Differentiation or Immune Destruction: Two Pathways for Therapy of Squamous Cell Carcinomas with Antibodies to the Epidermal Growth Factor Receptor

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ABSTRACT

We have carried out an immunohistochemical investigation of xenografts of epidermal growth factor receptor (EGFR)-overexpressing tumors that have been induced to regress by treatment with rat monoclonal antibodies (mAbs) to the human EGFR [ICR16 (IgG2a), ICR62 (IgG2b), and ICR64 (IgG1)]. When mice bearing xenografts of the HN5 squamous cell carcinoma were treated for 5 days with mAb ICR62 or ICR16, the antibodies were found to be localized uniformly on the tumor cell membranes. However, the foci of tumor cells that remained following treatment with ICR62 were smaller than with ICR16 and the former showed a more pronounced host mononuclear cell infiltrate. Examination of the few tumors that had not regressed completely and were still present as static nodules 77 days following the final treatment with anti-EGFR mAbs revealed significant levels of therapeutic mAb in the nonviable areas of the tumors. The microscopic areas of apparently viable tumor cells that did not stain when only secondary antibody was used stained positive when the sections were treated first with an anti-EGFR antibody. This suggests that loss of the target antigen was not a significant factor and that these residual cells might be eradicated by further treatment with mAb. Furthermore, the finding of keratinized areas in the tumors undergoing regression suggested that the carcinoma cells had undergone terminal differentiation following exposure to antibody. This possibility was supported by the finding that treatment of HN5 cells in vitro with mAbs ICR16, ICR62, or ICR64 resulted in the accumulation of cells in the G0-G1 phases of the cell cycle and expression of the terminal differentiation markers involucrin and cytokeratin 10. We found no evidence of apoptosis in such cells. We conclude that antibodies which block the binding of EGF and transforming growth factor α to the EGFR can inhibit the growth of EGFR-overexpressing tumors by directing terminal differentiation and that a further therapeutic benefit may be obtained via immunological mechanisms with rat IgG2b mAbs such as ICR62.

INTRODUCTION

There is increasing evidence to suggest that polypeptide growth factors and their receptors are involved not only in the regulation of normal cell proliferation and differentiation but also, when aberrantly expressed, in the pathogenesis of certain types of human malignancy (1–3). The EGFR3 and its ligands are one such example (4, 5). This receptor is a Mr 170,000 transmembrane glycoprotein with tyrosine kinase activity which transmits the mitogenic action of the EGF family of growth factors including EGF, TGFα, and amphiregulin (6–8). The binding of these ligands to the external domain of the EGFR initiates a number of early and delayed responses in the target cells leading ultimately to DNA synthesis and cell division (4, 6, 7). Overexpression of the EGFR has been reported in a number of human malignancies, including cancer of the breast, brain, bladder, head and neck, pancreas, and lung (1, 9–12). High levels of expression of this receptor also have been associated with poor survival in some of these patients (1, 12, 13). In addition, the histological and biological examination of human tumor biopsies and cell lines has shown that overexpression of the EGFR often is accompanied by the production of one or two of its ligands (TGFα and/or EGF) by the same tumors, suggesting that an autocrine loop may be responsible for growth of tumors of this type (14–17). Furthermore, since the ligand-induced activation of such cells acts primarily via receptors on the cell surface rather than intracellularly, such a system may form a suitable target for monoclonal antibody-directed therapy (1, 18–24).

We have described recently the production of a number of rat monoclonal antibodies raised against five distinct epitopes on the external domain of the human EGFR, using as an immunogen LICR-LON-HN5, a squamous cell carcinoma of head and neck; MDA-MB 468, a breast carcinoma cell line; or A431, an epidermoid carcinoma cell line (1, 25, 26). Our aim was to obtain a diverse population of antibodies to the human EGFR has been described previously (25, 26). ICR16 (IgG2b) was raised against the receptor on the squamous cell carcinoma HN5 while mAbs ICR62 (IgG2b), ICR61 (IgG2b), and ICR64 (IgG1) were raised against the receptor on the breast carcinoma cell line MDA-MB 468. Antibodies ICR16 and ICR62 bind to epitope C and antibodies ICR61 and ICR64 bind to another distinct epitope, D, on the external domain of the EGFR. Isotype-matched control antibodies included ALN/11/53 (IgG2a) and 11/160 (IgG2b), which are directed against a specific antigen on the rat sarcoma HSN (27), or RC1/4/74 (IgG1), an antibody directed against an idiotopic determinant on ICR16.4 The monoclonal antibody to cytokeratin 10, RKSE-60, was obtained from EuroPath, Ltd. (Cornwall, England). The mouse monoclonal antibody to involucrin (28) was a gift from Dr. Fiona Watt (Imperial Cancer Research Fund, London, England).

