EFFECT OF INSULIN ON INFLUENZA PRODUCTION IN HEK293 CELLS

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Mammalian cells are considered an alternative to eggs for the production of influenza vaccines. HEK293SF-3F6 is a suspension GMP cell line that grows in serum-free media, and influenza production has been achieved in flasks and large bioreactors. In order to boost influenza production, insulin was added to the cultures. This growth factor was chosen because it has cell survival effects, it acts on cellular signaling pathways that are exploited by the influenza virus and it is approved by regulatory agencies.

HEK293SF-3F6 cells were infected with H1N1/A/Puerto Rico/08/34 or H3N2/A/Aichi/8/68 in a 24-well microbioreactor cassette. Next, 5 to 100mg/L insulin was added. After 48hrs, supernatants were collected and hemagglutinin (HA) was quantified. HA is highly expressed at the surface of the virus, and it was quantified by dot blot using a pan-HA antibody developed in-house.

The HA concentration was increased by almost 2-fold with insulin: Depending on the media, the effective insulin concentration varied; with H1N1/A/Puerto Rico/08/34, 25 to 100mg/L insulin increased the yield in CD293 media, whereas concentrations between 5 and 25mg/L resulted in a similar increase in an in-house media (IHM-03). H3N2/A/Aichi/8/68 virus yield was increased with 25 mg/L insulin in CD293 media. Overall, a concentration of 25mg/L insulin provided an increase in influenza yield regardless of the media or viral strain used in HEK293SF-3F6 cells. A concomitant activation of signaling pathways associated with cell survival (PI3K-Akt pathway) was observed.

In conclusion, insulin is known to stimulate cell proliferation. Here we show that adding 25mg/L insulin is also an effective way of increasing influenza production and can easily be implemented in a vaccine bioprocess. Evaluation of the effect of insulin on the production of other viruses and viral vectors is in progress.

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