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EFFECTS OF GROWTH FACTORS ON CELL PROLIFERATION AND MONOCLONAL ANTIBODY PRODUCTION OF BATCH HYBRIDOMA CULTURES

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SUMMARY

Effects of growth factors such as EGF, FGF and IL-2 on cell proliferation and monoclonal antibody production in a hybridoma cell line adapted to a completely defined serum-free medium were determined in batch cultures. The results indicate that the presence of growth factors in the medium enhances the antibody secretion without significantly affecting the growth rate. The specific antibody secretion rate of cells grown in serum-free medium supplemented with growth factors was 35% higher than those grown in serum-free medium alone.

INTRODUCTION

Hybridomas are important cell lines used in the production of monoclonal antibodies which find widespread applications in areas such as therapy, diagnosis, tumor imaging and drug delivery. The particular hybridoma cell line TIB 131 was chosen as a model system for this study. Proliferation of mammalian cells is controlled by specific macromolecular regulatory factors termed 'growth factors'. Interleukin-2 (IL-2) is an important growth factor which stimulates proliferation in both human and murine T-lymphocytes, or T-cells. Normal B-cells also respond with modest proliferation to sources of IL-2 (Swain et al., 1983). Since TIB 131 is a B-cell hybridoma and since recombinant IL-2 was commercially available, we investigated IL-2 as a candidate for manipulating growth and antibody production by the hybridoma cell line. Effects of other readily available recombinant growth factors, in particular EGF and FGF, were also determined in the same study.

MATERIALS AND METHODS

CELL LINE

TIB 131, a mouse-mouse hybridoma was obtained from the American Type Culture Collection (ATCC; Rockville, MD). It secretes monoclonal antibodies which react with all classes of intermediate filaments. This hybridoma has been derived from a NS-1 mouse myeloma line (Pruss, 1981).

CELL CULTURE

The hybridoma cell line was adapted to a completely defined KSLM serum-free medium (Kawamoto et al., 1986). Briefly, the basal medium (RDF) consisted of a 2:1:1 mixture (by volume) of RPMI 1640, HAM'S F12 and DMEM. This was supplemented with insulin, transferrin, LDL, bovine serum albumin, oleic acid, mercaptoethanol, sodium selenite and aminoethanol.

Instead of direct introduction of the cells into the KSLM serum-free medium, a weaning procedure was adopted. In this procedure the KSLM serum-free medium was supplemented with 5% Equine serum in the first passage. During the subsequent passages, the serum concentration was reduced in a stepwise manner until the cells showed the original growth characteristics in the completely defined KSLM serum-free medium alone. As controls, cells were grown in DMEM supplemented with either 10% Equine serum or 10% FCS.

The cells were grown in T-flasks at 37°C in a humid atmosphere of 10% CO_2. The cells were subcultured every three days upon reaching the stationary phase (around 2 x 10^6 cells/ml). In one set of experiments with EGF alone, the cells were grown in serum-free medium supplemented with different EGF concentrations such as 1, 5, 10, 20, 50 and 100 ng/ml. In the second set of experiments, the cells were grown in serum-free medium supplemented with 10 ng/ml EGF, 10 ng/ml FGF, or 20 units/ml Interleukin-2 (IL-2). The cells were adapted to a particular growth factor concentration by cultivating them continuously for over 100 cell doublings in presence of that growth factor concentration. The adapted cells were then used to study the kinetics of growth and product formation as a function of different growth factors. All experiments, including the ones with serum were carried out within the same time span and thus were under comparable conditions. Samples were taken at regular intervals to monitor growth rate and antibody production. Total cell count was determined using a Coulter counter. Viability was obtained using the trypan blue exclusion method.

The IgG concentration in the culture medium was determined by using a modification of an ELISA procedure used in this laboratory (Meilhoc et al., 1988). In the modified assay, standard mouse IgG solutions (1 to 250 ng/mL) and two dilutions each of the cell-free IgG containing supernatants were prepared in KSLM serum-free medium instead of medium containing serum. An additional step of incubating the 96-well plate with 2% BSA solution at 37°C for 1 hour was carried out in order to prevent non-specific binding between the secreted IgG and the surface of the well. The absorbance of each sample measured at 405 nm using an ELISA reader (SLT Instruments) was proportional to the amount of IgG in the original sample.

RESULTS AND DISCUSSION

It was anticipated that, being a transformed cell line, TIB 131 could show a reduced or a loss of requirement for specific growth factors. The growth and product secretion kinetics for cells grown in the presence of different growth factors are summarized in Figures 1 and 2. The growth rate of cells grown in serum-free medium alone is almost the same as that of cells grown in serum-free medium supplemented with different growth factors or medium supplemented

with 10 % FCS.