MATERIALS AND METHODS

Cell Lines

The head and neck carcinoma cell line, LICR-LON-HN5, and the breast carcinoma cell line, MDA-MB 468, were cultured routinely in DMEM supplemented with 10% FCS, penicillin, streptomycin, and neomycin. For growth inhibition assays, the concentration of FCS in medium was reduced to 2% to minimize the effects of any growth factors present.

Monoclonal Antibodies

The preparation of rat monoclonal antibodies to the extracellular domain of the human EGFR has been described previously (25, 26). ICR16 (IgG2b) was raised against the receptor on the squamous cell carcinoma HN5 while mAbs ICR62 (IgG2b), ICR61 (IgG2b), and ICR64 (IgG1) were raised against the receptor on the breast carcinoma cell line MDA-MB 468. Antibodies ICR16 and ICR62 bind to epitope C and antibodies ICR61 and ICR64 bind to another distinct epitope, D, on the external domain of the EGFR. Isotype-matched control antibodies included ALN/11/53 (IgG2a) and 11/160 (IgG2b), which are directed against a specific antigen on the rat sarcoma HSN (27), or RC1/4/74 (IgG1), an antibody directed against an idiotopic determinant on ICR16.4 The monoclonal antibody to cytokeratin 10, RKSE-60, was obtained from EuroPath, Ltd. (Cornwall, England). The mouse monoclonal antibody to involucrin (28) was a gift from Dr. Fiona Watt (Imperial Cancer Research Fund, London, England).

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3. The abbreviations used are: EGF, epidermal growth factor receptor; TGFα, transforming growth factor α; mAb, monoclonal antibody; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; PBS, phosphate-buffered saline.

4. H. Modjtahedi and C. Dean, unpublished data.
Treatment of Mice Bearing Human Tumor Xenografts with Antibodies to the EGFR

Xenografts of human tumors were established in athymic (nu/nu) mice and treated with antibodies as described previously (1, 29). Briefly, the protocols used were as follows.

**MDA-MB 468 Xenografts.** Three groups of four mice were given inoculations in both flanks of 5 x 10⁶ tumor cells. On the day of tumor inoculation (day 0), one group of mice was given an i.p. injection of ICR62, the second was given ICR16, and the third was given a control antibody. Treatment with antibody (20 μg/dose) was continued for an additional 4 consecutive days and thereafter three times weekly until day 18 (total dose, 0.44 mg/mouse). Animals were observed for up to 100 days when the experiment was terminated and the tumors nodules remaining were excised, weighed, and fixed for histological examination (see below).

**HN5 Xenografts.** Since the treatment of athymic mice with antibody initiated at the time of tumor inoculation results in complete and permanent regression of these tumors (30), HN5 tumor xenografts were set up as described above but the commencement of treatment with antibodies to the EGFR was delayed until the tumors had reached a mean diameter of about 0.5 cm. In this experiment, groups of four to five mice were treated with antibodies ICR61 or ICR64, or with combinations of ICR61 plus ICR62 or ICR64 plus ICR62, pairs which do not compete for binding to the EGFR. In each case a second group of mice was treated with control antibodies or saline. Treatment with antibodies (200 μg/dose) was for 5 consecutive days and thereafter three times weekly until the day indicated in each experiment (total dose, 2.2 mg/mouse). Animals were observed for up to 100 days or killed when the tumors reached a mean diameter of 0.8–1.0 cm.

**Histological Examination of Human Tumor Xenografts following Treatment with Antibodies**

Two protocols were used for examination of tumor specimens. For routine histological examination, tumors were excised and samples were fixed in modified Methacarn (60% methanol, 30% inhibited, 10% glacial acetic acid) and then embedded in paraffin. Four-μm sections were cut and stained with hematoxylin and eosin.

