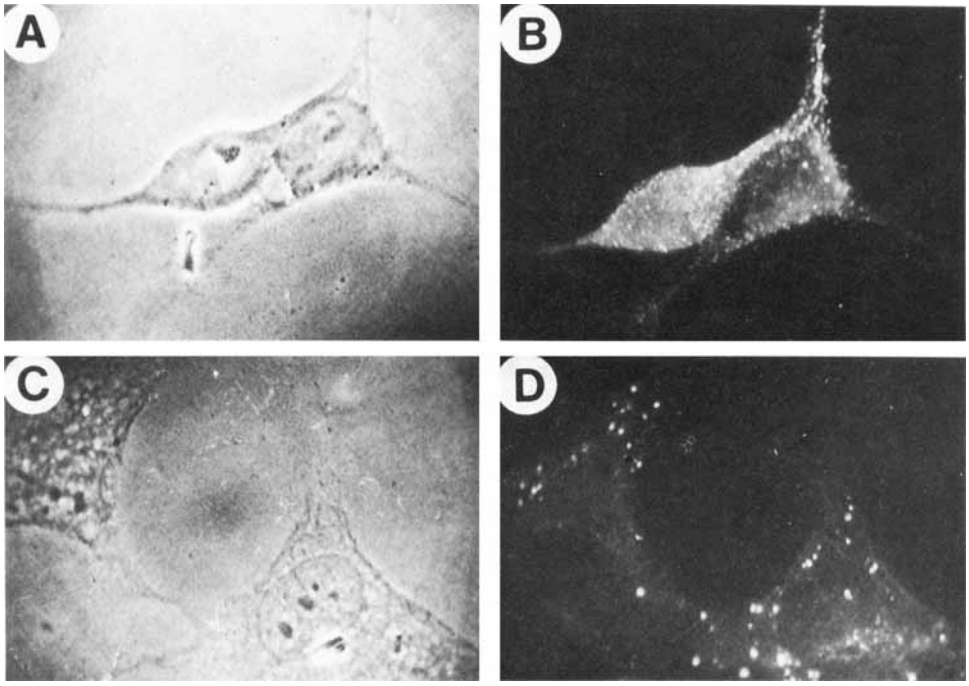

Fig. 3.Immunofluorescence visualization with EGFR1 of the EGF receptor in LNSV cells: LNSV cells were grown and treated as for Figure 2, except that they were incubated for 0 min (A and B) or 60 min (C and D) in the presence of 0.5 ml DMEM/0.1% BSA containing 10 mM methylamine HCl, and that rhodamine-conjugated rabbit antimouse IgG was used. A andC) phase-contrast. B and D) fluorescence. (×500).

studies using human-mouse somatic cell hybrids. Independently, two groups have fused EGF receptor-positive human cells with EGF receptor-negative mouse cells and measured the ability of the resultant hybrids to bind EGF [65,66]. The expression of EGF-binding ability was reported to segregate with human chromsome 7. Unfortunately the ligand-binding assays used could not distinguish between human and mouse receptors, and it is possible that chromosome 7 carried a gene which complemented a defect in the parental mouse cells rather than the structural gene for the human EGF receptor. The necessary discrimination between human and mouse EGF receptors could be made using EGFR1, which binds to human EGF receptor-positive cells but does not bind to mouse cells even if they have the capacity to bind EGF. When tested on a panel of hybrids a positive correlation was seen between EGFR1 binding and the presence of human chromosome 7 (Table II). Particularly compelling is the observation of positive reactions of EGFR1 with the hybrid clone 21 (Fig. 4); this hybrid contains human chromosome 7 as its only human genetic contribution. This result therefore allows the unambiguous assignment of the structural gene for the human EGF receptor to chromosome 7.

DISCUSSION

This paper presents the characterization of a member of a new class of monoclonal antibodies, which has been named EGFR1 and which is an IgG2b molecule.

TABLE II. Binding of EGFR1 to Human-Mouse Cell Hybrids

Hybrid[a]	Reference	Mouse parent	Human parent	Human chromosomes[b]	cpm Bound (± SD)[c] EGFR1	P3.X63.Ag8
3W4 C15	71	1R	Lymphocyte	7,10,11,12,14,15,17,21,X	3,627 ± 84	548 ± 23
HORP27R C14	72	1R	Lymphocyte	4,7,11,12,14,15,21,22	6,539 ± 497	1,205 ± 80
2WI-R70	71	1R	Lymphocyte	7,13,21,X	4,698 ± 657	1,298 ± 267
MOG7	73	RAG	Fibroblast	1,3,4,5,7,8,10,11,12,13,15,16,18,21,X	2,770 ± 67	562 ± 84
DUR4-3	74	1R	Fibroblast	3,5,10,11,12,13,14,15,17,18,20,21,22,X	962 ± 345	500 ± 228
HORL9D2	75	1R	Lymphocyte	X,11,15	1,005 ± 221	544 ± 47
-	71	1R	-	-	1,029 ± 83	1,120 ± 231
-		-	Fibroblast	All	11,627 ± 1,306	1,226 ± 15
-		-	Lymphocyte	All	1,519 ± 122	678 ± 45

