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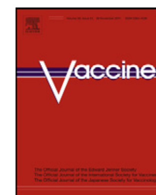

Process optimization and scale-up for production of rabies vaccine live adenovirus vector (AdRG1.3)

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ABSTRACT

Rabies virus is an important causative agent of disease resulting in an acute infection of the nervous system and death. Although curable if treated in a timely manner, rabies remains a serious public health issue in developing countries, and the indigenous threat of rabies continues in developed countries because of wildlife reservoirs. Control of rabies in wildlife is still an important challenge for governmental authorities.

There are a number of rabies vaccines commercially available for control of wildlife rabies infection. However, the vaccines currently distributed to wildlife do not effectively immunize all at-risk species, particularly skunks. A replication competent recombinant adenovirus expressing rabies glycoprotein (AdRG1.3) has shown the most promising results in laboratory trials. The adenovirus vectored vaccine is manufactured using HEK 293 cells.

This study describes the successful scale-up of AdRG1.3 adenovirus production from 1 to 500 L and the manufacturing of large quantities of bulk material required for field trials to demonstrate efficacy of this new candidate vaccine. The production process was streamlined by eliminating a medium replacement step prior to infection and the culture titer was increased by over 2 fold through optimization of cell culture medium. These improvements produced a more robust and cost-effective process that facilitates industrialization and commercialization. Over 17,000 L of AdRG1.3 adenovirus cultures were manufactured to support extensive field trials. AdRG1.3 adenovirus is formulated and packaged into baits by Artemis Technologies Inc. using proprietary technology. Field trials of AdRG1.3 rabies vaccine baits have been conducted in several Canadian provinces including Ontario, Quebec and New Brunswick. The results from field trials over the period 2006–2009 demonstrated superiority of the new vaccine over other licensed vaccines in immunizing wild animals that were previously difficult to vaccinate.

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1. Introduction

Rabies is an old disease with a historical background that goes back to ancient Egypt, however, it is still bringing new challenges to public health agencies. Rabies is a viral zoonosis caused by an RNA virus belonging to the family *Rhabdoviridae*, the *Lyssavirus* genus, capable of infecting all mammals. The virus is transmitted via saliva after animal bites, resulting in an acute infection of the nervous system. Cases of human rabies with established clinical symptoms are essentially fatal. Although curable if treated in a timely manner, rabies remains a serious public health problem in many developing countries, and the indigenous threat of rabies continues in developed countries because of wildlife reservoirs. Globally, there is an unacceptably high mortality of approximately 55,000 human cases of rabies each year [1]. Therefore, control of rabies in wildlife is a critical element in the strategy for prevention of rabies in humans and domestic animals.

In Ontario, Canada, the primary terrestrial vectors of rabies have been red foxes and striped skunks for the Arctic variant, and raccoons for the raccoon variants [2]. There was an average of 1500 confirmed cases of wildlife rabies per year between 1958 and 1990 in Ontario. The majority of those cases were in red foxes (46%) and striped skunks (20%) that were most likely infected with an Arctic variant of the rabies virus. Fortunately, rabies prevalence has been substantially reduced in Ontario as a result of wildlife oral vaccination campaigns conducted by the Ontario Ministry of Natural Resources (OMNR) since the early 1990s. In particular, rabies in foxes has been eliminated in certain parts of the province using the live attenuated ERA (Evelyn-Rokitnicki-Abelseth) vaccine [3], and the raccoon variant of rabies has been successfully eliminated from

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Ontario through a combination of vaccination by Trap-Vaccinate-Release (TVR) and the distribution of oral rabies vaccine baits containing vaccinia-rabies glycoprotein (V-RG) vaccine and other vaccination techniques [4]. There were only 16 confirmed cases in terrestrial mammals in 2009, and 50% of the confirmed cases were in skunks. However, the vaccines currently distributed to wildlife do not effectively immunize all at-risk species, especially skunks [4–6]. Consequently, alternative efficacious vaccines are needed to target skunks and other at-risk species [5,7].

The human adenovirus rabies glycoprotein (AdRG1.3) construct was prepared in the laboratory of Dr. Ludvik Prevec at the Department of Biology, McMaster University, Hamilton, Ontario, Canada [8]. The virus master seed and the cell line master bank were prepared and tested at Microbix Biosystems Inc. (Toronto, Ontario). In 1999, the virus master seed and the cell line master seed were transferred to Artemis Technologies Inc. (Guelph, Ontario) to establish working seeds and develop the production process. In laboratory trials conducted in collaboration with the Rabies Research and Development Unit, Ontario Ministry of Natural Resources, the AdRG1.3 construct has proved immunogenic via the oral route in skunks and other animals (unpublished data) and therefore is effective in a reservoir host that was previously difficult to vaccinate orally [8]. The key element in this vaccine candidate is a replication competent human adenovirus type 5 vector expressing rabies glycoprotein produced using an HEK 293 cell culture process. To evaluate the efficacy of this vaccine, a feasible and cost-effective large-scale cell culture production process had to be developed for manufacturing mass quantities of AdRG1.3 required for the field trials. Furthermore, the process had to be designed to meet all requirements for industrialization and commercialization of this vaccine candidate.

