Preventive, Diagnostic and Therapeutic Applications of Baculovirus Expression Vector System

Neeraj Kumar, Deepak Pandey, and Ashutosh Halder

Abstract

Different strategies are being worked out for engineering the original baculovirus expression vector (BEV) system to produce cost-effective clinical biologics at commercial scale. To date, thousands of highly variable molecules in the form of heterologous proteins, virus-like particles, surface display proteins/antigen carriers, heterologous viral vectors and gene delivery vehicles have been produced using this system. These products are being used in vaccine production, tissue engineering, stem cell transduction, viral vector production, gene therapy, cancer treatment and development of biosensors. Recombinant proteins that are expressed and post-translationally modified using this system are also suitable for functional, crystallographic studies, microarray and drug discovery-based applications. Till now, four BEV-based commercial products (Cervarix®, Provenge®, Glybera® and Flublok®) have been approved for humans, and myriad of others are in different stages of preclinical or clinical trials. Five products (Porcilis® Pesti, BAYOVAC CSF E2®, Circumvent® PCV, Ingelvac CircoFLEX® and Porcilis®PCV) got approval for veterinary use, and many more are in the pipeline. In the present chapter, we have emphasized on both approved and other baculovirus-based products produced in insect cells or larvae that are important from clinical perspective and are being developed as preventive, diagnostic or therapeutic agents. Further, the potential of recombinant adeno-associated virus (rAAV) as gene delivery vector has been described. This system, due to its relatively extended gene expression, lack of pathogenicity and the ability to transduce...
a wide variety of cells, gained extensive popularity just after the approval of first AAV-based gene therapy drug alipogene tiparvovec (Glybera®). Numerous products based on AAV which are presently in different clinical trials have also been highlighted.

9.1 Introduction

Baculovirus (family: Baculoviridae) derived its name from the Latin word “baculum” meaning “stick”. They are rod-shaped (30–60 × 250–300 nm) large enveloped viruses with circular, supercoiled double-stranded DNA genomes, approximately 80–180 kb in size. While most of the baculoviruses infect their natural host, i.e., butterflies and moths (Lepidoptera), few are also known to infect sawflies (Hymenoptera) and mosquitoes (Diptera) (King et al. 2011). They have not been linked with any disease in any organism outside the phylum Arthropoda (Kost and Condreay 2002). Baculoviruses are well known for their role as biopesticides and are efficient tools for heterogeneous protein production in insect cells (Summers 2006). Morphologically, these enveloped viruses have been classified into two phenotypes: occlusion-derived viruses (ODVs) that are embedded in paracrystalline matrix forming polyhedral occlusion bodies (OBs) which are responsible for horizontal transmission between insects and the budded viruses (BuVs) present in the haemolymph which spreads infection from cell to cell (Luckow and Summers 1988). Occlusion body morphology was initially used to define two major groups of baculoviruses: nucleopolyhedroviruses (NPVs) and the granuloviruses (GVs). NPVs obtain their envelop from host nuclear membrane and are occluded within main occlusion protein polyhedrin forming large (1–15 μm) polyhedral inclusion bodies, while GVs obtain their envelop from cell membrane to make oval-shaped single virion structure called granule or capsule with diameter in the range of 0.2–0.4 μm (King et al. 2011). NPVs are further distinguished as single nucleopolyhedrovirus or multiple nucleopolyhedrovirus based on the number of nucleocapsids in a polyhedral inclusion body (O’Reilly et al. 1994). OBs allow virions to remain infectious for long period due to their highly resistant and stable structure.

Baculovirus-infected insect cell expression system has been used for the routine production of recombinant proteins, including several proteins of therapeutic nature over the last three decades. The establishment of this system begins from the production of human beta interferon (INF-β), the protein normally not produced in the cultured human cells. It was produced with a recombinant Autographa californica multiple nucleopolyhedrovirus (AcMNV) by exploiting its polyhedrin promoter (Smith et al. 1983). In this system, the protein coding sequence of human interferon gene was linked to the AcNPV polyhedrin gene promoter. The interferon gene was inserted at different positions relative to the AcNPV polyhedrin transcriptional and translational signals. The interferon-polyhedrin hybrid plasmid was then transferred to infectious AcNPV expression vectors by recombination within S. frugiperda insect cells, where more than 95% of biologically active glycosylated interferon was produced in the secreted form.
At the same time, another group successfully expressed *Escherichia coli* β-galactosidase gene in insect cells by using this system. A 9.2 kb plasmid construct was made of β-galactosidase gene (1 kb) after fusion with the N-terminal region of the polyhedrin gene (1.2 kb) of AcNPV. Co-transfection of this fused plasmid construct with wild-type AcNPV genomic DNA (134 kb) was performed in order to insert the foreign gene into the polyhedrin gene of AcNPV genome by the process of homologous recombination. Finally, the recombinant viruses were selected as blue plaques in the presence of β-galactosidase indicator X-gal medium. These discoveries mark the beginning of baculovirus expression system, facilitating the engineering and improvement of baculovirus vectors, modification of the sugar moieties of glycoproteins expressed in insect cells and scale up of the cell culture processes.

A baculovirus expression vector (BEV) platform has been tailored by taking advantage of baculoviruses’ natural tendency to infect insect cells. There are almost 500 different types of baculoviruses, all of which specifically infect invertebrates. For laboratory research and manufacturing purposes, the most commonly studied baculovirus is *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) which is often considered as a prototype of baculoviruses. It has a double-stranded circular DNA genome of 134 kb inside a rod-shaped nucleocapsid of size 25 × 260 nm (Fauquet et al. 2005). Its large size genome gives sufficient ability to accommodate a large foreign DNA or multiple genes together.

Typically, recombinant BEVs are constructed by co-transfecting a mixture of transfer plasmid and modified non-infectious and linearized AcMNPV that lacks parental polyhedrin gene and a portion of ORF1629. Transfer plasmid contains the gene of interest (GOI), flanked upstream by strong polyhedrin or p10 promoter and downstream by an essential portion of ORF1629 of AcMNPV for high-level protein expression in insect cells. The transfer plasmid and modified linearized AcMNPV DNA undergo homologous recombination to generate de novo recombinant baculoviruses. After plating of these baculoviruses, single pure plaques of recombinant baculovirus are selected. Subsequently, this plaque is passaged through multiple rounds of insect cell infection to generate a high-titre stock. It creates a working virus bank (WVB) for utilization during downstream processes.

This system was further enhanced for manufacturing and commercialization purposes by multiple ways and technologies. Bacmid technology (Bac to Bac®, Life Technologies) was employed for the generation of recombinant AcMNPV genomes in bacterial host system *E. coli*. FlashbackTM (Oxford Expression Technologies Ltd.) and BacMagicTM (Merck) BaculoOneTM (PAA) technologies are used to avoid the bacterial sequences in the final vector or rapid production of multiple recombinant viruses in a one-step procedure. MultiBac system is being used for the synthesis of multisubunit protein complex and OmniBac as multigene transfer vector for universal generation of recombinant baculoviruses. Sleeping beauty or PiggyBac transposon system are being exploited in highly efficient seamless excision of transposons from the genomic DNA and for its potential to target integration events to desired DNA sequences. For the production of AcMNPV vectors and
recombinant proteins, the *Spodoptera frugiperda* Sf21 and its subclone Sf9 and High Five cell lines are being used. These insect cells exhibit several properties like rapid growth, stress resistance and robust expression of recombinant proteins that make them suitable for the production of clinical biologics and commercial products.

Initially, insect-derived baculovirus expression vector (BEV) was recognized as a safe system for routine production of recombinant proteins both in insect and mammalian cells. During the last three decades, it has emerged as an effective tool for research as well as various applications in the field of biotechnology. It has shown tremendous potential as preventive, diagnostic and therapeutic agent against a myriad of diseases in the form of vaccination, tissue engineering, stem cell transduction, viral vector production and gene therapy (Airenne et al. 2013). It has been extensively used for functional studies, crystallography, biosensors, protein microarray and drug discovery. All these applications are based on different baclovirus-derived products such as heterologous proteins, protein/antigen displayed on baculovirus particle surface, heterologous viral vectors and gene delivery vehicles for mammalian cells (van Oers et al. 2015). In this chapter, we have presented the application of these products from a clinical point of view in three main categories, viz. preventive, diagnostic and therapeutic agents. Most of the approved biomolecules produced by using baculovirus expression system in insect cells have been discussed. As thousands of other products are being developed by BEVs, it seems ineffectual to include the entire list under the ambit of the present chapter; however few among them have been mentioned to have an understanding about the scope of this powerful expression system in the near future.

### 9.1.1 BEVs As Disease Preventive Agents

BEV exhibits many characteristics that make it suitable for the production of heterologous proteins in insect cells. It can be easily handled in the BSL1/2 laboratories due to its harmless nature to nontarget organisms. These viruses are environmentally safe due to their instability outside the laboratory. It is used to produce high level of proteins in insect cells or larvae where the eukaryotic environment provides the appropriate post-translational modifications. BEVs host insect cells are mostly free of human pathogens and do not require controlled oxygenic environment for their growth. Insect cells can be grown into serum-free medium, and the heterologous protein production can be enhanced to the level of pilot plant or larger bioreactors. Therefore, the proteins obtained by the BEVs can be used as vaccines either in the form of heterologous subunit proteins or virus-like particles (VLPs) formed by subunit proteins of virus.

Subunit vaccines are relatively safe as they are devoid of virus genetic material but exhibit poor immunogenicity that might be due to incorrect folding of the target protein. Structural proteins of viruses such as capsid and envelop proteins assemble into particulate structure similar to the naturally occurring virus or subviral particles. Therefore, virus-like particles (VLPs) that are non-infectious and
non-replicating due to the absence of viral genetic material can be produced in heterologous system (Yamaji 2014). VLPs are highly effective in eliciting both humoral and cellular immune response because of their densely repeated display of viral antigens in right conformation (Roy and Noad 2008). VLPs comparatively exhibit wide spectrum of clinical applications such as prevention of disease as vaccines, diagnostics as antigens for the detection of antibodies and therapeutics in the form of therapeutic vaccines and delivery agents. The use of heterologous proteins and VLPs as preventive agents in the form of vaccines against different diseases is being described (Table 9.1).

