Baculovirus as a vaccine vector

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Abstract

Baculovirus is extensively utilized as an excellent tool for production of recombinant protein in insect cells. Baculovirus infects insects in nature and is non-pathogenic to humans. In addition to insect cells, baculovirus is capable of transducing a broad range of animal cells. Due to its biosafety, large cloning capacity, low cytotoxicity and non-replication nature in the transduced cells as well as the ease of manipulation and production, baculovirus has been utilized as RNA interference mediators, gene delivery vectors and vaccine vectors for a wide variety of applications. This article focuses on the utilization of baculoviruses as vaccine vectors to prepare antigen or subunit vaccines.

Baculoviruses are a diverse group of DNA viruses capable of infecting more than 600 insects, among which *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) is the best characterized and most extensively utilized. AcMNPV possesses a circular dsDNA genome of 134 kb, replicates in a bi-phasic fashion, and produces two virion phenotypes during infection from cell to cell throughout the animal, after infection is achieved by ODV in the midgut. Baculovirus is used as a vector in the construction of recombinant baculovirus, surface display vector, vaccine, VSVG, gp64 Baculovirus Surface Display by a gp64-Based Strategy

The wild-type baculovirus triggers the innate immunity and potentiates the adaptive immune response, protecting the host from infection by several virus types. Baculovirus is utilized as a vector vaccine candidate, in which the antigens can be expressed by the vector within the host cells and displayed on the baculovirus surface or plasma membrane. Baculovirus surface display, using the TM and CTD of gp64, has been achieved by fusion with the gp64 gene of baculovirus, with expression driven by either the p10 or polyhedrin promoter. The significance of baculovirus...
gp64 in virus budding has been employed for the surface display of exogenous peptides by inserting a heterologous gene-encoding protein fused in frame between the SS and TM of gp64. The fusion protein, after expression along with the native gp64, is translocated to the plasma membrane and incorporated into the baculoviral envelope. This surface display system has been extensively used to develop pseudotyped baculoviruses as a vaccine delivery platform. 35-39,42,43,50-52

The CTD of a viral glycoprotein plays a critical role in protein incorporation, membrane fusion, virus budding and virus morphology. 39-41 The function of CTDs may differ among different viruses. For example, both the native baculovirus gp64 and chimeric gp64 fused with the CTD from human respiratory syncytial virus (HRSV) fusion protein are efficiently incorporated into HRSt. 41 In contrast, human immunodeficiency virus (HIV) envelope protein could not be incorporated into ribosomes viral or vesicular stomatitis virus unless the CTD of HIV envelope protein is replaced with that of G protein of vesicular stomatitis virus (VSV). 42,43 A compelling finding from our group has demonstrated that the origin of CTD greatly influences the properties and vaccine efficacy of HA-pseudotyped baculovirus. 42 The differences in incorporation efficiencies may be due to the disparities in the palmitoylation in the CTD, a post-translational modification essential for targeting envelope proteins to lipid rafts, which are dynamic structures on cell surfaces and may act as selective concentration devices for proteins. 44,45 Furthermore, recent studies have shown that the SS and CTD of gp64 can enhance the display of influenza HA on baculovirus surface, while the gp64 transmembrane domain impairs HA display. 53 Collectively, these studies provide conclusive evidence that the origin of the CTD and TM exerts a profound effect on envelope protein incorporation, thereby influencing vaccine efficacy.

Baculovirus as a Bivalent Vaccine Vector

A bicistronic transfer vector, PfastBacDUAL, which contains two promoters (polyhedrin and p10 viral promoters) and cloning sites in two separate cassettes for simultaneously subcloning two foreign genes for co-expression, was also applied. 25 This approach has been used to simultaneously display two proteins on the viral envelope or plasma membrane. 25-27 The baculovirus vectors displaying two foreign proteins could be prepared as a bivalent vaccine candidate. The display of the baculovirus displaying the GP5 protein of respiratory syndrome virus (PRRSV) and the Cap protein of porcine circovirus type 2 (PCV2) on the viral envelope (via gp64 fusion) elicits high levels of anti-PRRSV GP5 and anti-PCV2 Cap neutralization antibodies and mediates cell immune response in swine. 25 This dual surface display system can be utilized as an alternative strategy in order to develop bivalent vaccines against mixed virus infections. To allow rapid selection of recombinant baculoviruses, the co-expression of enhanced green fluorescent protein (EGFP) and one or multiple viral proteins has been previously described in reference 60. For example, the co-expression of EGFP and hemagglutinin (HA) gene of HSN2 avian influenza virus (AIV) in insect cells allows rapid identification of the recombinant baculoviruses in SF-9 insect cells, eliminating cumbersome and time-consuming assays. The HA-encoding gene of AIV is expressed in host cells under the control of the polyhedrin promoter. The EGFP gene with cytomegalovirus (CMV) promoter is expressed under the control of the Ppromoter. Furthermore, the co-expression of EGFP and one or two viral proteins were also established. The EGFP is expressed in host cells as an indicator for rapid identification of recombinant baculoviruses while viral proteins are displayed on the baculovirus envelope or plasma membrane. 25,26,27,29,30 This system allows researchers to readily adjust the molar ratio of two expressed proteins. However, an inverse correlation may occur between amounts of two proteins because of two expressed genes competing with each other for protein synthesis within the cells.