Significant variation in antibody productivities is evident. The antibody productivity of cells grown in serum-free medium supplemented with different growth factors or medium supplemented with 10 % FCS was found to be around 35% higher than those grown in serum-free medium alone.

It has been observed previously that antibody titer was linearly correlated to the integral of viable murine hybridoma cell concentration, indicating that the specific secretion rate of the viable cells (Strain J4C2) is approximately independent of growth rate or stage of the culture (Renard et al., 1988). The kinetics of antibody secretion for cells grown in serum-free medium alone is compared with antibody production by cells grown in serum-free medium supplemented with IL-2 in Figure 3. Analysis of the data in Figure 3 shows an excellent agreement with the earlier model which is reflected in high linear correlation coefficient values of 0.98 or more. The same consistency with approximately constant antibody secretion rate per viable cell is seen with cells grown in serum-free medium supplemented with other growth factors as well as those grown in medium supplemented with 10 % FCS. The specific antibody secretion rates and specific growth rates for cells grown in medium supplemented with different growth factors or 10 % FCS are listed in Table 1. This data indicates that the presence of the growth factor in the medium increases the antibody productivity even though the growth rate seems to be relatively unaffected.

Table 1

Comparison of specific growth and antibody secretion rates as a function of medium composition

Medium composition	Specific growth rate, hr^{-1}	Specific antibody secretion rate, pg/viable cell/hr
KSLM ALONE	0.045	12.9
KSLM + EGF	0.048	15.6
KSLM + FGF	0.049	15.7
KSLM + IL-2	0.046	16.4
DMEM + 10 % FCS	0.048	15.3

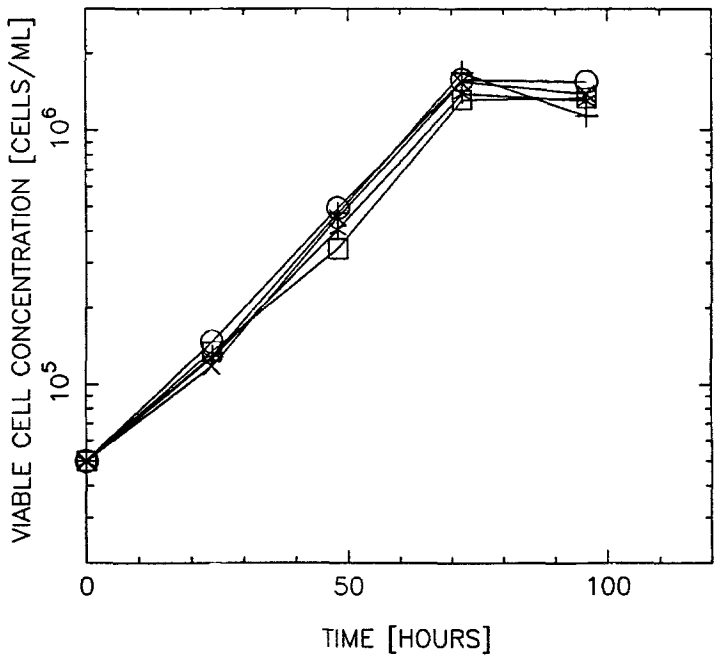

Figure 1: Viable cell concentration as a function of time and growth factors: no growth factors (□), DMEM with 10% FCS (○), 10 ng/ml EGF (×), 10 ng/ml FGF (+), 20 units/ml IL-2 (∗).

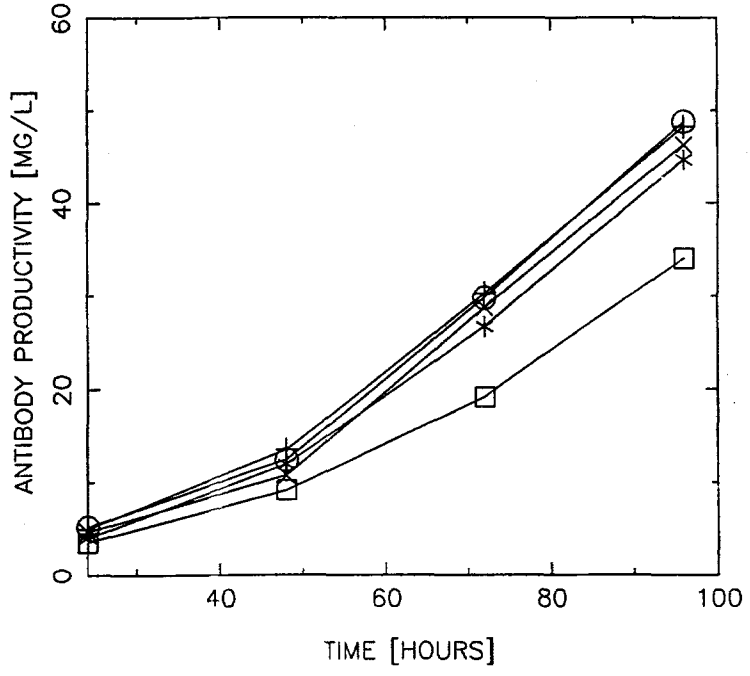

Figure 2: Antibody productivity as a function of time and growth factors: no growth factors (□), DMEM with 10% FCS (○), 10 ng/ml EGF (×), 10 ng/ml FGF (+), 20 units/ml IL-2 (∗).