A decade back, only two veterinary products were manufactured using BEVs to prevent classical swine fever in pigs. Now, five more new vaccines have been approved, two of which are for humans, and many more products are in the development phase. Here, approved vaccines as well as development of other vaccines in preclinical stages have been highlighted.

9.1.1.1 Veterinary Vaccines

(a) Subunit marker vaccine for classical swine fever: Classical swine fever virus (CSFV) infection invariably develops antibodies against virus envelop proteins E\textsuperscript{NS} and glycoprotein E2 and the non-structural protein NS3 in swine (Paton et al. 1991). However, injection of only glycoprotein E2 in pigs is reported to sufficiently provide protection to CSFV (Van Rijn et al. 1996). Therefore, a subunit vaccine has been produced on the basis of conserved glycoprotein E2 with a baculovirus vector in insect cells (Moormann et al. 2000). Glycoprotein E2 being expressed as envelop protein, its C-terminal transmembrane domain was removed to secrete it into the medium, and the residual baculovirus was inactivated with 2-bromoethyl-imminebromide. This vaccine was manufactured and commercialized as “Porcilis Pesti®” by MSD Animal Health. The same vaccine was also commercialized as “BAYOVAC CSF E2®/Advasurea” by Bayer AG/Pfizer Animal Health but was later discontinued.

(b) Virus-like particle (VLP)-based vaccine for porcine circovirus type 2: Porcine circovirus type 2 (PCV2) vaccine was developed based on VLPs. PCV2 is the primary causative agent of postweaning multisystemic wasting syndrome (PMWS) in swine. Two major open reading frames (ORF1 and ORF2) have been identified in PCV2. ORF2 encodes a major structural protein with type-specific epitopes and is found to be highly immunogenic. Therefore, ORF2 that encodes the capsid protein was used to develop the vaccine with a baculovirus in Tn5 insect cells (Liu et al. 2008). Insect Sf9 and Tn5 cells were infected with recombinant baculovirus AcPCV2-ORF2 that contains the complete PCV2 capsid protein. As compared to Sf9 insect cells, Tn5 expressed higher amount of PCV2 capsid protein as virus-like particles of size around 28-KDa. This vaccine was commercialized by two different names “Circumvent PCV and Porcilis PCV” in different geographical areas by MSD Animal Health (known as Merck Animal Health in the USA and Canada) (Felberbaum 2015). Vaccine for PCV2-based ORF2 was also commercialized as “Ingelvac CircoFLEX” by Boehringer Ingelheim Vetmedica Inc. (Desrosiers et al. 2009).
| Product name (company name, if any) | Targeted/used for | Expressed product | Used against | Product type | Development stage |
|-------------------------------------|------------------|-------------------|--------------|--------------|------------------|
| **Porcilis Pesti (MSD Animal Health)** | Pigs             | E2 glycoprotein   | Classical swine fever | Protein subunit/marker vaccine | Approved |
| **Bayovac CSF E2 (Bayer Biologicals/Pfizer Animal Health)** | Pigs             | E2 glycoprotein   | Classical swine fever | Protein subunit/marker vaccine | Approved |
| **Circumvent PCV (MSD Animal Health)** | Pigs             | Porcine circovirus ORF2 | Porcine circovirus type 2 | VLP vaccine | Approved |
| **Porcilis PCV (MSD Animal Health)** | Pigs             | Porcine circovirus ORF2 | Porcine circovirus type 2 | VLP vaccine | Approved |
| **Ingelvac CircoFLEX (Ingelvac)** | Pigs             | Porcine circovirus ORF2 | Porcine circovirus type 2 | VLP vaccine | Approved |
| **AcAs3-PPV-VLP** | Pigs             | Viral capsid protein VP2 | Porcine parvovirus (PPV) | VLP vaccine | Unapproved |
| **BTV-1/BTV-4 VLP** | Sheeps           | BTV serotype 1 and 4 | Bluetongue virus (BTV) | VLP vaccine | Unapproved |
| **AI-H5N3 VLP** | Ducks            | Subunits HA, NA and M1 | Avian influenza (AI) | VLP vaccine | Unapproved |
| **IDBV-VLP** | Chickens         | Capsid proteins VP2, VP3 and VP4 | Infectious bursal disease virus (IBDV) | VLP vaccine | Unapproved |
| **RHDV-VLP** | Rabbits          | Capsid proteins VP60 | Rabbit haemorrhagic disease virus (RHDV) | VLP vaccine | Unapproved |
| **SIV-VLP** | Primates         | Precursor protein Pr56gag | Simian immunodeficiency virus (SIV) | VLP vaccine | Unapproved |
| **Flublok (Protein Sciences)** | Humans           | Influenza HA      | Trivalent flu vaccine | Protein subunit vaccine | Approved |
| **Cervarix (GlaxoSmithKline)** | Humans           | Human papillomavirus L1 protein (serotypes 16 and 18) | Cervical cancer | VLP vaccine | Approved |
| **Ebola-VLP** | Humans           | Ebola VP40 and GP protein | Ebola virus | VLP vaccine | Preclinical |
| Product Name                        | Species | Antigen Description                  | Virus/Agent                        | Application | Status     |
|------------------------------------|---------|--------------------------------------|------------------------------------|-------------|------------|
| Bac-P1-3CD Humans EV71-P1 protein and 3CD protease | Humans | Enterovirus 71 VLP vaccine | Preclinical                         |             |
| VAI-VP705 (NIH/Meridian Life Science) | Humans | B19 VP1, VP2 | Parvovirus B19 VLP vaccine | Phase I/II  |            |
| NV-VLP (Baylor College of Medicine) | Humans | Capsid proteins NV CP | Norwalk virus (Nv) VLP vaccine | Phase I     |            |
| NV-VLP (Ligo Cyte Pharmaceuticals) | Humans | Capsid proteins NV CP | Norwalk virus (Nv) VLP vaccine | Phase I     |            |
| NV-VLP (Ligo Cyte Pharmaceuticals) | Humans | Capsid proteins NV CP | Norwalk virus (Nv) VLP vaccine | Phase I/Phase I/II |            |
| PV-VP1-VLP Humans Major capsid protein VP1 | Humans | Polyomavirus VLP vaccine | Preclinical                         |             |
| SARS-CoV-VLP Humans SP, EP and MP | Humans | Severe acute respiratory syndrome-associated coronavirus (SARS-CoV) VLP vaccine | Preclinical                         |             |
| SV40-VLP Humans VP1 or P1 and 3CD | Humans | Simian virus 40 (SV40) VLP vaccine | Preclinical                         |             |
| RV-VLP Humans VP2, VP6 and VP7 | Humans | Rotavirus VLP vaccine | Preclinical                         |             |
| HIV-VLP Humans Gag protein | Humans | HIV VLP vaccine | Preclinical                         |             |
| Influenza (Novavax) Humans A/California/04/09(H1N1) HA, NA | Humans | Influenza VLP vaccine | Phase II |             |
| Influenza (Novavax) Humans A/Brisbane/59/07(H1N1), A/Brisbane/10/07, B/Florida/04/06 (H3N2) | Humans | Influenza VLP vaccine | Phase IIa |             |
| RSV (Novavax) Humans RSV-F | Humans | RSV VLP vaccine | Phase I |             |

*Discontinued

*MSD Animal Health got the same products licensed by two names in different geographical areas*
(c) **VLP-based vaccine for porcine parvovirus (PPV):** PPV, a non-enveloped DNA virus, causes major reproductive failure in swine. Its viral capsid is made up of 50–60 molecules of VP2, the major structural protein that are being targeted for vaccine development. VP2 gene was expressed under the control of late p10 promoter of baculovirus and the LacZ gene under the control of *Drosophila hsp* 70 promoter. The recombinant baculovirus AcAs3-PPV was used to infect Sp21 insect cell line to express VP2 that leads to self-assembled empty PPV VLPs in serum-free medium for safety point of view (Maranga et al. 2002). Earlier, it was also produced in *Sf9* cells in the presence of serum proteins. However, its commercialization at large scale still needs more developmental efforts.

(d) **VLP-based vaccine for sheep bluetongue virus (BTV):** Bluetongue primarily causes disease in ruminants due to infection by BTV double-stranded RNA virus. Sheep generally shows more severe clinical signs than other cattle. Recombinant baculovirus expression system in *Sf9* insect cell lines shows great potential to develop VLP-based vaccine against BTV (de Diego et al. 2011). Monovalent and bivalent VLP vaccines are being developed for two serotypes 1 and 4 of BTV. BTV-1 exhibits more protection to virulent BTV live strain as compared to BTV-4. Earlier, VLP expressing capsid proteins VP2 and VP5 were developed by co-transfection of dual transfer vector DNA (pAcVC3/BTV10-2/BTV10-5) with wild-type AcNPV DNA in insect cells (French et al. 1990). Strong developmental efforts and further research are needed to commercialize robust and effective BTV vaccine.

Many more VLPs veterinary vaccines by baculovirus expression system in insect cells have been developed such as avian influenza (AI), (H5N3)-VLPs that consists of subunits haemagglutinin (HA), neuraminidase (NA) and matrix protein (M1) of AI virus for ducks (Prel et al. 2008); chimeric infectious bursal disease virus (IBDV)-VLPs consisting of structural protein VP2, VP3 and VP4 with varying degree of one of the capsid protein VP2 tagged with histidine of IBDV for chickens (Hu and Bentley 2001); rabbit haemorrhagic disease virus (RHDV)-VLPs made up of VP60 capsid protein for rabbits (Laurent et al. 1994); and simian immunodeficiency virus (SIV)-VLPs consisting of precursor protein Pr56<sup>ppg</sup> for vaccine testing in non-human primates (Yamshchikov et al. 1995).