All immunohistochemical studies of human tumor xenografts were performed by the indirect method using immunoperoxidase-conjugated Fab' (λ)abbit anti-rat immunoglobulin as the second reagent (Star 51; Serotec Ltd., Oxford, England). This antibody had been preadsorbed to remove all reactivity with rabbit anti-rat immunoglobulin. All immunohistochemical studies of human tumor xenografts were performed by the indirect method using immunoperoxidase-conjugated Fab' (λ)abbit anti-rat immunoglobulin as the second reagent (Star 51; Serotec Ltd., Oxford, England). This antibody had been preadsorbed to remove all reactivity with mouse immunoglobulins. Samples of tumor tissue were snap frozen in isopentane precooled to liquid nitrogen temperature. Tissue sections (5 μm thick) were cut on a cryostat and mounted on glass slides that had been coated with one or a combination of two anti-EGFR mAbs that bind to two different epitopes on the external domain of the human EGFR. When athymic mice bearing ICR5 xenografts were treated from day 7-24 with a combination of ICR62 plus ICR64 (total dose, 2.2 mg/mouse), on termination of the experiment (day 79) complete control was observed at 2 of 10 sites and the tumors nodules remaining were still undergoing regression (29). Histological examination of these tumor nodules showed the presence of few if any viable cells and the lesions were composed largely of scar tissues (Fig. 2A) compared with the progressively growing tumors treated with control antibody (Fig. 1A).

We also have examined HN5 tumor xenografts following treatment with one or a combination of two anti-EGFR mAbs that bind to two distinct epitopes on the external domain of the human EGFR. When athymic mice bearing ICR5 xenografts were treated from day 7-24 with a combination of ICR62 plus ICR64 (total dose, 2.2 mg/mouse), on termination of the experiment (day 79) complete control was observed at 2 of 10 sites and the tumors nodules remaining were still undergoing regression (29). Histological examination of these tumor nodules showed the presence of few if any viable cells and the lesions were composed largely of scar tissues (Fig. 2A) compared with the progressively growing tumors treated with control antibody (Fig. 1A).

**Flow Cytometric Analysis of Tumor Cells following Treatment with Antibody**

HN5 cells (5 x 10⁶) were plated onto glass coverslips in 24-well plates containing 1 ml DMEM plus 2% FCS. Following overnight incubation at 37°C, specific or control antibodies (25 μg/ml) or medium alone was added to the cultures and the cells were incubated for a further 3–4 days at 37°C. After two washes with PBS, the cells were fixed for 5 min in ice-cold methanol and then washed by incubation in PBS for 30 min. Mouse anti-cytokeratin 10 or anti-involucrin mAb diluted in PBS-0.5% BSA was added and the coverslips were incubated at 4°C for 1 h. After three washes, bound primary antibody was detected using fluorescein-conjugated sheep anti-mouse Ig (Amersham International, Amersham, United Kingdom). The coverslips were mounted in Hydromount:glycerol (1:1) and examined for green fluorescence using a Zeiss Axiosvert 100 microscope.

**RESULTS**

**Histological Examination of Regressing Tumors**

**Hematoxylin and Eosin Staining.** We have shown previously that when athymic mice bearing xenografts of the breast carcinoma MDA-MB 468 were treated from day 0–18 with a total dose of 440 μg of ICR16 (IgG2a), one-half of the tumors regressed completely but small static nodules persisted at the remaining sites when the experiment was terminated on day 100 (30). However, similar treatment with mAb ICR62 resulted in complete eradication of all tumors. Histological examination of hematoxylin and eosin-stained sections of the tumor nodules remaining following treatment with ICR16 showed that a few areas contained apparently viable tumor cells among the largely necrotic zones (Fig. 1B). The necrotic cells also showed substantial loss of cytoplasmic staining following treatment with ICR16 (Fig. 1B) compared with the progressively growing tumors treated with control antibody (Fig. 1A).

