Application of animal-free recombinant bioactive proteins to improve the growth performance of cell lines for viral vaccines

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Introduction

The use of mammalian cell culture for the production of both pharmaceutical proteins and viral vaccines has grown dramatically over recent decades. The increased demand for cell-derived products has been accompanied by rapid developments in process technology and radical changes in the design of culture media. The progressive availability of relevant bioactive proteins using recombinant DNA technology has allowed the replacement of animal-derived components in the production process; this has assisted the efforts of regulatory bodies to minimise the potential risk to product users. The use of bioactive proteins has also contributed to greater consistency in process performance and provided the scope to elevate process yields. Examples of typically used bioactive proteins are listed in Table 1.

Although individual proteins may have a significant effect on cell growth and productivity, the effect on performance can be enhanced further when particular proteins are used in combination. The industrial application of these proteins has largely focused on recombinant Chinese Hamster Ovary (CHO) cell lines producing pharmaceutical proteins. This poster presents the results of a preliminary study where the growth responses to a selection of bioactive proteins (Insulin-like growth factor, Epidermal growth factor, Transferrin and Albumin) were tested in three cell lines that are important in the production of viral vaccines.

Table 1. Examples of recombinant bioactive proteins routinely used to enhance cell culture

Insulin-like growth factor
Epidermal growth factor
Transforming growth factor
Transferrin
Albumin

Materials and methods

Cell lines:

1. Madin Darby Canine Kidney (MDCK)
2. VERO African Green Monkey Kidney (VERO)
3. Human Embryonic Kidney 293 (HEK 293)

Culture media:

Cells were maintained in Dulbecco's Modified Eagle's Medium / Ham's Nutrient Mixture F12 (DMEM /F12) supplemented with 2 mM GlutaMax™ and 10%(v/v) Foetal Bovine Serum.

Supplements:

Bioactive proteins tested and their final concentrations in media were:

LONG®R³ insulin-like growth factor-I (**LR3**): 100 µg/litre

LONG® Epidermal Growth Factor (**LONG EGF**): 20 µg/litre

CellPrime® rAlbumin AF-G (**AF-G**): 1 g/litre

CellPrime® rTransferrin AF (**rTf**): 5 mg/litre

LONG®R³ IGF-I is available from SAFC Biosciences, LONG EGF is available through Novozymes Biopharma, and CellPrime AF-G and rTransferrin are available through Millipore.

Batch culture:

Cells were harvested from tissue-culture flasks and washed twice with DMEM/F12 basal medium. Cells were counted then diluted in basal medium with 2 mM GlutaMax™. 180 µL of cell suspension containing 4,000 cells was seeded into the wells of a 96-well culture plate (Corning®CellBIND® Surface, Cat. 3300). Supplements were added to a final volume of 200 µL.

All conditions were tested in triplicate. The plates were incubated at 37 C, 5% CO_2 for 6 days.

Cell growth:

Cell growth was assessed by the CyQUANT NF Cell Proliferation Assay kit (Invitrogen). Results are expressed as Relative Fluorescence Units (RFU).

Conclusions

The combinations of bioactive proteins tested had a greater effect on cell growth of these cell lines than did the individual proteins.

The combination of LR3, LONG EGF and rTf showed strong growth enhancement across all three cell lines.

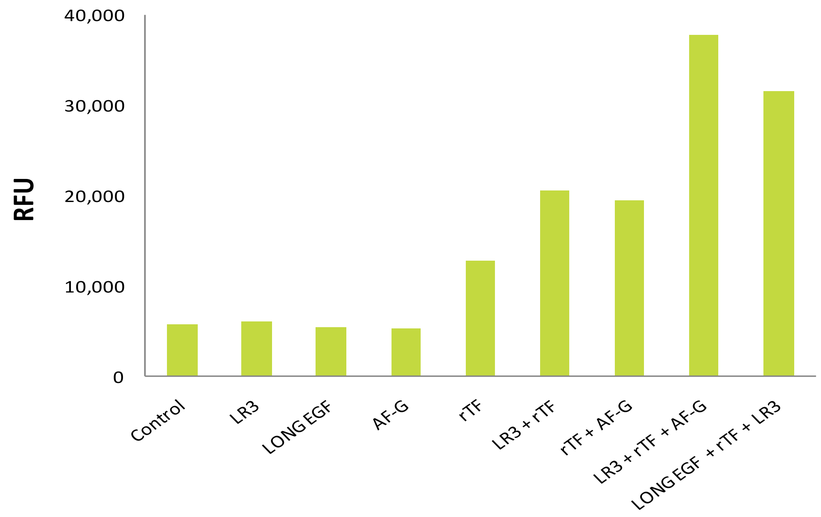
Further work will examine the most effective concentrations and combinations of these bioactive proteins for growth of the individual cell lines.