9.1.1.2 Human Vaccines

(a) **Subunit vaccine for influenza:** Influenza generally called as “the flu” is caused by RNA influenza viruses, designated from type A to C. Both type A and B influenza viruses possess haemagglutinin (HA) or neuraminidase (NA) glycoprotein spikes in their envelope which act as key antigens in the host immune response, therefore targeted for vaccine development. But HA and NA exhibit antigenic drift due to continuous mutations in the genetic material, and the vaccine based on these glycoproteins is required to be updated annually. However, type C influenza virus is not involved in annual influenza virus vaccine as they cause only mild respiratory disease in humans. The vaccine against influenza in *Spodoptera frugiperda* *Sf9* insect cells is developed by targeting dominant
surface glycoproteins HA of influenza virus. Recombinant viruses as vaccines are produced in Sf9 insect cells by co-transfecting linearized AcMNPV genomic DNA with the baculovirus transfer plasmids containing the HA gene. Recombinant plaques are selected on the basis of their morphology and virus stocks generated. These viral stocks are used for their amplification through passage in the fresh insect cell cultures. Commercial production of recombinant HA vaccine could begin within 45 days after identification of the new virus strain. It is commercialized by Protein Sciences Corporation by Flublok trademark (Cox and Hashimoto 2011).

(b) **VLP-based vaccine for human papillomavirus (HPV):** HPV infection causes mostly all forms of cervical cancer in women. HPVs are icosahedral viruses with double-stranded circular DNA codes for two classes of genes; early (E) and late (L). Early genes regulate replication, transcription and other biological processes, whereas late genes (L1 and L2) are responsible for structural components of the viral capsid. L1 capsid proteins are known to form virus-like particles, therefore targeted for vaccine development against HPV. L1-based vaccine in insect cells that shows remarkable safety profile and clinical efficacy from the genotypes HPV16 and 18 was commercialized by GlaxoSmithKline by the trademark Cervarix (Monie et al. 2008). It has been produced in *Trichoplusia ni* insect cell lines Hi-5 Rix4446 by using baculovirus expression system.

With the advent of successful cases of approved VLP-based vaccines, researchers are indeed redirecting their efforts for the development of such products. Therefore, a number of vaccines have been produced against many viral diseases in humans; however many of them are either in preclinical or clinical trial stages. Prominent VLPs that are made up of multimeric proteins expressed in insect *Sf9* cells include Ebola by VP40 and glycoproteins (Sun et al. 2009); enterovirus by P1 and 3CD (Chung et al. 2010); human parvovirus B19 by B19 VP1, VP2 (Roldão et al. 2010); Norwalk virus (Nv) by capsid proteins (Jiang et al. 1992; Ball et al. 1999; Atmar et al. 2011; Frey 2011); polyomavirus by VP1 (Montross et al. 1991); severe acute respiratory syndrome-associated coronavirus (SARS-CoV) by SP, EP, MP and EN (Mortola and Roy 2004) and simian virus 40 (SV40) by VP1 or P1 and 3CD (Kanesashi et al. 2003). VLPs for rotavirus were prepared by using two (VP2 and VP6) to three (VP2, VP6 and VP7) capsid proteins expressed both in *Sf9* and High Five insect cells. It has also been expressed in *Sf larvae* with two capsid proteins VP2 and VP6 (Roldão et al. 2010). Combinations of capsid proteins from different strains of influenza were used in both *Sf9* and High Five insect cells such as HA (H1N1) with M1 (H3N2) and HA (H3N2) with M1 (H1N1) to produce higher amount of influenza A-VLPs. Other influenza A-VLPs formed by co-expression of M1 and ESAT6-HA were produced only in High Five cells. Strain-specific influenza HA and M1 capsid proteins were used to prepare influenza A H1N1-VLPs and influenza A H3N2-VLPs in both the insect cells (Krammer et al. 2010; López-Macías et al. 2011). Respiratory syncytial virus (RSV) vaccine was produced by using RSV-F protein (Mazur et al. 2015; Neuzil 2016). HIV VLPs were produced by targeting gag protein in rodents and rhesus macaques for preclinical trials (Pillay et al. 2009; Wagner et al. 1996).
9.1.2 BEVs as Diagnostic Agents

Supposedly, both heterologous subunit proteins and VLP-based subunit vaccines can be used as vaccines as well as antigens for the detection of antibodies, given the condition that it satisfies the various diagnostic parameters like sensitivity, specificity, predictive values and likelihood ratios. These parameters have been well evaluated and found to be acceptable for diagnostic purposes for numerous BEV-derived products. However, commercialization of these vaccines/proteins demands further standardization and evaluation. Here, we have summarized some of the human as well as veterinary usage diagnostic molecules produced by BEV system in insect cells (Table 9.2).

Table 9.2  BEVs produced biomolecules as diagnostic agents

| Targeted/used for | Expressed product | Used to detect | Test type |
|------------------|------------------|----------------|----------|
| Rodents          | Recombinant nucleocapsid protein | Sendai virus | ELISA    |
| Swine            | G-protein        | Nipah virus (NiV) | ELISA    |
| Swine            | SVDV-VLP         | Swine vesicular diseases virus (SVDV) | ELISA    |
| Horse            | EIAV-core gag and p26 protein | Equine infectious anaemia virus (EIAV) | ELISA and agar gel immunodiffusion (AGID) |
| Horse            | HA               | Equine influenza strain LP/93 | ELISA    |
| Cattles          | VP7              | Bluetongue (BTV) and epizootic haemorrhagic disease (EHDV) | Antigen capture competitive ELISA (Ag Cap c-ELISA) |
| Pigs and goats   | VP1 capsid protein and 3C protease | Foot-and-mouth disease virus (FMDV)-type A | Blocking ELISA |
| Bovine           | Recombinant-F protein | Bovine respiratory syncytial virus (BRSV) | Immunofluorescence |
| Ducks            | E protein        | Tembusu virus (TMUV) | E-ELISA |
| Birds            | APMV2-HN         | Avian paramyxovirus type 2 | Haemagglutination inhibition (HI) test |
| Geese            | VP1              | Goose haemorrhagic polyomavirus (GHPV) | ELISA and haemagglutinin inhibition test |
| Humans           | Nucleocapsid protein (N) | Lassa virus | ELISA    |
| Humans           | E1, E2 and polyprotein precursor | Rubella virus (RV) | Enzyme immunoassay (EIA) and immunoblot |
| Humans           | Flagellar repetitive antigen (FRA) | Trypanosoma cruzi | ELISA    |
### Table 9.2 (continued)

| Targeted/used for | Expressed product | Used to detect | Test type                |
|-------------------|-------------------|----------------|-------------------------|
| Humans            | Glutamic acid decarboxylase (GAD65 and GAD67) | Insulin-dependent diabetes mellitus | Immunoassay |
| Humans            | Nucleocapsid protein of strain SR-11 | Hantavirus | Indirect immunofluorescence antibody (IFA) |
| Humans            | E2 protein | Human papillomavirus (HPV) | ELISA |
| Humans            | Hou/90 capsid | Human calicivirus (HuCV) | Immunoprecipitation and EIA |
| Humans            | Fragment of gG comprising residues 321–580 of HSV-2 | Herpes simplex virus (HSV) | Indirect ELISA |
| Humans            | Capsid proteins | Human caliciviruses (HuCVs) | EIA |
| Humans            | C-terminus truncated form of protein (Etr) | TBE complex virus | ELISA and immunoblot assay |
| Humans            | Recombinant Fel dl (rFel dl Ch1 + Ch2) | Cat allergen | Radioimmunoassay (RIA) and ELISA |
| Humans            | Recombinant human tissue TG (hu-rTG) | Autoantigen transglutaminase (TG) | ELISA |
| Humans            | Envelop glycoproteins gB, gD, gC, gE and gG | Herpes B virus (HBV) | ELISA |

### 9.1.2.1 Veterinary Applications

(a) **Rodent:** Recombinant nucleocapsid protein produced in baculovirus expression system-based enzyme-linked immunosorbent assay (ELISA) is reported to be more specific as compared to whole virion conventional ELISA for the detection of Sendai virus infection in rodents (Wan et al. 1995).

(b) **Swine:** Specific indirect ELISA method was developed for the detection of Nipah virus (NiV) infection in swine serum samples by cloning G-protein of NiV into pFASTBac HT vector (Eshaghi et al. 2004). Its further use as diagnostic reagent for humans needs to be explored. P1 and 3CD protein genes of swine vesicular disease virus (SVDV)-derived VLPs as antigens for detection of antibodies against SVDV in pigs by ELISA were also developed (Ko et al. 2005).

(c) **Horse:** Recombinant baculovirus expressing equine infectious anaemia virus (EIAV) core proteins Gag and p26 as antigens was found to possess high specificity and sensitivity in ELISA and agar gel immunodiffusion (AGID) to detect antibodies from infected horse sera (Kong et al. 1997). Haemagglutinin (HA)
protein of equine influenza strain, A/equine/La Plate/I/93 (LP/93), was produced in silkworm larvae with recombinant baculovirus for the detection of antibodies in horse sera by ELISA (Sugiura et al. 2001). Its efficiency was further tested by HA1 subunit of HA (Sguazza et al. 2013).

(d) Cattle: Baculovirus-derived antigen capture competitive ELISA (Ag Cap c-ELISA) for the diagnosis of bluetongue and epizootic haemorrhagic disease virus infection in cattle exhibits advantages in terms of easy production, standardization, less requirement of downstream processing and its non-infectious nature as compared to commercially available c-ELISA (Mecham and Wilson 2004). Blocking ELISA was developed by BEVs for the detection of antibodies against foot-and-mouth disease of cattle, pigs and goats by virus type A with a specificity of 99% (Ko et al. 2010). Bovine respiratory syncytial virus (BRSV) infection that causes lower respiratory tract disease in calves 1–3 months old can be detected by immunofluorescence analysis with recombinant F-protein as antigen (Pastey and Samal 1998).

(e) Bird: A variant of ELISA known as E-ELISA using eukaryotically expressed E protein as the antigen for the detection of Tembusu virus (TMUV) in ducks was developed with 93.2% specificity and 97.8% sensitivity (Yin et al. 2013). Recombinant avian paramyxovirus type 2 haemagglutinin (APMV2-HN) is found to be a useful alternative to APMV-2 antigens in haemagglutination inhibition (HI) test for the detection of APMV-2 infection in avians (Choi et al. 2014). Whole Sendai virus virion VLPs are being used as antigens for the detection of antibodies against virus for diagnostic purposes such as major capsid protein VP1 of goose haemorrhagic polyomavirus-VLPs for the detection of GHPV-specific antibodies in sera from flocks with haemorrhagic nephritis and enteritis of geese (HNEG) disease (Zielonka et al. 2006).