**Flow Cytometric Analysis of Human Tumor Cell Lines following Treatment with Antibody**

HN5 cells (7.5 x 10⁶) were seeded into 25-cm² Nunc flasks (Gibco Europe, Ltd., Irvine, Scotland) containing 15 ml DMEM plus 2% FCS. Then monoclonal antibody (25 μg/ml), EGF (10 ng/ml), or the equivalent amount of medium was added and the cultures were incubated for 4 days at 37°C. Flow cytometric analysis of the nuclei prepared from these cells was performed essentially as described by Ormerod (31). Briefly, a suspension of single cells from each flask was prepared in 200 μl of PBS, followed by the vigorous addition of 2 ml of ice-cold 70% ethanol-30% PBS. The cells were incubated for at least 30 min at 4°C, harvested by centrifugation, and resuspended in 700 μl PBS. Following the addition of 100 μl of RNase (1 mg/ml; Sigma) and 200 μl of propidium iodide (100 μg/ml; Sigma), the suspension was incubated first at 37°C for 30 min and then on ice for 90 min. The nuclei were analyzed using an Ortho Cytofluorograph 50HI equipped with a Spectra-Physics argon-ion laser producing 200 mW at 498 nm and an Ortho 2150 computer system (31).
could be found in all of the tumor lesions remaining in mice that had undergone treatment with the specific antibodies used either alone or in combination. These findings suggest that squamous differentiation of HN5 was an important consequence of treatment with EGFR-specific antibodies.

**Immunoperoxidase Staining.** An integral part of this study was to determine if any viable tumor cells remained after treatment with anti-EGFR antibody that continued to express high levels of the EGFR or if loss of this antigen could contribute to their escape. While none of the rat mAbs used in this study bind to the EGFR in formalin-fixed paraffin-embedded sections, they are all effective in staining the membranes of cryopreserved tissues. Strong membrane reactivity was obtained when frozen sections of HN5 tumor xenografts were stained with a specific antibody (Fig. 3A). No staining was obtained if treatment with a specific antibody was omitted or when the sections were pretreated with control antibody (Fig. 3B).
We have shown previously that when athymic mice bearing xenografts of the HN5, A431, or MDA-MB 468 tumors were treated with antibody ICR62, the tumors regressed more rapidly (and in most cases completely) compared with the same tumors treated with mAbs ICR16 or ICR64 (30). We have carried out an immunohistochemical investigation of regressing tumors to determine the reasons for the greater efficiency of antibody ICR62 in vivo. First, athymic mice bearing HN5 xenografts were treated for a short period (days 0–4) with either ICR16 or ICR62. On day 7 tumors were excised and cryosections were prepared and stained with peroxidase-conjugated F(ab')2 rabbit anti-rat IgG. The uniform staining of the tumor cell membranes with the second antibody (Fig. 4) shows that all of the tumor cells were coated with a specific rat antibody at this time. However, the total area of tumor remaining was smaller in the mice treated with ICR62 (mean tumor diameter, 27.5% of control) (Fig. 4B) compared with those treated with ICR16 (mean tumor diameter, 86% of control) (Fig. 4A). Also, the ICR62-treated tumors showed a more extensive host cell infiltrate (stained blue in Fig. 4B) surrounding the remaining viable tumor cells.

Finally, we have examined the residual nodules at day 101 after mice bearing established xenografts of the HN5 tumor were treated with ICR64 from day 7 to day 24. Despite the fact that the last treatment with antibody was given 77 days previously, staining of the sections with peroxidase-conjugated anti-rat Ig showed that significant amounts of rat mAb remained associated with the dead cells and the keratinized areas where tumor destruction was complete. Since the second antibody used was a F(ab')2 preparation that had been preadsorbed against mouse Ig, the staining could not have been due to nonspecific binding to mouse Ig or Fc-receptor-bearing cells. In contrast, the small areas of viable tumor did not stain, suggesting that either ICR64 had not reached these locations or that it had been lost from the cell membranes during cell proliferation (Fig. 5A). To determine if these cells still overexpressed the EGFR, consecutive sections were treated first with ICR64 and then with the peroxidase-conjugated second antibody. Here, the nests of viable cells stained positively, suggesting that loss of antigen was not a significant factor in the escape of these cells from antibody treatment (Fig. 5B).

**HN5 Cells Treated with Antibodies to the EGFR Undergo Terminal Differentiation**

The finding of keratin whorls in tumor nodules remaining after antibody treatment was of particular interest. We have shown previously that at concentrations above 5 nm, mAbs ICR16, ICR62, and ICR64 completely inhibit the growth of HN5 cells cultured in medium containing 2% FCS (25, 26). To investigate the possibility that terminal differentiation could be a pathway for tumor cell inactivation, we have determined the cell cycle characteristics of growth-arrested cells and examined them for expression of the differentiation markers involucrin (32-34) and cytokeratin 10 (35, 36).