Further work will also examine how these bioactive proteins influence the production of virus by these cell lines.

Results

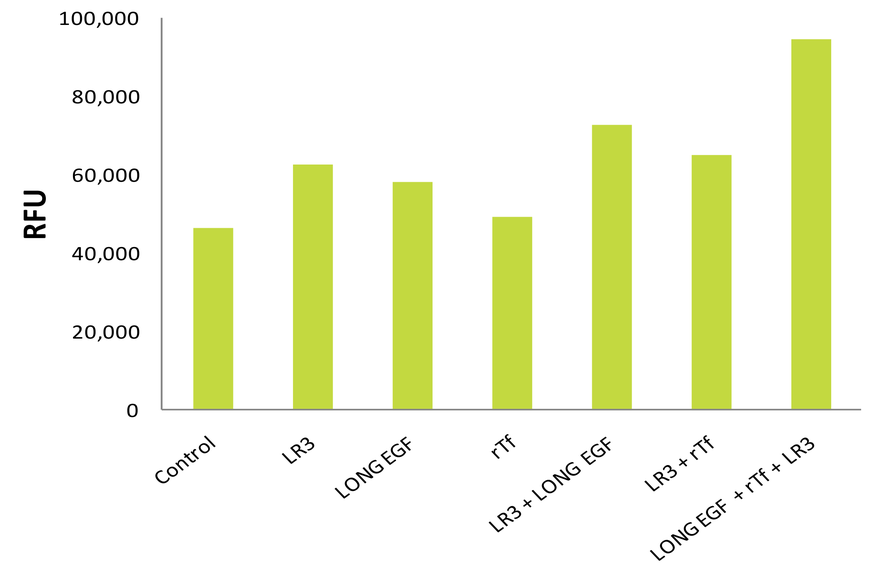
Effect of bioactive proteins on vaccine cell line growth when used individually and in combination

Fig. 1. MDCK cell growth



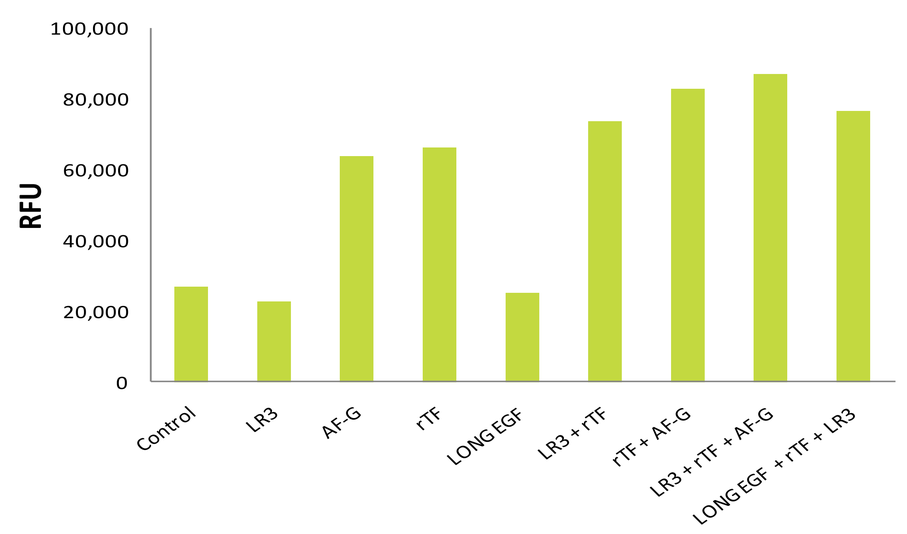
- MDCK cells (Fig.1) showed an increased growth response to media supplemented with transferrin. This response was enhanced by the addition of either LR3 (260% above control) or albumin (240% above control). The three components tested together increased the response further (560% above control), with a similar response observed when LONG EGF, LR3 and transferrin were tested in combination (450% above control).

Fig. 2. VERO cell growth



- VERO cells (Fig.2) showed an increased growth response when LR3 was tested in combination with either LONG EGF or transferrin. The strongest response was observed when all three proteins (LR3, LONG EGF and transferrin) were added together to the medium (105% above control culture growth), qualitatively similar to the response seen with MDCK cells.

Fig. 3. HEK 293 cell growth



- HEK 293 cells (Fig.3) showed increased growth with the single addition of either albumin or transferrin (140% and 150% respectively above control). Growth was enhanced further when LR3 plus transferrin were tested in combination (170% above control) and when albumin plus transferrin were tested in combination (210% above control). The greatest improvement to growth was observed when LR3, transferrin and albumin were added to the medium in combination (220% above control).

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