9.1.2.2 Application in Humans

Most of the recombinant proteins that are used as antigens have been expressed by baculovirus expression system in Sf9 insect cells unless otherwise stated. Some of them are mentioned here.

Lassa virus infection causes Lassa fever mainly endemic in West Africa. Recombinant nucleocapsid protein acts as antigen for the detection of antibodies in Lassa virus-infected patient sera by ELISA (Barber et al. 1990; Saijo et al. 2007). Rubella virus (RV) normally causes a self-limiting disease, but its infection during the first trimester of pregnancy may cause foetal damage. Therefore, serological diagnostic test was developed by expressing E1, E2 and polyprotein precursor of rubella virus as antigen for enzyme immunoassay (EIA) and immunoblot analysis of patient sera (Seppänen et al. 1991). Trypanosoma cruzi causes Chagas’ disease in Latin America. Flagellar repetitive antigen (FRA), part of T. cruzi-based improved diagnostic assay, was developed for Chagas’ disease (dos Santos et al. 1992). Full-length human glutamic acid decarboxylases (GAD65 and GAD67) with histidine tag were produced in their natural conformations for the development of an immunoassay for the diagnosis of insulin-dependent diabetes mellitus (Mauch et al. 1993). Hantavirus which causes haemorrhagic fever with renal syndrome (HFRS)
nucleocapsid protein of strain SR-11 (rNP-SR-Sf9) was used as antigen for the indirect immunofluorescence antibody (IFA) diagnostic test that detects three serotypes (hantan 76-118, SR-11 and Puumala) of hantavirus (Yoshimatsu et al. 1993). Purified human papillomavirus (HPV) E2 protein was used to develop ELISA to detect IgG and IgA responses in cervical neoplasia patients (Rocha-Zavaleta et al. 1997). Houston/90 (Hou/90) is a human calicivirus (HuCV) strain in one of the three clades of Sapporo-like HuCVs that cause acute gastroenteritis in children. The viral capsid gene of Hou/90 capsid was used as antigen for immunoprecipitation and EIA (Jiang et al. 1998). Herpes simplex virus (HSV) infection is caused by two viruses HSV-1 and HSV-2. Diagnostic test that can distinguish between two strains has been developed that utilizes both type-specific and type-common HSV antigens in a single-step assay format to perform accurate diagnosis (Burke 1999; Wald and Ashley-Morrow 2002; Liu et al. 2015). Eight different strains of human caliciviruses (HuCVs) capsid proteins have been used to develop antigen-antibody detection assay by EIAs that are highly specific (Jiang et al. 2000). Causative agent of tick-borne encephalitis (TBE), C-terminus truncated form of protein E (Etr) of TBE complex virus tagged with histidine was used to develop sensitive and specific ELISA as well as immunoblot assay to detect the TBE virus-specific antibodies in infected individuals (Marx et al. 2001). Fel dl, the major allergen from cats, consists of two polypeptide chains, chain 1 (ch1) and chain 2 (ch2), which are usually linked with a disulphide bond. Recombinant Fel dl (rFel dl Ch1 + Ch2) protein construct in which two chains are linked together with glycine/serine linker was used as more potent antigen than bacterial-derived proteins for the detection of IgE and IgG antibodies by radioimmunoassay (RIA) and ELISA (Guyre et al. 2002). Coeliac disease (CD) is characterized by the presence of autoantigen transglutaminase (TG). Recombinant human tissue TG (hu-rTG) expressed with baculovirus system was used as antigen for ELISA that showed a sensitivity of 100% and a specificity of 98.6% (Osman et al. 2002). The envelope glycoproteins: gB, gD, gC, gE and gG are thought to be the primary targets of IgG antibody response in patients with Herpes B virus (HBV) infection. Therefore, ELISA test was developed by using the cocktail of these recombinant glycoproteins along with other capsid proteins with high sensitivity and specificity (Perelygina et al. 2005). Similarly, the recombinant proteins in single or multiple subunits for the diagnosis of different types of viral infections in humans have been developed with baculovirus expression system in insect cells.

9.1.3 BEVs as Therapeutic Agents

BEVs express products like growth factors, cytokines, chemokines, enzymes, hormones and monoclonal antibodies that can be used for human therapeutic purposes. More recently, BEV has also been exploited as effective tool for gene therapy. For simplicity, the applications of these products have been divided into two major groups: biological drug therapy and gene therapy. Over thousands of such biomolecules have been developed till now in this system, few among them are discussed here (Table 9.3).
Table 9.3  BEVs produced biomolecules as disease therapeutic agents

| Therapy type       | Targeted for                      | Product type                        | Product name                  | Expressed product                | Development stage | Company name, if any |
|--------------------|-----------------------------------|-------------------------------------|--------------------------------|----------------------------------|-------------------|-----------------------|
| Immunotherapy      | Prostate cancer                   | Recombinant fusion protein          | Provenge or sipuleucel-T      | PAP-GM-CSF^                      | Approved          | Dendreon              |
| Immunotherapy      | Colorectal carcinoma              | Monoclonal antibodies               | Anti-GA733-2E                 | CO17-1A Mab (IgG2a)             | Unapproved        |                       |
| Immunotherapy      | Haematolymphoid cells             | Recombinant protein                 | Anti-Bcl-2-Mab                | B-cell lymphoma leukaemia-2 (Bcl-2) protein | Unapproved        |                       |
| Immunotherapy      | Rotavirus                         | Single-domain antibodies (sdAbs)   | 3B2 and 2KD1 antibodies       | Anti-VP6                         | Unapproved        |                       |
| Immunotherapy      | Breast cancer                     | Monoclonal antibodies               | mAb-BR55/mAb-BR55K            | HC and LC                        | Unapproved        |                       |
| Immunotherapy      | Antigen-presenting cells (APCs)   | Adjuvant antibody                   | APCH1 antibody                | Anti-MHC class II DR             | Unapproved        |                       |
| Immunotherapy      | Immune cells                      | Cytokine                            | IL-2                          | Human interleukin 2              | Unapproved        |                       |
| Immunotherapy      | Stem cells, macrophages           | Cytokine                            | hGM-CSF                       | Human granulocyte-macrophages colony-stimulating factor | Unapproved        |                       |
| Enzyme therapy     | Purine salvage pathway            | Enzyme                              | ADA                           | Human adenosine deaminase        | Unapproved        |                       |
| Therapy                      | Condition                        | Hormone          | Protein Source | Approved Status | Sponsor/Company                        |
|------------------------------|----------------------------------|-------------------|----------------|-----------------|----------------------------------------|
| Hormone therapy             | Hypoparathyroidism               | hPTH              | Human parathyroid hormone | Unapproved      |                                        |
| Growth factor therapy       | Wound healing                    | huEGF1            | Human epidermal growth factor | Unapproved      |                                        |
| Growth factor therapy       | Growth factor                    | huFGF2            | Human fibroblast growth factor 2  | Unapproved      |                                        |
| Growth factor therapy       | Growth factor                    | huKGF1            | Human keratinocyte growth factor 1  | Unapproved      |                                        |
| Growth factor therapy       | Alzheimer’s disease              | rhNGF             | Human prepro (beta) nerve growth factor | Unapproved      |                                        |
| Enzyme-gene therapy         | Familial lipoprotein lipase deficiency | Glybera or LPL<sup>547</sup> × transgene | Lipoprotein lipase transgene | Approved | UniQure                                |
| Protein gene therapy        | Haemophilia A                     | AAV-FVIII         | Factor VIII    | Unapproved      |                                        |
| Protein gene therapy        | Haemophilia B                     | AAV8-hFIX19       | Factor IX      | Phase I         | Spark Therapeutics                     |
|                             |                                  |                   |                |                 |                                        |
|                             |                                  |                   |                |                 | AskBio009 (AAV8)                        | Phase I/II | Baxalta US Inc.                        |
|                             |                                  |                   |                |                 | scAAV 2/8-LP1-hFIXco                    | Phase I    | St. Jude Children’s Research Hospital  |
|                             |                                  |                   |                |                 | AAV2-hFIX16                              | Phase I    | Spark Therapeutics                     |
| Enzyme-gene therapy         | Leber congenital amaurosis        | AAV2-hRPE65v2     | Retinoid isomerohydrolase | Phase III       | Spark Therapeutics                     |
|                             |                                  |                   |                |                 | rAAV2-CB-hRPE65                          | Phase I/II | Applied Genetic Technologies Corp (continued) |
| Therapy type | Targeted for | Product type | Product name | Expressed product | Development stage | Company name, if any |
|--------------|--------------|--------------|--------------|-------------------|-------------------|---------------------|
| Enzyme-gene therapy | Leber’s hereditary optic neuropathy | Transgene | AAV2-ND4 | NADH-ubiquinone oxidoreductase chain 4 | Phase I | John Guy, University of Miami |
| Enzyme-gene therapy | Age-related macular degeneration | Transgene | AAV2-soluble Flt1 | Soluble fms-like tyrosine kinase | Phase I | Genzyme, a Sanofi Company |
| Enzyme-gene therapy | Canavan disease | Transgene | AAV-ASAP | Aspartoacylase | Unapproved | |
| Growth factor-gene therapy | Alzheimer’s disease | Transgene | AAV-NGF or CERE-110 | Beta-nerve growth factor | Phase I | Sangamo Therapeutics (Ceregene) |
| Enzyme-gene therapy | Parkinson’s disease | Transgene | AAV2-GAD | Glutamic acid decarboxylase | Phase II | Neurologix, Inc. |
| Protein gene therapy | | | AAV2-NTN or CERE-120 | Neurturin | Phase II | Ceregene |
| Enzyme-gene therapy | | | AAV-hAADC-2 | Aromatic L-amino acid decarboxylase | Phase I | Genzyme, a Sanofi Company |
| | | | AAV2-hAADC | | Phase I | Voyager Therapeutics |
| Therapy Type               | Disease/Condition                        | Transgene  | Vector          | Therapy Type               | Disease/Condition                        | Transgene  | Vector          | Phase | Sponsor/Institution                          |
|---------------------------|------------------------------------------|------------|----------------|---------------------------|------------------------------------------|------------|----------------|-------|---------------------------------------------|
| Protein gene therapy      | Duchenne muscular dystrophy              | Transgene  | rAAV2.5-CMV-minidystrophin | Mini-dystrophin               | Phase I                                  | Asklepios BioPharmaceutical, Inc.        |
| Protein gene therapy      | Becker muscular dystrophy                | Transgene  | rAAV1.CMV.huFollistatin344 | Follistatin                 | Phase I                                  | Nationwide Children’s Hospital           |
| Protein gene therapy      | Limb girdle muscular dystrophy           | Transgene  | AAV1-gamma-sarcoglycan | Gamma-sarcoglycan           | Phase I                                  | Genethon                                |
| Protein gene therapy      | Spinal muscular atrophy                  | Transgene  | scAAV9.CB.SMN    | Survival motor neuron      | Phase I                                  | AveXis, Inc.                            |
| Enzyme-gene therapy       | Acute intermittent porphyria             | Transgene  | rAAV2/5-PBGD    | Porphobilinogen deaminase  | Phase I                                  | Digna Biotech S.L.                      |
| Enzyme-gene therapy       | Alpha 1-antitrypsin deficiency           | Transgene  | rAAV1-CBhAAT    | Alpha 1-antitrypsin        | Phase II                                 | Applied Genetic Technologies Corp       |
| Enzyme-gene therapy       |                                           | Transgene  | rAAV2-CBhAAT    |                           | Phase I                                  | University of Massachusetts, Worcester  |
| Enzyme-gene therapy       |                                           | AAVrh.10halpha1AT |                           |                           | Phase I                                  | Adverum Biotechnologies, Inc.            |
| Enzyme-gene therapy       | Aromatic amino acid decarboxylase        | Transgene  | AAV2-hAADC      | Aromatic L-amino acid decarboxylase | Phase I/II                              | National Taiwan University Hospital      |
| Protein gene therapy      | Choroideremia                            | Transgene  | rAAV2.REP1      | Rab-escort protein 1       | Phase I                                  | University of Oxford                    |

