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ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR MONOCLONAL ANTIBODIES AS POTENTIAL ANTI-CANCER AGENTS

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Summary—The EGF receptor is a potential target for antitumor therapy, because it is expressed at high levels on many human tumor cells and appears to be involved in autocrine stimulation of cell growth in a number of experimental studies. Anti-EGF receptor MAbs, which block ligand binding, can prevent the growth in culture of cells that are stimulated by EGF or TGF-α. Growth of human tumor xenografts bearing high levels of EGF receptors is also inhibited. A Phase I trial in patients with squamous cell carcinoma of the lung has demonstrated the capacity of a single dose of 120 mg anti-EGF receptor MAb to localize in such tumors and to achieve saturating concentrations in the blood for more than 3 days, without causing toxicity.

BACKGROUND

Our laboratory is exploring the hypothesis that monoclonal antibodies (MAbs) against the epidermal growth factor (EGF) receptor may be useful as antitumor agents. The rationale for selecting growth factor receptors as targets for anti-cancer therapy is compelling. Growth factors are required for cell proliferation in culture and are produced in an autocrine fashion by many types of cells. Receptors for growth factors are expressed in abnormally high numbers on a variety of tumor cells, often with poor prognostic implications. The location of these receptors in the plasma membrane makes them readily accessible to MAbs and other potential blocking agents.

Evidence for extracellular autocrine stimulation of the EGF receptor

It is possible that malignant cells dependent upon autocrine growth factor stimulation might be able to achieve receptor activation intracellularly through union of the factor with its receptor prior to their expression on the cell surface membranes, as has been demonstrated for the interaction of the *sis* oncogene product with the PDGF receptor in murine fibroblasts transfected with *sis* [1]. We obtained evidence that autocrine stimulation of A431 cells by TGF-α results, at least in part, from binding of the cell-derived growth factor to receptors after they are expressed on the cell surface, rather than via an intracellular pathway [2]. When cells were cultured in the continuous presence of saturating concentrations of MAbs that can block binding of TGF-α to the EGF receptor, receptor phosphorylation decreased to 30% of control levels, concurrent with a reduction in cell proliferation rate. In another experiment, A431 cells were pulse labeled for 15 min with ^{35}S-labeled cysteine followed by a cold chase, and the appearance of phosphorylated receptor was determined by immunoprecipitation with anti-phosphotyrosine MAb. Autophosphorylation of tyrosines in labeled receptors was first detectable after 55 min, and peaked at 4 h. These time intervals correlate closely with the reported rates of initial and maximal appearance of newly synthesized EGF receptors on the surface of A431 cells.

Studies with anti-EGF receptor MAbs in cultured cells

Our laboratory has produced a panel of MAbs against the human EGF receptor. 225 IgG1 and 528 IgG2a are similar in that they bind to the receptor with affinity comparable to the natural ligand ($K_d = 2$ nM), compete with EGF binding, precipitate the receptor and block EGF-induced tyrosine kinase activity [3–6]. The MAbs can block EGF- and TGF-α-induced stimulation of growth rate. This has been demonstrated in cultures of human foreskin

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fibroblasts which grow with a slow doubling time of 5–7 days in serum-free medium supplemented only with insulin and transferrin. The marked increase in proliferation rate that is induced by addition of EGF can be blocked in a concentration-dependent manner by concurrent addition of antireceptor MAb [4]. Similar effects are observed when a variety of tumor cell lines that are stimulated by EGF or TGF-α are cultured in the presence of MAb.

When MAbs 225 IgG1 or 528 IgG2a are added to cultures of A431 human epidermoid carcinoma cells, at a concentration of 2–20 nM, the rate of proliferation is reduced by 50–80% [3, 4, 7]. A comparable direct inhibitory effect has been observed against other tumor cell lines bearing high numbers of EGF receptors, including breast adenocarcinoma cell line MDA-468 [8] and colon adenocarcinoma cell line DiFi [9]. Inhibition of proliferation has also been observed with colon cell lines bearing normal numbers of EGF receptors [10, 11]. We believe that this inhibition results from MAb-mediated interruption of autocrine stimulation by TGF-α produced in these cells. The observed heterogeneity of responses to anti-EGF receptor MAbs in studies of normal and malignant human breast cell lines demonstrates that dependence upon autocrine or paracrine sources of EGF or TGF-α is not a fixed physiologic state for all mammary cells [8, 12–14].

