

**EFFECTS OF SERUM AND
GROWTH FACTORS ON
HEK 293 PROLIFERATION
AND APOPTOSIS, AND
ADENOVIRUS PRODUCTIVITY**

Angela Buckler and Mohamed Al-Rubeai

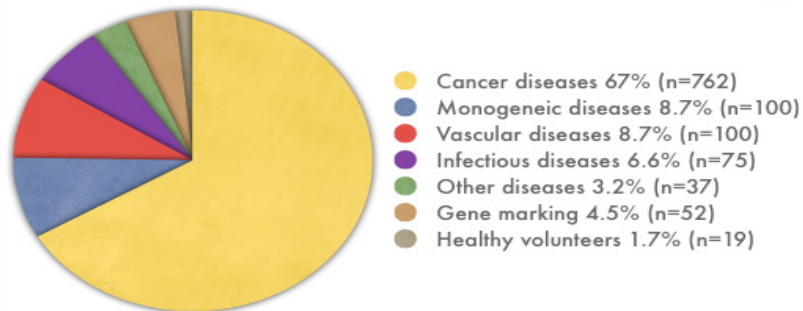


INTRODUCTION

- GENE THERAPY
- ADENOVIRUS
- SERUM
- SERUM FREE MEDIA
- INSULIN-LIKE GROWTH FACTORS
- RESULTS
 - EFFECT OF FCS ON CELL PROLIFERATION
 - EFFECT OF FCS ON VIRUS INFECTIVITY AND PRODUCTIVITY
 - EFFECT OF INSULIN-LIKE GROWTH FACTORS ON CELL PROLIFERATION
 - EFFECT OF INSULIN-LIKE GROWTH FACTORS ON VIRUS PRODUCTIVITY
- CONCLUSIONS

GENE THERAPY

Indications Addressed by Gene Therapy Clinical Trials

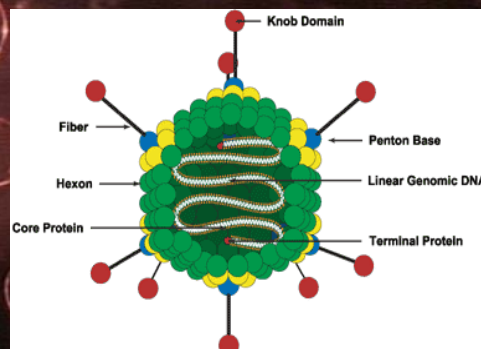


The Journal of Gene Medicine, © 2006 John Wiley and Sons Ltd

www.wiley.co.uk/genmed/clinical

ADENOVIRUSES

- Clinical Trials
- Belong to family *Adenoviridae*
- Double stranded DNA virus with non enveloped icosohedral capsid



SERUM FREE MEDIA

- Supplemented with hormones, growth factors, attachment factors and proteins, etc.
- Advantages over serum containing media
 - Simplified and better defined media
 - Reduced degree of contaminants
 - Elimination of potential source of infectious agents
 - Lower cost
- Cell growth and virus productivity?

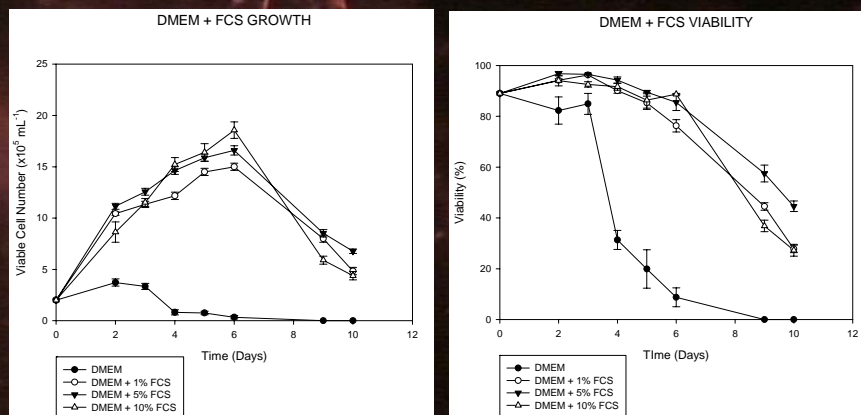
INSULIN-LIKE GROWTH FACTORS

- Growth factors such as insulin essential for cell proliferation
- Recombinant version is used in serum free media
- IGF-1 and insulin have very similar amino acid sequences and tertiary structures
- LONG (tm) R3 developed by substituting the third amino acid of IGF-1 with an arginine and adding a thirteen amino acid N-terminal extension