(continued)
| Therapy type                  | Targeted for               | Product type | Product name                        | Expressed product                                      | Development stage | Company name, if any                                                                 |
|------------------------------|----------------------------|--------------|-------------------------------------|--------------------------------------------------------|-------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Enzyme-gene therapy          | Chronic heart failure      | Transgene    | AAV1-CMV-SERCA2a                    | Sarcoplasmic reticulum calcium ATPase                  | Phase II          | Imperial College London, Assistance Publique—Hôpitaux de Paris and Celladon Corporation                                        |
| Protein gene therapy         | Gastric cancer             | Transgene    | AAV-DC-CTL                          | Carcinoembryonic antigen                               | Phase I           | Tianjin Medical University Cancer Institute and Hospital                                                                       |
| Enzyme-gene therapy          | HIV                        | Transgene    | AAV-2 HIV vaccine (tgAAC09)         | Gag, protease and reverse transcriptase parts          | Phase I           | International AIDS Vaccine Initiative                                                                                           |
| Antibody-gene therapy        |                            | rAAV1-PG9DP  |                                     | PG9 antibody                                            | Phase I           | International AIDS Vaccine Initiative                                                                                           |
| Receptor-gene therapy        | Inflammatory arthritis     | Transgene    | tgAAC94                             | TNFR:Fc fusion gene                                    | Phase I/II        | Targeted Genetics Corporation                                                                                                 |
| Protein gene therapy         | Late infantile neuronal ceroid lipofuscinosis | Transgene | AAVirh.10CUCLN2 | Neuronal ceroid-lipofuscinosis 2 | Phase I/II        | Weill Medical College of Cornell University                                                                                     |
| Protein gene therapy         |                            | AAV2CUhCLN2  |                                     | Neuronal ceroid-lipofuscinosis 2                       | Phase I           | Weill Medical College of Cornell University                                                                                     |
| Trinucleotide-gene therapy   | Pompe disease              | Transgene    | rAAV1-CMV-GAA                       | Normal GAA                                             | Phase I/II        | University of Florida                                                                                                           |
9.1.3.1 Biological Drug Therapy

BEVs have been utilized as eukaryotic expression vectors in insect cells for the production of therapeutic or immunotherapeutic proteins such as monoclonal antibodies, cytokines and chemokines, growth factors, etc. that require post-translational modifications, more importantly glycosylation. The baculovirus expression system has been accepted as one of the most efficient and powerful technologies for the production of biological recombinants in terms of achievable quantity, purity and ease of the eukaryotic processing (Luckow and Summers 1988). Therapeutic recombinant protein production is considered as an essential section of the emerging biotechnology industries. This system has the potential for the development of high commercial value industry.

(a) Immunotherapy: Over the years, numerous tumor immunotherapies achieved early-stage successes but failed in clinical trials Phase-III (Goldman and DeFrancesco 2009). Baculovirus-derived Dendreon’s Provenge (Seattle; sipuleucel-T) for prostate cancer is among the first therapeutic cancer vaccines to complete Phase-III trial successfully and to receive FDA approval. Provenge (Sipuleucel-T) is an autologous active cellular immunotherapy that has shown evidence of reducing the risk of death among men with metastatic castration-resistant prostate cancer (Kantoff et al. 2010). It consists of autologous peripheral-blood mononuclear cells (PBMCs), including antigen-presenting cells (APCs), which have been activated ex vivo with a recombinant fusion protein (PA2024). The fusion protein PA2024 contains prostate antigen, prostatic acid phosphatase which is fused to an immune-cell activator called granulocyte-macrophage colony-stimulating factor. PA2024 is produced by BEV in Sf21 insect cells.

Monoclonal antibody CO17-1A was prepared against colorectal cancer cells by using pFastBac vectors (Park et al. 2011). The BEVs expressed proteins that are being utilized for the production of monoclonal antibodies against Bcl-2 (B-cell lymphoma leukaemia-2). It is an integral membrane oncoprotein that regulates programmed cell death (apoptosis) in haematolymphoid cells (Reed et al. 1992). Single-domain antibodies (sdAbs) that are prepared against rotavirus infection are also known as nanobodies or VHHs. They have characteristic high stability, solubility and very high affinity for their antigens. These antibodies were first produced in the insect larvae Trichoplusia ni which serve as living bio-factories for the production of these biomolecules (Gómez-Sebastián et al. 2012). Anti-breast cancer monoclonal antibodies (mAb) BR55, with or without fusion with KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum retention signal, were prepared. The heavy chain (HC) and light chain (LC) genes of mAb BR55 were cloned in pFastBac Dual vector under the control of polyhedrin (P_{ph}) and p_{10} promoters, respectively, in Sf9 insect cells (Lee et al. 2014). Antibody response was enhanced against two recombinant subunit vaccines by tagging the vaccines with adjuvant recombinant single-chain antibody APCH1. It recognizes the MHC Class II DR and produced in Trichoplusia ni insect cells (Gil et al. 2011).
Human interleukin 2 (IL-2) was prepared in insect larvae of *T. ni* by placing the IL-2 gene under p10 promoter of BEV (Pham et al. 1999). Human granulocyte-macrophages colony-stimulating factor (hGM-CSF) was prepared in *Bombyx mori* (silkworm) nuclear polyhedrosis virus (BmNPV) (Shi et al. 1996). Other cytokines and chemokines are being produced by using this expression system in a similar manner.

Recently, the intravesical instillation of transgene devoid baculovirus is found to elicit local immune stimulation by upregulating a set of Th-1-type cytokines in orthotopic bladder tumours in mice (Ang et al. 2016). However, the application of such strategy for non-muscle invasive bladder cancer (NMIBC) in humans is awaited.

(b) **Enzyme and hormonal therapy:** Enzyme human adenosine deaminase, a key purine salvage enzyme required for immune competence, has been produced both in *Trichoplusia ni* and *Spodoptera frugiperda* insect cells as well as larvae. This enzyme possessed specific activity of 70 units/mg in crude homogenate that is 70–350 times higher than its two most abundant natural sources thymus and leukemic cells. Such biologically active, inexpensive, rapid and huge production of the enzymes by this baculovirus system opens up the avenues for other biologically active molecules. Human parathyroid hormone (hPTH) was produced both in *Bombyx mori* cells and larvae. Both of the host systems have been reported to be suitable for efficient synthesis and secretion of the correctly processed hPTH (Mathavan et al. 1995). Similarly, recombinant full length human growth hormone (hGH) was produced in *Bombyx mori* nuclear polyhedrosis virus (vBmhGH) (Sumathy et al. 1996).

(c) **Growth factors therapy:** Growth factors are naturally signalling molecules required for myriads of biological processes for which the requirement of consistent, cost-effective and clinically efficient technologies is indispensable. Wound healing is one of such complex biological processes that requires the collaborative efforts of different tissues, cells and molecules. The repair process of wounds after injury is initiated by the release of various growth factors (GFs). GFs act as functional messenger molecules between cells which control the cellular processes in the regulatory network and sometimes require recombinant protein therapies. Currently, wound healing is being focussed on GFs and/or human skin substitutes, required for decreasing healing time by modifying inflammation and accelerating the proliferative phase. The beneficial effects of GFs to attract different kinds of cells at the site of wound healing have been demonstrated by many studies. Wider clinical and commercial applications of such GFs depend on their scalable cost-efficient production. BEVs have been successful in unblocking the bottlenecks for such inevitabilities. Three fully functional human GFs, the human epidermal growth factor 1 (huEGF1), the human fibroblast growth factor 2 (huFGF2) and the human keratinocyte growth factor 1 (huKGF1), have been produced with BEVs in *Trichoplusia ni* insect larvae (Dudognon et al. 2014). The expression of huKGF1 was found to be enhanced further when it was expressed by tagging it with human antibody IgG fragment crystallisable region (Fc).
Human prepro (beta) nerve growth factor that has been suggested as a therapeutic agent for the treatment of Alzheimer’s disease was produced in insect cells as recombinant virus, mature human beta nerve growth factor (rhNGF). It was found to be biologically active in cholinergic cell survival (Barnett et al. 1990). Similarly, different strategies are being worked out with BEVs in insect cells or larvae for biologically active, cost-effective, therapeutic and commercial scale production of numerous highly variable molecules.