Studies with anti-EGF receptor MAbs in xenografts

In earlier studies, *in vivo* effects of treatment with anti-EGF receptor MAbs were assayed against xenografts of human A431 tumors in athymic mice. Administration of either 225 IgG1 or 528 IgG2a MAb intraperitoneally (i.p.), beginning concurrent with tumor cell implantation subcutaneously (s.c.), caused a dose-dependent inhibition of tumor growth [15]. A concentration of 2 mg twice weekly resulted in total suppression of tumor growth, which persisted for 3 months after completion of a 3-week course of therapy. Comparable inhibition of xenograft tumor growth was observed with three additional tumor cell lines which were also inhibited in culture, but was not observed against other tumor cell lines that were not inhibited in culture. The observation that a number of EGF receptor-bearing xenografts were not inhibited by MAbs suggests that the antibodies were active against a subclass of tumors with particular susceptibility to blockage of the receptor. We postulate that the characteristic defining the susceptible subclass of tumor cells is the production of TGF-α and response to it in an autocrine fashion [16]. It should be noted that the evidence for physiologic effects of anti-EGF receptor MAbs does not rule out the possible concurrent activity of these antibodies as immune effector agents.

A series of experiments were carried out to determine whether anti-EGF receptor MAbs could selectively image tumors bearing elevated levels of EGF receptors [17]. Tumor cells were injected into athymic mice s.c., and anti-EGF receptor MAb 225 IgG1, conjugated with diethylenetriaminepentaacetic acid (DTPA) to ^{111}In, was administered i.p. when tumor size reached 50–100 mg. As a control for these studies, ^{111}In was conjugated to MAb KS1/4S-1, which is an IgG1 that does not bind to human cells. Antibody doses ranged from 100 to 200 μg, with about 150 μCi radioactivity. Mice injected with labeled MAb 225 showed excellent localization of s.c. A431 xenografts (with high receptor levels), while animals injected with labeled control MAb KS1/4S-1 and animals bearing xenografts of MCF-7 breast adenocarcinoma cells (with low receptor levels) showed poor tumor images. For A431 xenografts, the tumor uptake 3 days after injection of labeled MAb was 28 ± 2% Injected Dose/g, which was more than 4 times the uptake in blood or liver. For the MCF7 xenografts maximal uptake was observed at 8 days, and MAb 225 did not show preferential uptake in the tumor cells.

Clinical trial with anti-EGF receptor MAb

A Phase I trial of anti-EGF receptor MAb therapy was initiated with 225 IgG1, which is less likely to activate complement and inflammatory mechanisms than 528 IgG2a [18, 19]. By selecting 225 IgG1 for our clinical trial, we were able to design a more pure test of the hypothesis that an antibody can exert antitumor effects *in vivo* by directly affecting a physiologic function of the antigen, in this case, the EGF receptor. The goals of this Phase I trial were to define the toxicity and pharmacokinetics of ^{111}In-labeled 225 IgG1 in patients, and to determine if ^{111}In-labeled 225 IgG1 localizes to sites of squamous carcinoma of the lung, which was selected for study because many reports have demonstrated increased EGF receptors in these tumors.

Eligible patients had Stage III or IV carcinoma of the lung, with a performance status

>60% (Karnofsky), and they were first offered and/or received treatment with courses of radiation or chemotherapy. Patients in groups of 3 received 1-h infusions of MAb. The first group received 1 mg of unlabeled MAb as a single injection. Subsequent groups received 4 mg MAb labeled with 5 mCi ^{111}In, and 16, 36, 116 or 296 mg unlabeled MAb.

Most importantly, we have not observed any toxicity in patients treated with up to 300 mg of anti-EGF receptor MAb. Imaging studies on patients who received 40 mg MAb or more revealed visualization of each primary tumor, and could detect all presumed sites of metastatic disease with diameter more than 1 cm by CT scan or X-ray. Percent injected dose in the tumor at 72 h determined by area of interest scanning with a γ-camera was 3.4% in patients who received 120 mg MAb. As expected from previous studies utilizing In-labeled MAbs, there also was extensive uptake in the liver. The serum concentration of ^{111}In-labeled 225 IgG1 was more than 40 nM for more than 3 days, when the administered dose was escalated to 120 mg and above. This level of MAb could saturate EGF receptors if it were achieved in tissues. All pateints produced human anti-mouse antibodies.

These observations suggest that 225 IgG1 may be useful for imaging tumors which bear increased numbers of EGF receptors, and they indicate that therapy with antireceptor MAbs, or with immunoconjugates of the MAbs, are worthwhile areas for clinical investigation in the future. The data indicate that patients tolerated the presence of saturating concentrations of an EGF receptor-blocking agent in their blood for a period of more than 3 days without side effects. Our studies with athymic mouse xenografts suggest that therapeutic intervention with antireceptor MAb will require prolonged exposure of tumor cells to blockade. Future approaches include administration of multiple doses of hybrid MAbs containing human constant regions, production of small polypeptide analogues that bind to and block receptors and use of immunoconjugates of radionuclides or toxins attached to receptor-binding molecules.

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