[a]Several of the human-mouse hybrids have been recloned and analyzed since the original reference given. Notice the expression of the human EGF receptor in hybrids derived from receptor-negative human lymphocytes.

[b]The human chromosomal contribution of the hybrids was deduced from karyotypic, antigenic, and isozymic analysis. References to the techniques and markers used are given in [70].

[c]EGFR1 and P3.X63.Ag8 binding were measured using the conditions described in Table I, footnote a.

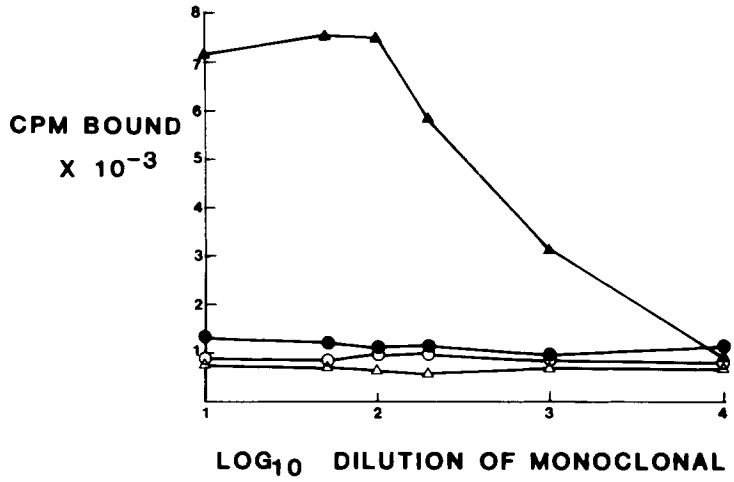

Fig. 4. Titration of EGFR1 against the chromosome 7-containing human-mouse cell hybrid clone 21 and EGF receptor-positive mouse cells: Binding of EGFR1 monoclonal and 12E7 monoclonal (control) to clone 21 cells and mouse 3T3K cells was measured using the indirect radioimmunoassay as described [68]. The hybrid clone 21 was made by Croce et al [64], and karyotypic and isozymic analysis have been used to demonstrate the presence of chromosome 7 as the only human genetic material present in this hybrid [69 and E. Solomon, personal communication]. The monoclonal antibody 12E7 recognizes a human antigen controlled by the X-chromosome and is used here as the negative control [68]. The initial concentration of EGFR1 and 12E7 was 2mg/ml. EGFR1 titrated against clone 21 (▲), and 3T3K (●); 12E7 titrated against clone 21 (△), and 12E7 (○).

The A431 antigen recognized by the EGFR1 monoclonal antibody has an apparent molecular weight of approximately 175,000, is a cell-surface molecule which can be specifically cross-linked to EGF, exhibits an EGF-stimulated protein kinase activity, is coded for by a gene on human chromosome 7, binds to EGFR1 in a number of human cell lines so as to correlate with EGF binding, and shows EGF-dependent internalization in A431 cells and human fibroblasts. We therefore conclude that EGFR1 is directed against an antigenic site on the human EGF receptor. EGFR1 is not mitogenic for human fibroblasts (unpublished data). The properties of EGFR1 thus make it particularly suitable for studying receptor internalization and biosynthesis, and preliminary data suggest that this reagent will enable the purification of the EGF receptor from A431 cells and human placenta for sequencing and other studies. Such properties, taken together with those of a different anti-EGF receptor monoclonal recently reported [38], which is an IgM molecule and is mitogenic for human fibroblasts, demonstrate the great potential of monoclonal antibodies for studies of the EGF receptor. The characterization of additional types of monoclonals is now in progress.

The procedure for isolating anti-EGF receptor monoclonals is substantially improved by use of a screening assay involving binding of hybridoma supernatants to a human-mouse cell hybrid containing chromosome 7, on which the EGF-receptor gene is located. The principle of this technique should be applicable to the isolation of monoclonal antibodies against other cell surface molecules, provided that the chromosomal location of the gene is known and the appropriate cell hybrid is available.

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