9.1.3.2 Gene Therapy

Today, gene therapy potential has reached to the point whereby it can be exploited to treat many diseases that were earlier thought to be untreatable. The requisite modalities for such gene drugs such as safety, generation, immune response, duration of expression and the gene delivery capacity are being successfully realized by baculovirus-based vectors. Baculoviruses have been found to deliver genes into a wide range of vertebrate cells and species. However, the exact mechanism of entry of baculovirus in to the host cells is still not fully understood. Recently, phagocytic-like mechanism of entry into mammalian cells was found to be more convincing than pinocytosis (Long et al. 2006). Baculovirus progeny production occurs in two forms, budded virus (BuV) and occlusion-derived virus that only differ in their envelopes. BuV derives its envelop from cell membrane and spreads the infection within host, whereas occlusion-derived virus envelop is derived from nuclear membrane and spreads infection between hosts. BuV is the most widely used form in biotechnology that enters the insect and other hosts through endocytosis mechanism, although the tenet of exact endocytic mechanism still needs to be build.

AcMNPV is the prototype of baculoviruses and widely used for different applications including gene therapy. It is able to transduce both dividing and non-dividing mammalian cells and activates the transgene in the target cells that it carries under the control of specified promoter. It indicates that the nucleocapsid of the baculovirus transports its genome across the intact host cell nuclear membrane through nuclear pore complex. However, the detailed molecular mechanism of baculovirus transduction in mammalian cells demands further investigation for efficient gene delivery. BEVs gene delivery capability have been exploited in understanding the mechanism of vertebrate cell transduction, preclinical studies, vaccination, cartilage and bone tissue engineering, cancer gene therapy, assay development, drug screening and generation of other gene therapy vectors (Airenne et al. 2013). We would like to emphasize on the use of recombinant adeno-associated vectors (rAAV) as gene therapy tools which are highly important from bioprocess and therapeutic perspective.

BEVs-Derived Recombinant Adeno-Associated Viruses (rAAVs) for Gene Therapy

Recombinant AAVs that carry therapeutic DNA turn out to be the attractive gene delivery vectors because of their suitability for in vivo gene therapy potential, relatively long-term gene expression, lack of pathogenicity and ability to transduce wide variety of both dividing and non-dividing cells. Nine different serotypes of rAAVs are used for gene therapy whereby each serotype exhibits different
propensity for tissue-specific infection and infection kinetics (Zincarelli et al. 2008). The major limitation of low production quantity was addressed recently by optimizing the BEVs platforms and adjusting different parameters such as multiplicity of infection, cell density and fermentation mode that produced up to $10^4$ vector genomes per litre (Mena et al. 2010).

The strategy for rAAV production requires the production of three AAV capsid proteins, VP1, VP2 and VP3. These capsid proteins assemble within BEV-transduced insect cells to produce icosahedral VLPs (Aucoin et al. 2007). More efficient rAAVs require co-infection of insect cells with three different kinds of baculoviral vectors. The first one is Bac-Rep, expressing the major AAV replication enzymes Rep 78 and Rep 52 essential for AAV genomic replication and packaging, respectively. Second is Bac-Cap, expressing the AAV virion capsid proteins (VP1, VP2 and VP3), and third is Bac-GOI, expressing the gene of interest flanked by AAV inverted terminal repeat elements required for the rescue, replication and packaging of the heterologous gene. Co-infection with these three vectors in insect cells produces efficiently replicated and encapsulated single-stranded AAV vector genome (Weyer and Possee 1991). Further enhancement of AAV in terms of stability, robustness, scalability and high-titre production involves both Rep and Cap protein expression from a single baculovirus (Bac-Rep Cap), i.e. expression of both Rep 78 and Rep 52 transcription from a single mRNA and genetic modifications of the original Bac-Rep and Bac-Cap constructs (Virag et al. 2009). The development of such robust gene delivery vehicles was based on the fact that AAV genome is efficiently replicated in $Sf9$ and $Sf21$ insect cell lines in a Rep-dependent fashion. Some of the diseases that are being targeted by gene therapy using rAAV are discussed below:

(a) **Gene therapy against lipoprotein lipase deficiency (LPLD):** It is a rare autosomal recessive genetic and metabolic disorder in which inactivation of familial lipoprotein lipase enzyme occurs due to mutation in gene LPL. Functional lipase is required for plasma triglyceride hydrolysis under normal condition. Inactivated enzyme results into hypertriglyceridemia characterized by frequent abdominal pain and fatty deposits in the skin and retina that in severe cases can lead to fatal pancreatitis, diabetes and onset of cardiovascular diseases. Earlier therapies targeted to lower the plasma triglycerides have not been proved much effective. Alipogene tiparvovec (also known as AAV1-LPL$^{S447X}$ in the early phases of clinical trial) is the first adeno-associated virus (AAV)-mediated gene therapy manufactured by UniQure that got market authorization and government approval in Europe. It is an AAV1 (serotype 1) vector expressing naturally occurring variants of LPL transgene, LPL$^{S447X}$ linked with improved lipid profile and is commercialized by the name of Glybera (Gaudet et al. 2010). It is injected through intramuscular route in the patients that results in natural gain of function of LPL gene variants to muscle tissues. Glybera use significantly lowers plasma triglycerides by increasing the lipoprotein lipase enzyme activity.

The major concern for using such vector-based gene therapy is to prevent both humoral and cell-mediated immune response elicited against viral capsid
proteins that may impact the efficacy and safety of these drugs. Intramuscular injections of Glybera has been proved clinically safe and efficient drug that does not elicit any additional systemic and local immune response harmful for humans. This approach was found to be relevant and promising for the treatment of thousands of single gene disorders. Similar strategies are being investigated in diverse range of therapeutic areas, and many products for the treatment of human diseases are in different stages of clinical development. These AAV gene therapy drugs at different clinical development phases are being discussed here.

(b) **Haemophilia**: It is a blood clotting disorder caused by the mutation in the clotting factor IX gene. Presently, four clinical trials are going on that involve rAAV serotype 2 or 8, designed to express factor IX.

Haemophilia A, the most common severe inherited bleeding disorder caused by mutation in factor VIII gene, is significantly more problematic for this treatment because of a larger size of cDNA that prevents in achieving the adequate level of transgene expression and elicits the anti-factor VIII immune response (High et al. 2014).

(c) **Retinal degeneration**: Recombinant AAV has been used to treat a number of animal models but is limited by carrying capacity, slow onset of expression and limited ability to transduce some of the retinal cell types from the vitreous. Next-generation AAVs have been produced to address these issues by creation of self-complementary AAV vectors for faster onset of expression and specific mutations of self-exposed residues to increase transduction. Such vectors were further improved for broader applicability and advantageous characteristics by directed evolution through an iterative process of selection (Day et al. 2014). Age-related macular degeneration (AMD) that leads to the central vision loss in elderly individuals due to choroidal neovascularization is marked by proliferation of blood vessels and retinal pigment epithelial (RPE) cells. It leads to photoreceptor death and fibrous disciform scar formation. Treatment of AMD patients requires neutralization of vascular endothelial growth factor (VEGF) for which expression of modified soluble Flt1 receptor was designed and expressed in AAV2-sFLT01 vector. Presently, this study is in Phase 1 trial (MacLachlan et al. 2011). Leber congenital amaurosis (LCA) is an autosomal recessive blinding disease that occurs due to mutations in RPE65 gene. Sub-retinal administration of AAV2-hRPE65v2 has been reported both safe and efficient for at least 1.5 years after injection. Currently six clinical trials, either in stage 1 or 2, are going on to treat this retinal disease (Simonelli et al. 2010).

(d) **Neurological diseases**: rAAV has been used as an effective gene delivery system for the treatment of central or peripheral nervous system with almost no adverse effects in many clinical trials. First time, its clinical use in the human brain has been used to treat Canavan disease, a childhood leukodystrophy also known as Van Bogaert-Bertrand disease caused by the deficiency of enzyme aspartoacylase (ASAP). It involves neurosurgical administration of approximately 10 billion infectious particles of recombinant adeno-associated virus (AAV) containing the aspartoacylase gene (ASPA) directly to the affected
regions of the brain (Janson et al. 2002). To treat Alzheimer’s disease, transfer of gene encoding nerve growth factor (NGF), which is essential for healthier nerve cells, is transduced by an adeno-associated nerve growth factor (CERE-110) (Bakay et al. 2007). Transduction of glutamic acid decarboxylase (GAD) and trophic factor neurturin was assessed successfully in different Phase 1 and 2 clinical trials for the treatment of Parkinson’s disease (Marks et al. 2010; Kaplitt et al. 2007).

(e) Duchenne muscular dystrophy (DMD): DMD is a severe recessive X-linked muscle disorder caused by mutations in gene encoding dystrophin. Gene therapy to treat DMD is a challenge due to the large size of DMD gene. However, alternative gene delivery strategies like exon skipping, trans-splicing, micro- and mini-dystrophin in Phase II/III clinical trials have been found to be promising (Jarmin et al. 2014).

A number of Phase I/II/III clinical trials are underway for the treatment of numerous diseases such as acute intermittent porphyria, alpha 1-antitrypsin deficiency, aromatic amino acid decarboxylase deficiency, Becker muscular dystrophy, choroideremia, chronic heart failure, gastric cancer, HIV, inflammatory arthritis, late infantile neuronal ceroid lipofuscinosis, Leber’s hereditary optic neuropathy, limb girdle muscular dystrophy, macular degeneration, Pompe disease, spinal muscular atrophy, etc. (Felberbaum 2015).

The future prospectives of baculovirus gene delivery applications in stem cell transduction, cancer gene therapy and cartilage and bone tissue engineering are also quite optimistic. Great interest in regenerative medicine begins with the advancement in identification, isolation and derivation of human stem cells, specifically the generation of human-induced pluripotent stem cells. Prolonged expression of transgenes has been demonstrated in multiple multipotent stem cells such as mesenchymal, neural, umbilical cord, bone marrow, adipose tissue, human embryonic stem cells (hESCs) and pluripotent stem cells. These baculoviruses have also been customized for stable gene expression in stem cells by genomic integration for downstream therapeutic applications, for example, deriving unlimited numbers of genetically corrected functional adult cells for cell replacement therapy (Kotin et al. 1991).

