Development of a chemically defined medium for virus vaccine production in a duck suspension cell line

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Introduction

A project was initiated to improve the production process for viral vaccines through the use of the EB66 cell line derived from duck embryonic stem cells and proprietary to Valneva. The primary goal of this project was to develop a chemically defined medium that could be used in a monophasic process while maintaining the growth and production of a broad spectrum of different viruses.

As with many viral cell culture-based production processes, viral production in EB66 cells is biphasic, requiring separate media for the cell proliferation phase and the virus production phase (Fig 1). Hence, the process includes two different basal medium formulations, with several separate additives or feeds required. While these processes are serum-free, they are not considered chemically defined.

Many cell lines employed in vaccine production are obligate attachment cells, requiring non-defined additives or even serum to maintain successful culture conditions. However, EB66 cells grow in serum-free suspension culture at high cell density, allowing for much easier and more efficient scale-up, as well as virus production levels comparable to attachment cells (Fig 2).

Material and methods

An initial growth-only screen of 20 different medium formulations, producers, and/or fortifications of existing media was performed to identify the most promising formulations to support good cell growth, viability, consistent morphology and level of cellular aggregation. Cell counts and viability were determined using flow cytometry. Screened formulations were assessed for viral production with representatives of secreted and non-secreted viruses, namely paramyxovirus and poxvirus. Viral titer was determined via standard TCID50 methods. Scale-up in bioreactor level was also evaluated, prior to selection of a final formulation.

Discussion and conclusions

Using non-optimized monophasic production processes, viral titer was comparable to the standard biphasic production process, where serum-free media are used. EB66 cells banked in other serum-free media recover from cryopreservation quickly, and readily adapt to serum-free media. EB66 cells banked in other serum-free media are used. EB66 cells banked in other serum-free media recover from cryopreservation quickly, and readily adapt to serum-free media. EB66 cells banked in other serum-free media recover from cryopreservation quickly, and readily adapt to serum-free media. EB66 cells banked in other serum-free media recover from cryopreservation quickly, and readily adapt to serum-free media. EB66 cells banked in other serum-free media recover from cryopreservation quickly, and readily adapt to serum-free media. EB66 cells banked in other serum-free media recover from cryopreservation quickly, and readily adapt to serum-free media. 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constant for at least fifteen passages. Working cell banks made with HyCone CDM4Avian medium have worked well, exhibiting rapid recovery from cryopreservation. The CDM4Avian formulation and the monophasic approach are currently being investigated with several other viruses with encouraging results. Overall, CDM4Avian medium is the first chemically defined medium for EB66 cells that fulfils all critical requirements needed to grow viruses in a modern cell culture system.