**Growth-arrested HN5 Cells Accumulate in G0-G1.** Flow cytometric analysis of nuclei following treatment of HN5 cells with either antibodies to the EGFR (156 nm ICR16 or ICR62) or with the ligand EGF (10 nm) is illustrated in Fig. 6. After treatment with doses of anti-EGFR mAbs, which completely inhibited the growth of HN5 cells, the numbers of cells in S and G2-M had decreased substantially compared with the controls growing in medium alone, and most of the cells were arrested in G0-G1. Treatment with EGF at a concentration (10 nm), which also inhibited (but not completely) the growth of HN5 cells, resulted in a decrease in the percentage of cells in both S and G2-M phases of the cell cycle, but to a lesser extent than after treatment with the mAbs. We did not see any evidence for DNA fragmentation in a pre-G1 peak, indicative of apoptosis (Fig. 6A).

**Growth-arrested HN5 Cells Synthesize Markers of Terminal Differentiation.** When HN5 cells were incubated for 4 days with 156 nm anti-EGFR mAbs, most of the cells were found to express the differentiation marker cytokeratin 10 as visualised by immunofluorescence with mAb RKSE-60 (Fig. 7). The proportion of positive cells varied with the anti-EGFR antibody used, the most effective being ICR64, and by day 4 the majority of the treated cells were strongly positive for this differentiation marker (Fig. 7). In addition, the majority of cells expressing cytokeratin 10 were larger than the cyto-

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**Fig. 6.** Flow cytometric analysis of DNA in nuclei obtained from HN5 cells following 4 days incubation in vitro in DMEM-2% FCS alone or in DMEM-2% FCS containing anti-EGFR mAbs (156 nm) or EGF (10 nm). A, DNA histograms; B, percentage of cells in each phase.
mAb-INDUCED DIFFERENTIATION OR IMMUNE DESTRUCTION OF SCC

Fig. 7. Examination of HN5 tumor cells following treatment for 4 days in vitro with 156 nM of anti-EGFR antibody ICR64 (A–D) or control antibody (E and F) and then stained for expression of the terminal differentiation markers cytokeratin 10 and involucrin by indirect immunofluorescence. A, C, and E, phase contrast microscopy; B, D, and F, Immunofluorescence of samples A, C, and E, respectively. A and B, stained with anti-cytokeratin 10; C and D, stained with anti-involucrin; E and F, control HN5 cells stained with anti-cytokeratin 10.

keratin-negative cells. Similar results were obtained when the treated HN5 cells were stained with an antibody to another differentiation marker, involucrin (Fig. 7D).

Essentially identical results were obtained when HN5 cells were treated with ICR16 or ICR62 and examined for cytokeratin 10 and involucrin expression (data not shown). However, neither differentiation marker was expressed by cells that had been grown to near confluence in medium alone or with control antibody (Fig. 7F) although these cultures contained approximately 10 times more cells than in the wells treated with antibodies to the EGFR.

DISCUSSION

The results of recent studies have shown that overexpression of the EGFR is a major feature of certain types of human malignancy and that this in turn is associated with a poorer survival in such patients (for review, see Ref. 1). We have produced and characterized 21 rat monoclonal antibodies raised against five distinct epitopes on the external domain of the human EGFR in order to choose the best mAb or combination of mAbs for potential therapeutic and diagnostic application (reviewed in Ref. 1). Of these antibodies, ICR64 directed against epitope D, and ICR16 and ICR62, both directed against epitope C, were (in that order) the most effective at inhibiting ligand binding and in vitro growth of squamous cell carcinomas overexpressing the EGFR. However, ICR62 was the most effective of the three antibodies in inducing the regression of xenografts of such tumors growing in athymic mice.

Here we describe the results of an immunohistological examination of the events occurring in tumors during regression where athymic mice bearing established xenografts had been undergoing mAb therapy. Our aim was: (a) to investigate the mechanism by which antibodies to the EGFR inhibited tumor growth; (b) to determine if viable tumor cells were present in the residual tumor nodules following treatment with antibody; and if so (c) to determine whether loss of antigen expression was a significant factor in the escape of these tumor cells.