De-differentiated chondrocytes transduced with baculovirus vector (Bac-CB) expressing bone morphogenetic protein-2 (BMP-2) result into sustained expression of BMP-2 with passaged chondrocytes in vitro. It was further improved by co-expression of transforming growth factor beta with baculovirus vectors (Chen et al. 2008). These chondrocytes were further used to grow cartilage-like tissues in rotating shaft bioreactors that demonstrated the potential of baculovirus in cartilage tissue engineering, but their clinical utility in humans is yet to be proved.

Bac-CB-based BMP-2 transduction into human bone marrow-derived mesenchymal stem cells (BMSCs) is also demonstrated to directing ontogenies of naïve BMSCs. Implantation of these transduced cells induced ectopic bone formation in nude mice and promoted calvarial bone repair in immunocompetent rats (Chuang et al. 2009). For massive repairing of bone, sustained expression of genes promoting
osteogenesis (BMP-2) and angiogenesis (VEGF) in adipose-derived stem cells (ASCs) was performed by dual baculovirus vector system. Transplantation of these ASCs in NZW rabbit resulted in accelerated healing, improved bone quality and angiogenesis. Same technique was also tested in rabbits, and the results altogether support the viability of baculoviruses for stem cell engineering and bone formation (Luo et al. 2011).

The propensity of baculoviruses for effective high-level transgene expression has been exploited for cancer gene therapy. Baculovirus vectors have been tailored with suicide, tumour suppressor, pro-apoptotic, immune-potentiating and anti-angiogenesis genes and studied in animal tumour models under in vivo conditions in many anticancer strategies (Luo et al. 2012; Wang and Balasundaram 2010). Recently, stem cells transduced with suicide genes have proved beneficial for curbing primary, solid and metastatic tumours (Zhao et al. 2012).

Today, baculovirus technology has matured to the level that it can be used for plethora of applications. The studies conducted on model organisms in the context of therapeutic applications are encouraging and support further development of baculoviruses from preclinical applications to clinical trials and for human diseases treatment. A deeper and holistic understanding of antigenic and target cell transduction molecular mechanisms will be helpful in enhancing the clinical utility of this unique and powerful gene delivery system.

References

Airenne KJ et al (2013) Baculovirus: an insect-derived vector for diverse gene transfer applications. Mol Ther 21(4):739–749
Ang WX et al (2016) Local immune stimulation by intravesical instillation of baculovirus to enable bladder cancer therapy. Sci Rep 6:27455
Atmar RL et al (2011) Norovirus vaccine against experimental human Norwalk Virus illness. N Engl J Med 365(23):2178–2187
Aucoin MG et al (2007) Virus-like particle and viral vector production using the baculovirus expression vector system/insect cell system. In: Baculovirus and Insect Cell Expression Protocols. Humana, New York, pp 281–296
Bakay RA et al (2007) Analyses of a phase 1 clinical trial of adeno-associated virus-nerve growth factor (CERE-110) gene therapy in Alzheimer’s disease: 866. Neurosurgery 61(1):216
Ball JM et al (1999) Recombinant Norwalk virus–like particles given orally to volunteers: phase I study. Gastroenterology 117(1):40–48
Barber GN, Clegg J, Lloyd G (1990) Expression of the Lassa virus nucleocapsid protein in insect cells infected with a recombinant baculovirus: application to diagnostic assays for Lassa virus infection. J Gen Virol 71(1):19–28
Barnett J et al (1990) Human β nerve growth factor obtained from a baculovirus expression system has potent in vitro and in vivo neurotrophic activity. Exp Neurol 110(1):11–24
Burke RL (1999) Herpes simplex virus diagnostics. Google Patents
Chen H-C et al (2008) Combination of baculovirus-expressed BMP-2 and rotating-shaft bioreactor culture synergistically enhances cartilage formation. Gene Ther 15(4):309–317
Choi K-S et al (2014) Baculovirus expression of the avian paramyxovirus 2 HN gene for diagnostic applications. J Virol Methods 198:12–17
Chuang C-K et al (2009) Xenotransplantation of human mesenchymal stem cells into immunocompetent rats for calvarial bone repair. Tissue Eng A 16(2):479–488
Chung C-Y et al (2010) Enterovirus 71 virus-like particle vaccine: improved production conditions for enhanced yield. Vaccine 28(43):6951–6957
Cox MM, Hashimoto Y (2011) A fast track influenza virus vaccine produced in insect cells. J Invertebr Pathol 107:S31–S41
Day TP et al (2014) Advances in AAV vector development for gene therapy in the retina. In: Retinal degenerative diseases. Springer, New York, pp 687–693
de Diego ACP et al (2011) Characterization of protection afforded by a bivalent virus-like particle vaccine against bluetongue virus serotypes 1 and 4 in sheep. PLoS One 6(10):e26666
Desrosiers R et al (2009) Use of a one-dose subunit vaccine to prevent losses associated with porcine circovirus type 2. J Swine Health Prod 17(3):148–154
dos Santos CND et al (1992) Trypanosoma cruzi flagellar repetitive antigen expression by recombinant baculovirus: towards an improved diagnostic reagent for Chagas’ disease. Biotechnology 10:1474–1477
Dudognon B et al (2014) Production of functional active human growth factors in insects used as living biofactories. J Biotechnol 184:229–239
Eshaghi M et al (2004) Nipah virus glycoprotein: production in baculovirus and application in diagnosis. Virus Res 106(1):71–76
Fauquet CM et al (2005) Virus taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses. Academic, London
Felberbaum RS (2015) The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors. Biotechnol J 10(5):702–714
French T, Marshall J, Roy P (1990) Assembly of double-shelled, viruslike particles of bluetongue virus by the simultaneous expression of four structural proteins. J Virol 64(12):5695–5700
Frey S, Treanor JJ, Atmar RL, Topman D, Chen WH, Ferreira J (2011) Phase 1 dosage escalation, safety and immunogenicity study of a bivalent norovirus VLP vaccine by the intramuscular route. In Annual meeting of the infectious diseases society of America, Boston, 2011
Gaudet D et al (2010) Review of the clinical development of alipogene tiparvovec gene therapy for lipoprotein lipase deficiency. Atheroscler Suppl 11(1):55–60
Gil F et al (2011) Targeting antigens to an invariant epitope of the MHC Class II DR molecule potentiates the immune response to subunit vaccines. Virus Res 155(1):55–60
Goldman B, DeFrancesco L (2009) The cancer vaccine roller coaster. Nat Biotechnol 27(2):129–139
Gómez-Sebastián S et al (2012) Rotavirus A-specific single-domain antibodies produced in baculovirus-infected insect larvae are protective in vivo. BMC Biotechnol 12(1):59
Guyre P et al (2002) Recombinant cat allergen, Fel d1, expressed in baculovirus for diagnosis and treatment of cat allergy. Google Patents
High K et al (2014) Current status of haemophilia gene therapy. Haemophilia 20(s4):43–49
Hu YC, Bentley WE (2001) Effect of MOI ratio on the composition and yield of chimeric infectious bursal disease virus-like particles by baculovirus co-infection: Deterministic predictions and experimental results. Biotechnol Bioeng 75(1):104–119
Janson C et al (2002) Gene therapy of Canavan disease: AAV-2 vector for neurosurgical delivery of aspartoacylase gene (ASPA) to the human brain. Hum Gene Ther 13(11):1391–1412
Jarmin S et al (2014) New developments in the use of gene therapy to treat Duchenne muscular dystrophy. Expert Opin Biol Ther 14(2):209–230
Jiang X et al (1992) Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol 66(11):6527–6532
Jiang X et al (1998) Baculovirus-expressed Sapporo-like calicivirus capsid self-assembles into virus-like particles (VLPs) and can be used to detect antibody in children† 854. Pediatr Res 43:148–148
Jiang X et al (2000) Diagnosis of human caliciviruses by use of enzyme immunoassays. J Infect Dis 181(Supplement 2):S349–S359
Kanasashi S-N et al (2003) Simian virus 40 VP1 capsid protein forms polymorphic assemblies in vitro. J Gen Virol 84(7):1899–1905
Kantoff PW et al (2010) Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 363(5):411–422
Kaplitt MG et al (2007) Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson’s disease: an open label, phase I trial. Lancet 369(9579):2097–2105

King AM et al (2011) Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Elsevier, London

Ko Y-J et al (2005) Noninfectious virus-like particle antigen for detection of swine vesicular disease virus antibodies in pigs by enzyme-linked immunosorbent assay. Clin Diagn Lab Immunol 12(8):922–929

Ko Y-J et al (2010) Use of a baculovirus-expressed structural protein for the detection of antibodies to foot-and-mouth disease virus type A by a blocking enzyme-linked immunosorbent assay. Clin Vaccine Immunol 17(1):194–198

Kong XG et al (1997) Application of equine infectious anemia virus core proteins produced in a baculovirus expression system to serological diagnosis. Microbiol Immunol 41(12):975–980

Kost TA, Condreay JP (2002) Innovations—biotechnology: baculovirus vectors as gene transfer vectors for mammalian cells: biosafety considerations. Appl Biosaf 7(3):167–169

Kotin RM et al (1991) Mapping and direct visualization of a region-specific viral DNA integration site on chromosome 19q13-qter. Genomics 10(3):831–834

Krammer F et al (2010) Trichoplusia ni cells (High FiveTM) are highly efficient for the production of influenza A virus-like particles: a comparison of two insect cell lines as production platforms for influenza vaccines. Mol Biotechnol 45(3):226–234

Laurent S et al (1994) Recombinant rabbit hemorrhagic disease virus capsid protein expressed in baculovirus self-assembles into viruslike particles and induces protection. J Virol 68(10):6794–6798

Lee JH et al (2014) Expression of recombinant anti-breast cancer immunotherapeutic monoclonal antibody in baculovirus–insect cell system. Entomol Res 44(5):207–214

Liu L-J et al (2008) Efficient production of type 2 porcine circovirus-like particles by a recombinant baculovirus. Arch Virol 153(12):2291–2295