Human adenovirus type 5 is the most characterized among the wide variety of human and non human adenoviral vectors, and its biology is well described [9]. Adenovirus vectors are highly efficient for gene transfer in a broad spectrum of cell types and species. Moreover, adenoviruses often induce humoral, mucosal and cellular immune responses to antigens encoded by the inserted foreign genes. Consequently adenovirus vectors have been widely investigated for gene delivery in numerous clinical protocols and they are still considered among the most effective vaccine vectors [10–12]. Over the last ten years there has been a set-back in gene therapy clinical protocols, particularly adenovirus-based protocols, following a death of patient [13], whereas recent discouraging results from the HIV vaccine STEP trial [14,15] raised concerns in using the adenovirus vector antigens in the case of HIV. Overall thousands of patients have been treated or vaccinated in clinical protocols using adenovirus vector contributing to generate solid clinical data demonstrating a good safety profile.

Methods of adenovirus production using HEK 293 cells vary from standard tissue culture plasticware to bioreactors using serum-containing or serum-free media cultures. The production of adenovirus is affected by a number of factors including cell line physiology, cell culture conditions and virus construct, and has been reviewed in several publications [16–18]. In general, nutrient limitation and/or accumulation of inhibitory metabolites are still the main factors limiting the yield of adenovirus [19]. A complete medium replacement at the time of infection has been commonly used as a strategy to reduce the nutrient limitations and/or metabolite accumulation in batch operation [16]. However, detailed information on the scale up of production process to large scale (*e.g.* more than 100 L) is limited.

Scale-up of the production process and improvement of product yield while maintaining product quality attributes are key success factors for manufacturing a large quantity of AdRG1.3 for field trials. Also, for successful commercialization, the production cost of the vaccine must be as low as possible to maintain its economical viability. This study describes the results of a series of experiments designed to improve the yield of AdRG1.3 culture and the robustness of the production process, and to reduce the production cost through optimization of cell culture media and fine-tuning of process parameters. Also, the study demonstrates the successful scale up of the production process in three different sizes of bioreactors up to 500 L.

2. Materials and methods

2.1. Cells, virus, sera and media

HEK 293 N3S cells with a population doubling level of 19.7 were provided by Artemis Technologies Inc. (Ontario, Canada). This 293 N3S cell, which was adapted for growth in suspension by passaging 293 cells in nude mice, expresses adenovirus 5 early region 1 (E1) antigens and is permissive for adenovirus 5 host range mutants defective in E1 functions [20]. The cells were maintained with basal media + 5% Foetal Bovine Serum (FBS) at 25 mL in 125 mL plastic shake flasks (Corning, NY), and agitated at 100 rpm on an orbital shaker at 37 °C and 5% CO_2 humidified incubator. They were subcultured to 0.3×10^6 cells/mL, three times a week. New cells were thawed every 2 months to prepare new cultures.

The adenovirus, AdRG1.3, is a replication competent recombinant human adenovirus type 5 (Ad5) expressing a rabies glycoprotein and accompanying SV40 polyA addition sequences within an E3 deletion [8]. AdRG1.3 Master Seed + 3 (MS + 3) was provided by Artemis Technologies Inc. and used for infection of cultures in shake flasks. AdRG1.3 MS + 3 was also amplified by infecting 293 N3S cultures grown in IHM-02 medium + 5% FBS in 2 L shake flasks or 20 L bioreactor to produce AdRG1.3 MS + 4. The amplified viral stock was aliquoted in 250 mL conical tubes or 1000 mL bottles with a respective seed volume of 150 mL or 800 mL each, stored at −80 °C, and used for infection of cultures in large-scale bioreactors.

Four commercial basal media, DMEM, DM-, Hybridoma-SFM (all from Invitrogen Corp., Grand Island, NY) and Ex-Cell Vpro (SAFC Biosciences, St. Louis, MO), were used for evaluation of the cell growth and virus production. The formulation of DMEM and DM- is similar except that the concentrations of some amino acids and vitamins are lower in the DM- medium. Both Hybridoma-SFM and Ex-Cell Vpro are commercial proprietary media and their formulations are not publicly available. Two proprietary media developed in-house, namely IHM-01 and IHM-02, were also used in the study. These in-house media were DM/F12 base media supplemented with potential limiting nutrients identified in published literatures and through extensive screening tests. IHM-01 and IHM-02 media were either prepared in house or outsourced to media manufacturing companies (Lonza, Walkersville, MD; Gibco, Grand Island, NY).

Canadian origin FBS was purchased from PAA Laboratories Inc. (Etobicoke, Ontario, Canada) and Sigma–Aldrich (Oakville, Ontario, Canada). The sera were tested for cell growth and virus production before the purchasing.

2.2. Small-scale process development

Small-scale experiments were all performed in 125 mL shake flasks (Corning, NY) with a working volume of 25 mL. The seeding density was around 2×10^5 cells/mL, and cultures were grown in basal media + 2% or 5% FBS to various cell densities. The culture was then infected with AdRG1.3 MS + 3 with or without a complete medium exchange before infection, depending on the experimental design. A multiplicity of infection of 10 infectious viral particles per cell (MOI = 10), as determined from preliminary experiment results and previous reports [21], was used for all

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