The results of our studies have shown that the nodules remaining at up to 82 days following the last treatment with antibodies to the EGFR consisted largely of necrotic and keratinized areas with few viable cells present (Figs. 1 and 2). In another study (37) it was reported that treatment of an established human colorectal tumor xenograft with high doses of the mouse anti-EGFR mAb 225 (2 mg, twice weekly) resulted in complete regression of these tumors by 2–3 weeks (37). This study also showed that by day 7 most of the cells were necrotic, and after 14–21 days most tumor cells had been replaced by connec-
tive tissues (37). In the present investigation, the presence of keratinizing areas was of particular interest and this finding pointed to a previously undescribed effect of treatment with antibodies to the EGFR, namely, that prolonged receptor blockade can induce terminal differentiation of the squamous carcinoma cells. We have investigated this function of antibodies to the EGFR by screening the anti-EGFR mAb-treated cells for expression of the terminal differentiation markers involucrin and cytokeratin 10 (Fig. 7). Furthermore, we also noted that cells expressing these differentiation markers were of larger size as previously shown for cultured human keratinocytes (33). The results of our flow cytometric analysis of HN5 cells following treatment with anti-EGFR mAbs showed that, as would be anticipated for differentiated cells, they were arrested in the G0-G1 phases of the cell cycle. Taken together these results suggest, for the first time to our knowledge, that antibodies against the EGFR which block growth factor-receptor interaction may inhibit the growth of EGFR-overexpressing tumors by inducing differentiation. In agreement with our findings, Rodeck et al. (20) have shown that growth inhibition of A431 cells by a mouse antibody to the EGFR (mAb 425) results in a decrease in the percentage of cells in the S and G2-M phases of the cell cycle and a increase in the percentage of cells in the G0-G1 phases of the cell cycle. A similar mode of action also was suggested recently by Bacus et al. (38) for the effect of growth-inhibitory antibodies directed against the external domain of the HER-2/c-erbB-2 receptor, which, like EGFR, belongs to the type I growth factor receptor family (4). This study also provided evidence for a relationship between the in vivo antitumor activity of anti-HER-2/c-erbB-2 mAbs and their capacity to induce differentiation of breast cancer cells in vitro (38).

Two other interesting findings came from the immunohistochemical staining of the tumor specimens. First, it was clear that the increased rate of tumor regression seen during treatment with ICR62 was due to rapid loss of tumor cells and, in addition, more infiltrating host cells were observed surrounding the tumor foci. As discussed above, although not the most effective inhibitor of growth in vitro, antibody ICR62 was the most effective inducer of the regression of three different human carcinomas grown as xenografts in athymic mice (30). These results point to a role for host immune effector functions in vivo. Indeed, it is well documented that rat antibodies to the IgG2b isotype, like murine IgG2a and human IgG1, are the most effective at mediating antibody-dependent cell-mediated cytotoxicity with Fc-receptor-bearing effector cells and in activating the complement cascade (20, 30, 39, 40).

Second, we were surprised to find that in the nodules remaining following treatment of the HN5 tumor with ICR64, cell membranes in the necrotic areas still retained the antibody that had been given 77 days earlier. This unexpected finding points to the stability of the immune complexes formed and is consistent with the results of experiments in vitro which indicate that the immune complexes formed on the binding of these antibodies to HN5 cells are stable and not rapidly internalized or shed from the cells. The small number of viable cells remaining, which did not stain with the anti-rat Ig receptor, had not lost their expression of the EGFR since they were positive when restained using ICR64 as the first reagent. We conclude that antigenic modulation was not a significant factor in the escape of viable cells from antibody treatment and, providing antibody access was satisfactory, these cells might be susceptible to further treatment with antibody. This possibility is under investigation.

For clinical application it is important to recognize that the EGFR also is expressed on some normal human tissues but the level of expression is much lower than on the corresponding tumor cells (9-11). However, in our experience tumors which express 105 or less receptors are much less sensitive to antibody treatment than are the EGFR-overexpressing tumor cells (1, 26). Indeed, Mendelsohn et al. (41) in a preclinical study with chimpanzees, have reported that treatment with a total dose of 650 mg of antibody produced no toxicity. In addition, in a phase I clinical trial where patients with lung carcinoma were treated with doses of up to 300 mg of the mouse antibody 225, observers noted no untoward effects in the patients (41, 42). Currently, the rat antibody ICR62 is undergoing a phase I clinical trial at the Royal Marsden Hospital in London, in patients with squamous cell carcinoma of the head and neck or lung cancer. Again, no untoward effects have been observed in patients given doses of up to 100 mg of this antibody. On the basis of our data we conclude that the best antibodies for clinical application will be those which are directed at the correct epitope and are also the most effective at inducing terminal differentiation and recruiting and activating host immune effector functions (43).

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