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Mitogenic Response to Epidermal Growth Factor: Relationship to Number, Affinity, and Down-Regulation of EGF Receptors in Three Murine Embyro Cell Lines

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Swiss 3T3 and C3H-M2 cells have a greater mitogenic response to epidermal growth factor (EGF) than do C3H-10T½ cells. The latter cell line, however, has a number of EGF receptors per cell intermediate between the two cell lines that have a more vigorous response to EGF. Scatchard analysis of binding data indicate that all three cell lines have one class of EGF receptor, with indistinguishable affinity for the ligand. When exposed to 10-nM EGF all three cell lines "down-regulate" their EGF receptors with the same time course, and to the same percentage of initial receptors.

Key words: down regulation, epidermal growth factor, epidermal growth factor receptor, mitogenesis

Nondividing Swiss 3T3 cells can be stimulated to reenter the cell cycle after treatment with epidermal growth factor (EGF) [1]. Continuous exposure to EGF is required to achieve maximal response in a population of quiescent 3T3 cells, although only a small percentage of the available EGF receptors need be occupied for optimal mitogenic response [2]. In both human fibroblasts and Swiss 3T3 cells exposure to EGF leads to "down regulation" of the EGF receptor [2,3]. Nearly 80% of the EGF receptors can be removed from the Swiss 3T3 cell surface as a consequence of exposure to the growth factor. Maximal "down regulation" occurs after only 4 h of exposure to saturating levels of EGF. In contrast, mitogenic response is severely impaired if EGF is removed after this brief exposure [2].

We have recently shown that murine embryo cell lines of varying origins have both qualitative and quantitative differences in their responses to a panel of mitogens [4]. Such variation could be the result of a variety of factors, including differences in receptor number, receptor affinity, or the nature of transmembrane signaling for the mitogenic

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stimulus. Receptor modulation as a consequence of mitogen stimulation of clonal cell lines can currently only be examined in the EGF system.

In this report we describe the relationship between EGF-induced cell division in three murine embryo cell lines and i) EGF receptor number, ii) EGF receptor affinity, and iii) EGF receptor down-regulation.

METHODS

Cells

Swiss 3T3 cells are the same as in our previous studies [1, 2, 4, 5]. The C3H-derived lines M2 [6] and 10T½ [7] were obtained from Drs Hans Marquart (Sloan-Kettering) and William Benedict (University of Southern California), respectively. These two lines have been used extensively for chemical carcinogenesis studies. We have recently characterized their response to a number of known mitogens [4].

Cell Culture

All cells were grown in Dulbecco's Modified Medium (DME, Gibco), supplemented with 5% fetal calf serum (Rheis). Mitogen stimulation studies were performed in 60-mm plates (Falcon) using the confluent cell culture method described previously [2, 5]. EGF was purified by the method of Savage and Cohen [8]. Methods for preparation of ^{125}I-EGF binding curves, and down-regulation protocols are similar to our previous studies [2, 5].

RESULTS

Stimulation of Cell Division by EGF

In a previous study [4] we found that EGF was unable to stimulate cell division in C3H-10T½ cells as well as in Swiss 3T3 cells, although the response of the two cell lines to additional serum was equivalent. These observations were consistent through a number of experiments (Fig 1). The 10T½ cell line consistently showed a markedly poorer response to EGF (10 ng/ml) than did either M2 or Swiss 3T3 cells. Increased concentrations of EGF (100 ng/ml) did not alter these results [4]. The *maximal* response of C3H-10T½ cells, when the concentration of EGF is not the limiting factor, is well below the response of Swiss 3T3 cells.

Binding of EGF; Receptor Number and Affinity

We measured the binding of ^{125}I-EGF to confluent monolayer cultures of the three cell lines, to determine if receptor number or affinity might play a limiting role in the mitogenic response of 3T3 cells. All three cell lines bound ^{125}I-EGF with essentially the same kinetics. Saturation values, however, differed for the three cell lines (Fig 2). When these data were analyzed by Scatchard plots (Fig 3), they indicated that all three cell lines had one class of EGF receptors with the same affinity (2.7×10^{-9}M). The number of receptors per cell, however, varied for the three cell lines. Swiss 3T3, the cell line with the greatest mitogenic response, had the lowest number of receptors per cell (60,000). The greatest number of receptors per cell (120,000) was present on the M2 cell line. Although the 10T½ cell line had the poorest mitogenic response (Fig 1), there were an intermediate number of EGF receptors per cell (84,000).

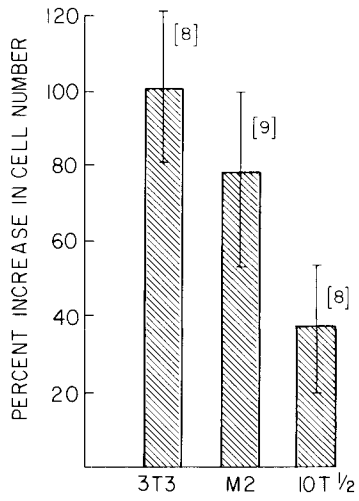

Fig 1. Mitogenic response of Swiss 3T3, M2, and 10T½ cells to EGF. Cells were grown to confluence as described previously [2, 4, 5]. After a constant cell number was established, EGF (10 ng/ml) was added, and cell numbers were counted at days 3 and 4. Data are expressed as the percentage increase in cell number per dish relative to untreated controls. A 100% increase thus means a doubling in cell number relative to unrelated controls. Error bars indicate standard deviations; numbers in brackets are the number of experiments for each cell line.