Baculovirus Utilized as Antigen Display Vectors and Gene Delivery Vectors by a VSV G-Based Strategy

With the exception of the gp64-based display, expression of vesicular stomatitis virus (VSV) G protein, 46-48 influenza virus neuraminidase, 49 single chain antibody fragments, 50 and Spodoptera exigua multiple nucleopolyhedrovirus F protein 51 in insect cells also leads to incorporation of the protein into baculovirus envelope. Among these strategies, display of VSVG or heterologous proteins via the VSVG anchor seems to be the most widely adopted strategy, enhancing baculovirus transduction in vitro and in vivo. 52-55 Wang and coworkers constructed a pseudotyped baculovirus with the VSVG protein on the envelope, a construct which was used as a vector to construct recombinant baculovirus co-expressing GP5 and M protein of PRRSV, under the transcriptional control of two independent cytomegalo virus immediate early enhancer/promoters. The resultant recombinant baculovirus efficiently expressed PRRSV GP5 and M protein in mammalian cells. Intramuscular injection of the recombinant baculoviruses induced the production of PRRSV-specific neutralizing antibodies and gamma interferon under dose-dependent pattern. 56 Furthermore, baculovirus was also used as a novel and attractive gene delivery vehicle for mammalian cells.

Polyspecific Vectors Carrying Multiple Expression Cassettes for Simultaneous Display and Expression of Multiple Proteins

The multiprotein complexes uncovered in cells have increased the need for improved heterologous protein production in order to investigate their molecular structure and function. Genes driven by AcNPV late promoters are abundantly expressed, as well as genetically processed and targeted in insect cells. 57 Expression of multiple genes in one cell can be completed by co-infection with numerous viruses, each carrying a single foreign gene. However, the logistical demand of maintaining several viruses at known titers and defined relative expression levels renders reproducing large-scale multi-protein production nearly impossible. Previous work has reported that construction of polyspecific vectors carrying multiple separate expression cassettes allows to simultaneously display or expression of multiple proteins. 58-60 Schematic illustration of polyspecific vectors carrying three
or five expression cassettes is shown in Figure 1. Therefore, infection with one baculovirus expressing all heterologous genes greatly simplifies virus handling, resulting in reproducible expression and higher protein yield as compared to multiple single-gene viruses. Furthermore, a simple and versatile system, Multibac, was established for generating recombinant baculovirus DNA to express numerous proteins or protein complexes consisting of many subunits. This system using transfer vectors that contain a multiplication module can be nested to facilitate assembly of polycistronic expression cassettes, thereby minimizing requirements for unique restriction sites.

Baculovirus is an attractive and convenient tool for use as a vaccine expression/delivery vector. Compared with other known viral vector, baculovirus has been extensively employed as antigen expression or display vectors for production of antigen or vaccines. In addition to the protein expression/display, baculovirus will likely gain growing popularity as therapeutic vaccine and regenerative medicine as well as an RNA interference mediator. We envisage that baculovirus will likely gain growing popularity as therapeutic vaccine and gene delivery vectors of the future.

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Figure 1. Schematic illustration of polycistronic vectors carrying multiple expression cassettes. The vectors carrying three (A) or five (B) expression cassettes are shown. The pBaculovirus Dual display vector contains three expression cassettes. The sequences coding for gp64 SS-His6-A target gene-gp64 TM-gp64 CTD and gp64 SS-His6-B target gene-gp64 TM-gp64 CTD, which can display two different proteins on viral envelope or plasma membrane under the control of baculovi- rus p10 and polyhedrin promoters. The pBaculovirus multiple display vector carries five expression cassettes as shown in the diagram. The target gene can be expressed under the control of the Pp10 promoter or polyhedrin promoters while and EGFP gene with cytosmeagerulins promoter is ex- pressed under control of the polyhedrin promoter. The EGFP is expressed in host cells for rapid identification of recombinant baculovirus while target proteins are displayed on the baculovirus envelope or plasma membrane.

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