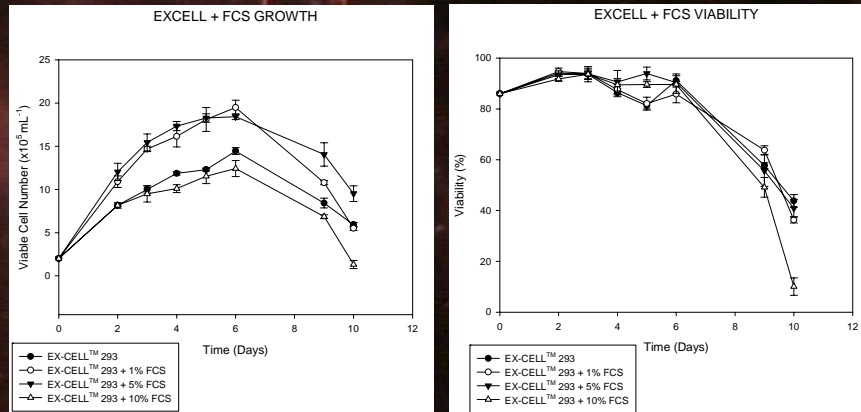
IGF BINDING PROTEINS

- Most cell types secrete IGF binding proteins (IGFBP) in various forms
- IGFBP inhibit activity of IGF-1 by requisitioning the peptide and preventing interactions between IGF-1 and IGF-1 receptor
- LONG (tm) R3 avoids this due to amino acid substitution
- LONG (tm) R3 has 100x reduced affinity for IGF binding proteins, making it a more potent growth factor which elicits similar responses to IGF-1 and insulin but at much lower concentrations

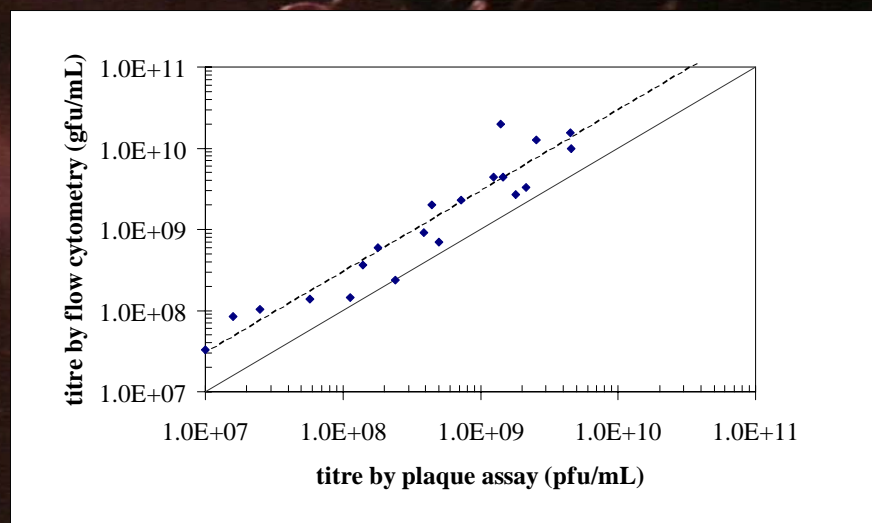
Effect of serum concentration on cell number and viability



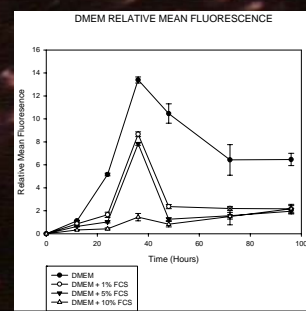
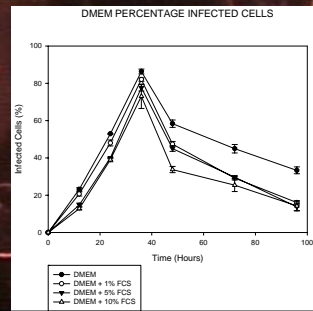
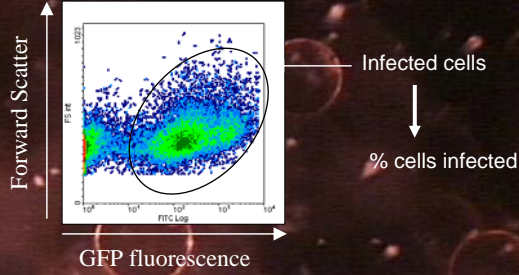
Effect of serum addition to serum-free media on cell number and viability



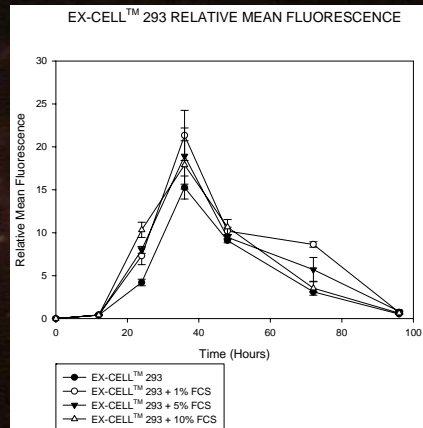
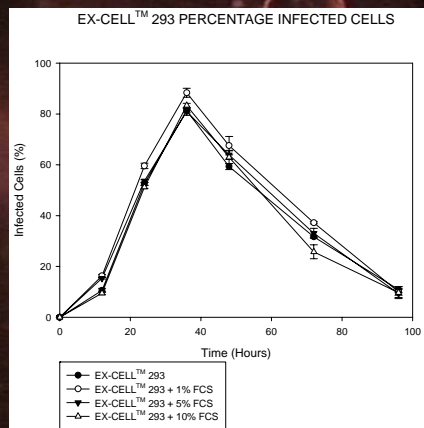
Flow cytometry vs Plaque assay



