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Mitogenic Effects of Murine Serum and Fibroblast Growth Factor on EGF Nonproliferative Variants of 3T3

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The enhanced ability of murine serum to support growth of 3T3 cells, when compared with fetal calf serum, is also evident on variants of 3T3 cells lacking the ability to bind epidermal growth factor (EGF). Variant 3T3 cell lines unable to bind EGF also retain a mitogenic response to fibroblast growth factor.

Key words: epidermal growth factor, fibroblast growth factor, cell division, mitogenesis, growth control

Over a decade ago Holley and Kiernan [1] reported that mouse serum was more active than calf serum in supporting the growth of 3T3 cells; cells could reach equivalent saturation densities in medium supplemented with a lower percentage of mouse serum. These authors also described a protease-sensitive, heat-stable growth factor that binds to DEAE cellulose and is present in urine. Epidermal growth factor (EGF), a small polypeptide with similar characteristics [2], is also found in urine [3], and is mitogenic for 3T3 cells [4]. Radioimmunoassay data have indicated that murine sera have circulating levels of EGF in the range required for mitogenesis of 3T3 cells [3]. It is possible that the presence of murine EGF is the cause of the enhanced activity of mouse serum for 3T3 mitogenesis. Direct measurement of bovine EGF in sera is complicated by cross-reactivity problems, however, making the resolution of the problem by this means difficult.

We have recently isolated variant 3T3 cell lines that 1) lack a mitogenic response to EGF, 2) respond to serum, and 3) are unable to bind radioactive EGF [5]. If murine EGF is the primary agent responsible for the enhanced proliferative stimulation of murine vs fetal calf serum, these EGF nonresponsive variants should not have a reduced requirement for murine sera relative to bovine sera, in contrast to their parental 3T3 cells.

Our EGF nonproliferative variants all respond to other mitogens [5]. In a recent study [6] we have demonstrated that the only mitogen we tested that rivals EGF in both ubiquity and potency of action for murine embryo cell lines is fibroblast growth factor (FGF), a polypeptide mitogen isolated from bovine pituitary [7]. In this report we describe the response of 3T3 and EGF nonresponsive 3T3 variants to 1) murine and fetal calf serum and 2) EGF and FGF.

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METHODS

EGF was purified from salivary glands of male mice, using the procedure of Savage and Cohen [8]. FGF was purchased from Collaborative Research (Waltham, MA). Cells were grown as described previously [4–6] in Dulbecco's Modified Eagle's medium (DME, Gibco) supplemented with fetal calf serum (FCS, Reheis) or mouse serum from Swiss-Webster mice (Hilltop Laboratories). All growth curves were carried out in Linbro 17 mm multiwell trays. Cells were plated in DME + 5% FCS, then switched to appropriate media after 24 h. Duplicate wells were counted as described elsewhere [6]. The isolation and characterization of the EGF nonproliferative ("pro$^-$") variants NR1, NR4, and NR6 of 3T3 have also been previously reported [5].

RESULTS

Response of 3T3 and 3T3-NR4 to Murine and Fetal Calf Sera

Cells were plated in 17 mm wells in DME + 5% FCS at a density of approximately 1×10^5 cells/well, then switched at 24 h to media containing the serum supplements shown in Figure 1. Cell counts were monitored daily, and saturation density was plotted as a function of serum concentration. For the data presented in Figure 1, we calculated that the saturation density of 3T3 cells increased 5.7×10^4 cells for each 1% increase in fetal calf serum concentration. In contrast, a 1% increase in murine serum increased the saturation density by 2.1×10^5 cells in the linear portion of this curve. Murine serum is thus 3.6

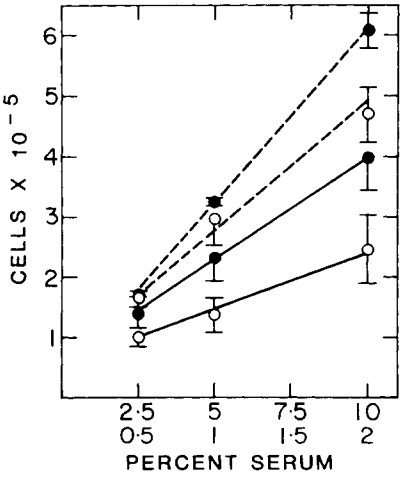

Fig. 1. Saturation densities of 3T3 and 3T3 (NR-4) cells in various concentrations of fetal calf or mouse serum. Cells were plated in DME + 5% FCS. Twenty-four hours later the DME + 5% FCS was removed and the cells were switched to the media indicated. Data are the averages of four wells counted over a 24 h period after saturation was reached. The upper numbers on the ordinate are concentrations of fetal calf serum (FCS); the lower numbers are concentrations of mouse serum (MS). (•– –•), FCS and 3T3; (○– –○) MS and 3T3; (•——•) FCS and 3T3 (NR-4); (○——○), MS and 3T3 (NR-4).

TABLE I. Saturation Densities of 3T3 and EGF Nonproliferative Variants in the Presence of EGF and FGF*

Cell line	5% Serum	10% Serum	EGF (10 ng/ml) + 5% serum	FGF (100 ng/ml) + 5% serum
3T3	3.41 (100)	6.24 (183)	6.88 (202)	8.69 (255)
NR-1	0.92 (100)	1.40 (152)	1.07 (116)	3.16 (343)
NR-4	1.86 (100)	2.94 (158)	1.70 (91)	4.03 (231)
NR-6	1.35 (100)	2.10 (155)	1.37 (101)	4.31 (219)

*All data are expressed as cells $\times 10^{-5}$ per 17 mm well.
Numbers in parentheses are percentages of the 5% serum control.
Plating, changing of media, growth of cells, and determination of saturation density were as described in Figure 1.

times more potent than fetal calf serum as a growth-promoting agent for 3T3 cells. When a similar type of analysis of the saturation density data for 3T3 (NR4) is carried out, murine serum is 2.9 times as potent as fetal calf serum in promoting the growth of the EGF nonresponsive variant.

Response of 3T3 and EGF Pro$^-$ Variants to EGF and FGF

The cell lines indicated in Table I were plated at 1×10^5 cells/well in DME + 5% FCS. After 24 h the medium was replaced by those media shown in the table. Cells were grown to their maximal densities in the various media and counted in stationary phase.

DISCUSSION

The nonresponsive variants selected by our colchicine procedure attain, in all cases, a saturation density lower than 3T3 (cf Table I). One possible reason for this is that the selective technique employed may also tend to select for a more rigidly "contact-inhibited" or "density-dependent inhibited" cell.

Mouse serum is nearly three times as effective as fetal calf serum in promoting the growth of a 3T3 variant lacking a receptor for EGF. Since the relative efficacy of murine serum vs fetal calf serum for the parent (EGF responsive) 3T3 line is only marginally (3.6-fold) greater, our data suggest that the presence of EGF is not the primary factor responsible for the enhanced ability of murine serum to support the growth of 3T3 cells.

FGF is at least effective on the EGF pro$^-$ variants as it is on the parental 3T3 cell in increasing the saturation densities reached in 5% FCS. These data suggest that 1) there are distinct cellular receptors for EGF and FGF, and 2) loss of the EGF receptor does not diminish the capacity of 3T3 variants to respond to an unrelated polypeptide mitogen.

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