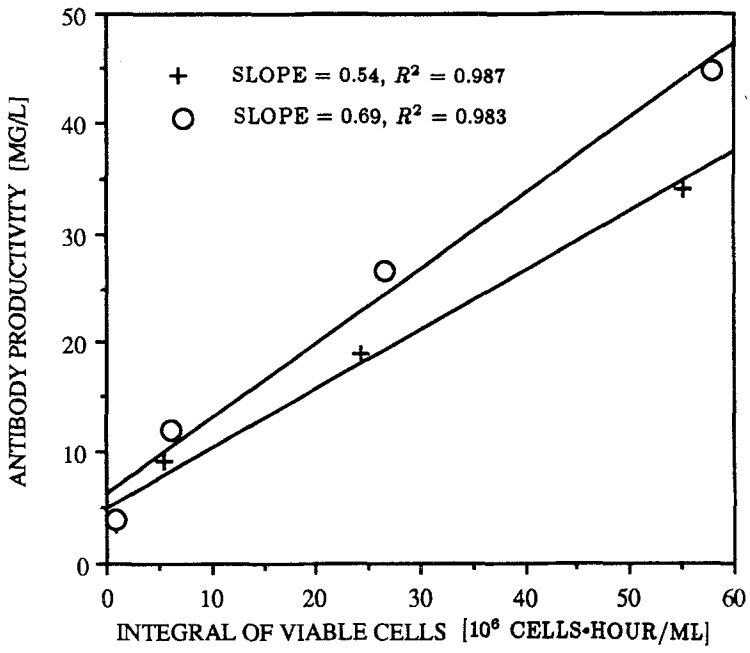

Figure 3: Kinetics of antibody secretion: KSLM alone (+), KSLM + IL-2 (○).

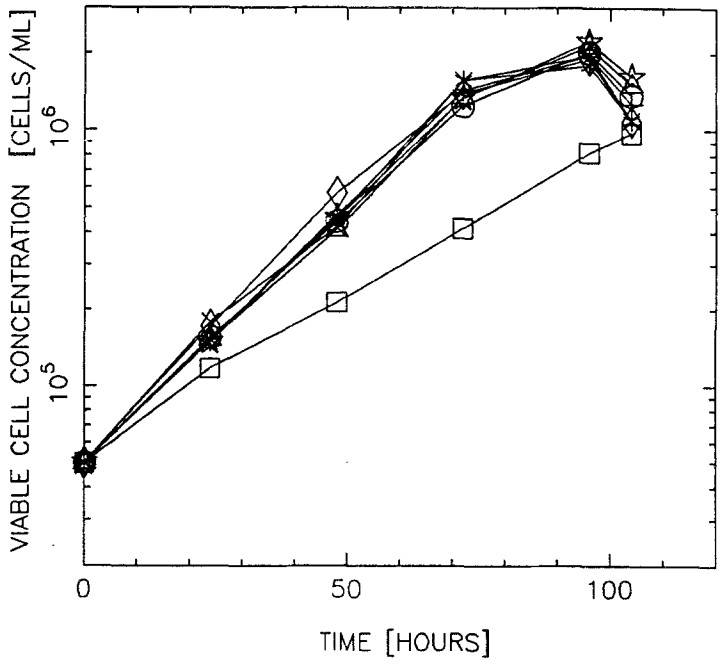

Figure 4: Viable cell concentration as a function of time and EGF concentrations: no EGF (◇), DMEM with 10% Equine serum (□), 1 ng/ml EGF (+), 5 ng/ml EGF (*), 10 ng/ml EGF (○), 20 ng/ml EGF (×), 50 ng/ml EGF (△), 100 ng/ml EGF (*).

In order to investigate whether the enhancement in antibody secretion was dependent on the concentration of growth factor in the medium, we carried out dose-response experiments with EGF as described earlier in the Materials and Methods section. There was no significant difference in the growth rates among different samples as seen in Figure 4. However, once again, the antibody productivities and, consequently, the specific antibody secretion rates were significantly higher in cells grown in serum-free medium supplemented with EGF as compared to cells grown in serum-free medium alone (data not shown).

These results indicate that, under conditions considered here, growth factors play a stimulatory role in the antibody production process without having a significant effect on cell growth.

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