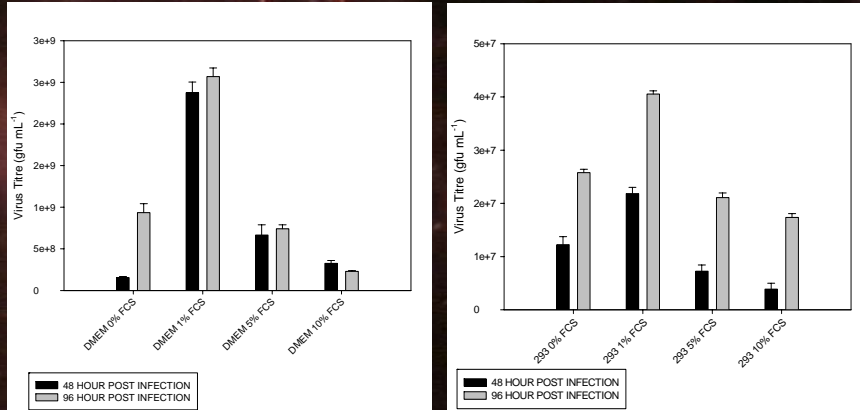
Effect of serum on infectivity



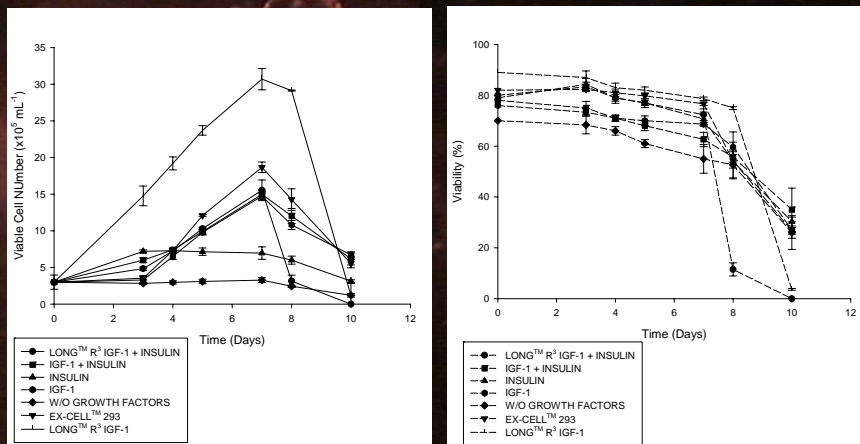
Effect of serum addition to serum-free media on infectivity



Higher virus titre obtained in low serum concentration

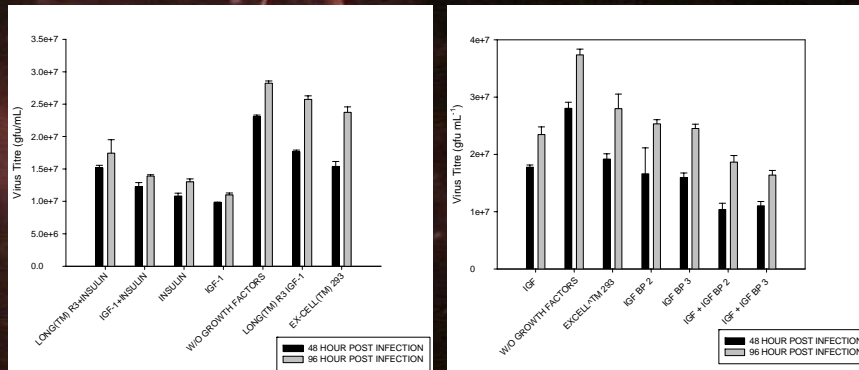


Long R3 promotes cell growth



Long R3 significantly increases growth rate, max cell number and IVC (Time integral of viable cell concentration)

Higher virus titre can be obtained in simple media



The presence of growth factors are inhibitory
 Long R3 has the least inhibitory effect compared to other growth factors
 Basic media is more suitable for infection

CONCLUSIONS

- Serum is required for cell growth in
- Serum decreases virus productivity in a dose dependent fashion
- Small quantity of serum is required for optimal virus productivity
- In serum free culture LONG™ R³ IGF-1 promotes cell growth more effectively than IGF-1 and Insulin
- No growth factors in media produces higher virus titres; may be due to the absence of IGF binding proteins

Acknowledgements

Terrell Johnson (SAFC Biosciences)

Animal Cell Technology Group (Birmingham)

Questions?

