Development of Serum Free Medium and Optimization of Porcine Rotavirus Vaccine Production

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Abstract

Serum is a potential source of bacterial, mycoplasmal and viral contamination, and it has a possibility of the introduction of serum proteins, prion and pyrogens into the final vaccine product. For porcine Rotavirus vaccine production, it is necessary to develop serum free medium which do not cause those problems. A new serum free medium was developed for porcine Rotavirus vaccine based on DMEM, and the performance of developed serum free medium was evaluated in terms of Vero cell growth and Rotavirus vaccine production. The cell density, grown in serum free medium developed, was similar with that in serum supplemented medium. Also, it was higher than that in other commercially available serum free medium. The productivity of Rotavirus vaccine using serum free medium developed and optimum production strategies will be also discussed.

Introduction

Rotaviruses, the major pathogen of severe dehydrating diarrhea in young of numerous species including human worldwide, are non-enveloped viruses formed by three concentric layers of protein that enclose a genome of double-stranded RNA. In developed countries, rotavirus is responsible not only for heavy medical and societal costs but also heavy loss of young animals including swine. Thus, the development and implementation of a safe and effective rotavirus vaccine has been a global goal for both public health and disease control of livestock. The maintenance of most mammalian cell lines in culture requires the addition of serum to the culture medium. The elimination of serum from mammalian cell culture is desirable since serum is expensive and a source of contaminants, e.g. viruses, mycoplasma or prions. And the use of serum in the cultures presents some disadvantages, such as safety and difficulty of approval. In this study, we developed a new serum free medium for porcine Rotavirus vaccine based on DMEM, and the

performance of developed serum free medium was evaluated in terms of Vero cell growth and Rotavirus vaccine production.

Materials and Methods

Vero cells (VERO-ATCC-CCL81), the host cell for Rotavirus vaccine production, were propagated at 37 °C in 5% CO_2 with RPMI 1640 (Gibco, Grand Island, NY) supplemented with 5%/3% fetal bovine serum (FBS, Hyclone). And then the cells were trypsinized, collected and washed twice with phosphate buffered saline (PBS). Cells were inoculated at 1×10^5 cells/ml with various serum free media in T-25 flask. After 72h/96h of cultivation in each medium, cell density was measured by trypan blue dye exclusion method. Various commercially available serum free media and serum-free medium developed based on DMEM were tested in terms of cell density and Rotavirus vaccine production. Rotavirus used in this study was purchased from ATCC and virus titers were determined by plaque assay.

Results and Discussion

Figure 1 and 2 show the comparison of cell densities at 72h/96h obtained from various serum free media with respect to serum supplemented medium. Three serum free media developed and four commercially available serum free media were screened for the proliferation of Vero cells. Vero cells were pre-adapted with RPMI 1640 supplemented with 3% FBS. As shown on the Figure 1, the cell density (1.6×10^6cells/ml) in serum free medium III was higher than that in RPMI 1640 supplemented with 3% FBS (1.3×10^6cells/ml) at 96h. Also, it was higher than that in other commercially available serum free media. Figure 2 shows the comparison of cell density of Vero cells pre-adapted with RPMI 1640 supplemented with 5% FBS. Although cells supplemented with 5% serum showed higher cell density than that with 3% serum, the figure showed that the density of cells pre-adapted up to 3 % showed better growth than that of cells pre-adapted up to 5 % in most serum free media. Therefore free adaptation is also a important factor to develop a serum free media for Rotavirus vaccine production.

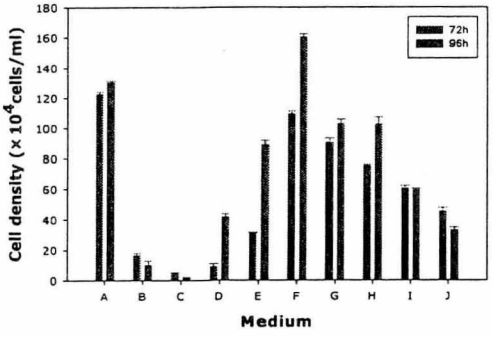

Fig. 1. The Cell Density in various Medium. Vero cells were pre-adapted with RPMI 1640 supplemented with 3% FBS.
A: Serum(3%) RPMI, B: W/0 FBS RPMI
C: W/0 FBS DMEM, D: SFM I (developed),
E: SFM II (developed), F: SFM III
(developed), G: EX cell 301 SFM, H: CHO
-Protein free, I: MDBK SFM, J: VP SFM

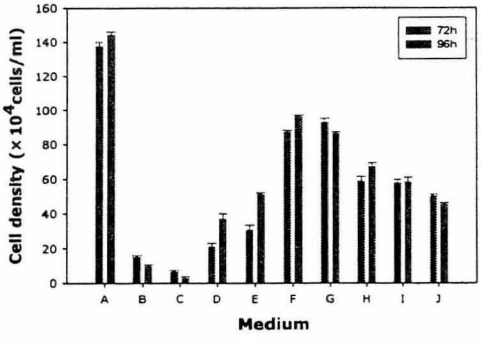

Fig. 2. The Cell Density in various Medium. Vero cells were pre-adapted with RPMI 1640 supplemented with 5% FBS.
A: Serum(5%) RPMI, B: W/0 FBS RPMI
C: W/0 FBS DMEM, D: SFM I (developed),
E: SFM II (developed), F: SFM III
(developed), G: EX cell 301 SFM, H: CHO
-Protein free, I: MDBK SFM, J: VP SFM

Summary

In this study, an optimum serum free medium for the Vero cell growth were screened to produce Rotavirus vaccine. It was confirmed that the cell density grown in serum free medium developed was higher than that in serum supplemented medium and in other commercially available serum free media. The productivity of Rotavirus vaccine employing serum free medium developed and optimum vaccine production strategies will be also discussed.

References

1. Sánchez-San Martín C, López T, Arias CF, López S (2004), Characterization of Rotavirus Cell Entry. Journal of Virology, 78(5): 2310-2318.
2. Victoria Pando, Pavel Ia, Carlos F. Arias and Susana López (2002), Influence of Calcium on the Early Steps of Rotavirus Infection. Virology, 295(1): 190-200.
3. Jong Hwa Park, Ki Woong Lee and In Sik Chung (1998), Improved propagation of human rotavirus from cell cultures of rhesus monkey kidney cells using medium supplemented with DEAE-dextran, dimethyl sulfoxide and cholesterol. Biotechnology Techniques, 12(1): 7-10.
4. Gyun Min Lee, Eun Jung Kim, No Soo Kim, Sung Kwan Yoon, Yong Ho Ahn, Ji Yong Song (1999), Development of a serum-free medium for the production of erythropoietin by suspension culture of recombinant Chinese hamster ovary cells using a statistical design. Journal of Biotechnology, 69: 85-93.