Liu T et al (2015) Production of a fragment of glycoprotein G of herpes simplex virus type 2 and evaluation of its diagnostic potential. Singap Med J 56(6):346

Long G et al (2006) Functional entry of baculovirus into insect and mammalian cells is dependent on clathrin-mediated endocytosis. J Virol 80(17):8830–8833

López-Macías C et al (2011) Safety and immunogenicity of a virus-like particle pandemic influenza A (H1N1) 2009 vaccine in a blinded, randomized, placebo-controlled trial of adults in Mexico. Vaccine 29(44):7826–7834

Luckow VA, Summers MD (1988) Trends in the development of baculovirus expression vectors. Nat Biotechnol 6(1):47–55

Luo W et al (2011) Baculovirus vectors for antiangiogenesis-based cancer gene therapy. Cancer Gene Ther 18(9):637–645

Luo W et al (2012) Development of the hybrid Sleeping Beauty-baculovirus vector for sustained gene expression and cancer therapy. Gene Ther 19(8):844–851

MacLachlan TK et al (2011) Preclinical safety evaluation of AAV2-sFLT01—a gene therapy for age-related macular degeneration. Mol Ther 19(2):326–334

Maranga L et al (2002) Large scale production and downstream processing of a recombinant porcine parvovirus vaccine. Appl Microbiol Biotechnol 59(1):45–50

Marks WJ et al (2010) Gene delivery of AAV2-neurturin for Parkinson’s disease: a double-blind, randomised, controlled trial. Lancet Neurol 9(12):1164–1172

Marx F et al (2001) Diagnostic immunoassays for tick-borne encephalitis virus based on recombinant baculovirus protein expression. J Virol Methods 91(1):75–84

Mathavan S et al (1995) High-level production of human parathyroid hormone in Bombyx mori larvae and BmN cells using recombinant baculovirus. Gene 167(1):33–39

Mauch L et al (1993) Baculovirus-mediated expression of human 65 kDa and 67 kDa glutamic acid decarboxylases in SF9 insect cells and their relevance in diagnosis of insulin-dependent diabetes mellitus. J Biochem 113(6):699–704
Mazur NI et al (2015) Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. Lancet Respir Med 3(11):888–900
Mecham J, Wilson W (2004) Antigen capture competitive enzyme-linked immunosorbent assays using baculovirus-expressed antigens for diagnosis of bluetongue virus and epizootic hemorrhagic disease virus. J Clin Microbiol 42(2):518–523
Mena JA et al (2010) Improving adenov-associated vector yield in high density insect cell cultures. J Gene Med 12(2):157–167
Monie A et al (2008) Cervarix™: a vaccine for the prevention of HPV 16, 18-associated cervical cancer. Biol Targets Ther 2(1):107–113
Montross L et al (1991) Nuclear assembly of polyomavirus capsids in insect cells expressing the major capsid protein VP1. J Virol 65(9):4991–4998
Moormann RJ et al (2000) Development of a classical swine fever subunit marker vaccine and companion diagnostic test. Vet Microbiol 73(2):209–219
Mortola E, Roy P (2004) Efficient assembly and release of SARS coronavirus-like particles by a heterologous expression system. FEBS Lett 576(1-2):174–178
Neuzil KM (2016) Progress toward a respiratory syncytial virus vaccine. Clin Vaccine Immunol 23(3):186–188
O’Reilly DR, Miller LK, Luckow VA (1994) Baculovirus expression vectors: a laboratory manual. Oxford University Press on Demand, New York
Osman AA et al (2002) Production of recombinant human tissue transglutaminase using the baculovirus expression system, and its application for serological diagnosis of coeliac disease. Eur J Gastroenterol Hepatol 14(11):1217–1223
Park D-Y et al (2011) Optimization of expression conditions for production of anti-colorectal cancer monoclonal antibody CO17-1A in baculovirus-insect cell system. Hybridoma 30(5):419–426
Pastey MK, Samal SK (1998) Baculovirus expression of the fusion protein gene of bovine respiratory syncytial virus and utility of the recombinant protein in a diagnostic enzyme immunoassay. J Clin Microbiol 36(4):1105–1108
Paton D et al (1991) An ELISA detecting antibody to conserved pestivirus epitopes. J Virol Methods 31(2-3):315–324
Perelygina L et al (2005) Production of herpes B virus recombinant glycoproteins and evaluation of their diagnostic potential. J Clin Microbiol 43(2):620–628
Pham M-Q et al (1999) Human interleukin-2 production in insect (Trichoplusia ni) larvae: effects and partial control of proteolysis. Biotechnol Bioeng 62(2):175–182
Pillay S et al (2009) Optimization of chimeric HIV-1 virus-like particle production in a baculovirus-insect cell expression system. Biotechnol Prog 25(4):1153–1160
Prel A, Le Gall-Recule G, Jestin V (2008) Achievement of avian influenza virus-like particles that could be used as a subunit vaccine against low-pathogenic avian influenza strains in ducks. Avian Pathol 37(5):513–520
Reed JC et al (1992) A strategy for generating monoclonal antibodies against recombinant baculovirus-produced proteins: application to the Bcl-2 oncoprotein. Anal Biochem 205(1):70–76
Rocha-Zavaleta L et al (1997) Differences in serological IgA responses to recombinant baculovirus-derived human papillomavirus E2 protein in the natural history of cervical neoplasia. Br J Cancer 75(8):1144
Roldão A et al (2010) Virus-like particles in vaccine development. Expert Rev Vaccines 9(10):1149–1176
Roy P, Noad R (2008) Virus-like particles as a vaccine delivery system: Myths and facts. Hum Vaccin 4(1):5–12
Saijo M et al (2007) Development of recombinant nucleoprotein-based diagnostic systems for Lassa fever. Clin Vaccine Immunol 14(9):1182–1189
Seppänen H et al (1991) Diagnostic potential of baculovirus-expressed rubella virus envelope proteins. J Clin Microbiol 29(9):1877–1882
Sguazza GH et al (2013) Expression of the hemagglutinin HA1 subunit of the equine influenza virus using a baculovirus expression system. Rev Argent Microbiol 45(4):222–228
Preventive, Diagnostic and Therapeutic Applications

Shi X et al (1996) Expression of biologically active human granulocyte-macrophage colony-stimulating factor in the silkworm (Bombyx mori). Biotechnol Appl Biochem 24(3):245–249

Simonelli F et al (2010) Gene therapy for Leber’s congenital amaurosis is safe and effective through 1.5 years after vector administration. Mol Ther 18(3):643–650

Smith GE, Summers M, Fraser M (1983) Production of human beta interferon in insect cells infected with a baculovirus expression vector. Mol Cell Biol 3(12):2156–2165

Sugiura T et al (2001) Serological diagnosis of equine influenza using the hemagglutinin protein produced in a baculovirus expression system. J Virol Methods 98(1):1–8

Sumathy S, Palhan VB, Gopinathan KP (1996) Expression of human growth hormone in silkworm larvae through recombinant Bombyx mori nuclear polyhedrosis virus. Protein Expr Purif 7(3):262–268

Summers MD (2006) Milestones leading to the genetic engineering of baculoviruses as expression vector systems and viral pesticides. Adv Virus Res 68:3–73

Sun Y et al (2009) Protection against lethal challenge by Ebola virus-like particles produced in insect cells. Virology 383(1):12–21

van Oers MM, Pijlman GP, Vlak JM (2015) Thirty years of baculovirus–insect cell protein expression: from dark horse to mainstream technology. J Gen Virol 96(1):6–23

Van Rijn P et al (1996) Classical swine fever virus (CSFV) envelope glycoprotein E2 containing one structural antigenic unit protects pigs from lethal CSFV challenge. J Gen Virol 77(11):2737–2745

Virag T, Cecchini S, Kotin RM (2009) Producing recombinant adeno-associated virus in foster cells: overcoming production limitations using a baculovirus–insect cell expression strategy. Hum Gene Ther 28(8):807–817

Wagner R et al (1996) Safety and immunogenicity of recombinant human immunodeficiency virus-like particles in rodents and rhesus macaques. Intervirology 39(1-2):93–103

Wald A, Ashley-Morrow R (2002) Serological testing for herpes simplex virus (HSV–1) and HSV-2 infection. Clin Infect Dis 35(Supplement 2):S173–S182

Wan C-H et al (1995) Expression of Sendai virus nucleocapsid protein in a baculovirus expression system and application to diagnostic assays for Sendai virus infection. J Clin Microbiol 33(8):2007–2011

Wang S, Balasundaram G (2010) Potential cancer gene therapy by baculoviral transduction. Curr Gene Ther 10(3):214–225

Weyer U, Possee RD (1991) A baculovirus dual expression vector derived from the Autographa californica nuclear polyhedrosis virus polyhedrin and p10 promoters: co-expression of two influenza virus genes in insect cells. J Gen Virol 72(12):2967–2974

Yamaji H (2014) Suitability and perspectives on using recombinant insect cells for the production of virus-like particles. Appl Microbiol Biotechnol 98(5):1963–1970

Yamshchikov GV et al (1995) Assembly of SIV virus-like particles containing envelope proteins using a baculovirus expression system. Virology 214(1):50–58

Yin X et al (2013) Detection of specific antibodies against Tembusu virus in ducks by use of an E protein-based enzyme-linked immunosorbent assay. J Clin Microbiol 51(7):2400–2402

Yoshimatsu K, Arikawa J, Kariwa H (1993) Application of a recombinant baculovirus expressing hantavirus nucleocapsid protein as a diagnostic antigen in IFA test: cross reactivities among 3 serotypes of hantavirus which causes hemorrhagic fever with renal syndrome (HFRS). J Vet Med Sci 55(6):1047–1050

Zhao Y et al (2012) Targeted suicide gene therapy for glioma using human embryonic stem cell-derived neural stem cells genetically modified by baculoviral vectors. Gene Ther 19(2):189–200

Zielonka A et al (2006) Generation of virus-like particles consisting of the major capsid protein VP1 of goose hemorrhagic polyomavirus and their application in serological tests. Virus Res 120(1):128–137

Zincarelli C et al (2008) Analysis of AAV serotypes 1–9 mediated gene expression and tropism in mice after systemic injection. Mol Ther 16(6):1073–1080