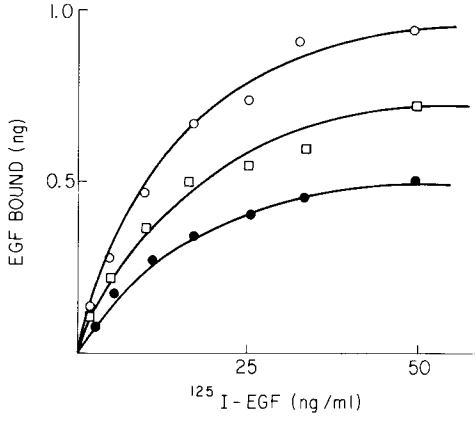

Fig 2. Binding of ^{125}I-EGF to murine embryo cell lines. Cells were grown to confluence in 35-mm plates. Binding assays were done for 90 min on triplicate plates at 22° for each point. Data shown are "specific binding," ie, binding not competed by excess (5μg/ml) unlabeled EGF. Details of the binding assay have been described previously [2, 5], M2, (○); 10T½, (□); 3T3 (●).

Down-Regulation of EGF Receptors in Response to Ligand

The three cell lines were exposed to saturating levels of EGF for various lengths of time in order to determine both the rate and degree of receptor down-regulation in response to EGF. Despite differences in the initial number of EGF receptors, the rate of their loss was essentially identical in all three cell lines (Fig 4). The three cell lines appeared to

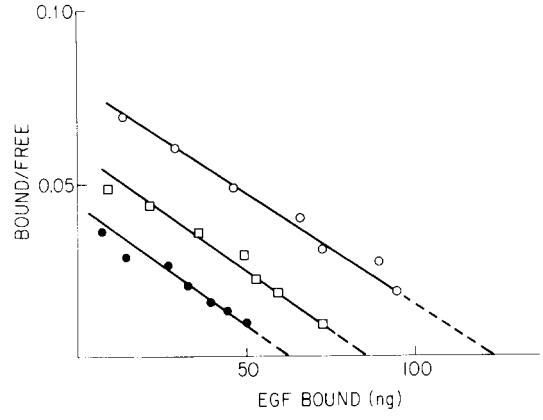

Figure 3. Scatchard analysis of binding of ^{125}EGF to murine embryo cell lines. Symbols as in Figure 2.

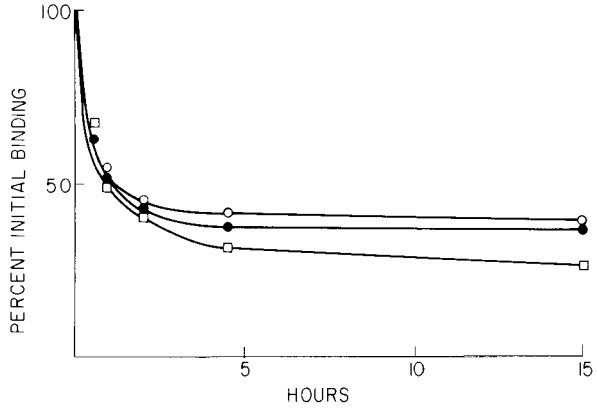

Fig 4. Down-regulation of the EGF receptor on murine embryo cell lines. For each point three confluent 35-mm plates of cells were exposed for the indicated times of 60 ng/ml of EGF in complete medium at 37°C. Cells were washed and incubated an additional 4.5 hours at 37°C with three changes of medium and then assayed for EGF receptors. Experiments were designed so that all receptor assays on a single cell line were performed simultaneously. Data are expressed as percentage of initial binding of ^{125}I-EGF for each cell line. Symbols as in Figure 2.

down-regulate to about the same extent on a percentage basis, despite differences in the total number of EGF receptors per cell initially present.

DISCUSSION

The cell line with the poorest mitogenic response (10T½) has a number of receptors per cell intermediate between that of two cell lines (3T3, M2) with greater mitogenic responses. Since the affinities of the EGF receptors on the three cell lines are indistinguishable, these data suggest that, under the conditions used for these mitogenic studies, the number of EGF receptors per cell does not play a limiting role in the reduced responsive-

ness of the 10T½ cell line to EGF-induced cell division. This is not simply a dose-response alteration; the same mitogenic relationship among the three cell lines was observed with increased concentrations of EGF (data not shown). Although 10T½ cells become growth-arrested at a lower cell density in 5% serum-supplemented medium, their mitogenic response to serum is as potent as that of Swiss 3T3 cells [4]. The 10T½ cell's limited response to EGF-induced mitogenesis is thus not due to a reduced cellular potential for cell division in response to all mitogenic stimuli, nor to a limiting number of EGF receptors.

"Down-regulation" of the EGF-receptor at saturating concentrations of EGF occurred with the same time dependence for all three cell lines. The concentration of EGF used for the initiation of down-regulation (60 ng/ml) will evoke a maximal mitogenic response in all three cell lines. It is of interest to note that the same percentage of EGF receptors is down-regulated (and perhaps internalized; see Refs 2, 3) in the three cell lines, despite differences in the number of receptors per cell. The best-responding line (3T3) consequently loses less EGF-binding activity than either 10T½ or M2 cells. Removal of EGF after an 8-h period, when down-regulation is complete (Fig 4), results in a severely impaired mitogenic response [2]. These data suggest that the rapid, massive loss of growth factor-binding activity from the cell surface in response to initial exposure to EGF is not sufficient to trigger a mitogenic response to EGF in murine embryo cell lines, and may even be disadvantageous.

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