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Baculovirus display: A novel tool for vaccination

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Abstract
Baculoviruses are enveloped DNA viruses that infects insect mainly from the order Lepidoptera. The virus is replicated and transcribed in the nucleus of infected cells. They only infect insects in nature and are non-pathogenic to vertebrates. The name Baculovirus derives from the Latin word baculum, which refers to the rod-shaped morphology. They have large, circular double stranded DNA genomes that range from 80–180 kbp and encode 90–180 genes. Among the numerous baculoviruses, *Autographa californica* multiple nucleopolyhedron virus (AcMNPV) is the most widely studied and used in biotechnology. These are extensively utilized as an excellent tool for production of recombinant protein in insect cells. They are also used in the development of strategies for displaying foreign peptides and proteins on the virus surface as well as on mammalian cell. This is achieved by fusing the protein of interest with the major viral envelope glycoprotein (Gp64). The surface displaying of antigenic epitopes make viruses efficient vaccine vehicles capable of mounting a strong specific immune response. The baculovirus expression vector system (BVES) is an excellent tool for the production of recombinant vaccine. Many subunit vaccines have been expressed in this system. Thecapability to transduce a wide variety of mammalian cells leads to the emergence of baculovirus as a novel vector for *in-vivo* and *in-vitro* gene delivery. 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, they has been utilized as gene delivery and vaccine vectors for a wide variety of application.

Keywords: Metagenomics, DNA, genome, CFUs

Introduction
The Baculoviridae form a family of arthropod-specific large double stranded DNA viruses that infect insect larvae. The family is divided into four genera, which reflects the co-evolution of these viruses and the insects they infect. Baculo viruses classified in the genera Alpha baculo virus and Beta baculo viruses infect the larvae of lepidopteran insect species. Gamma baculo viruses infect larvae of hymenopteran insect species and the single known virus classified as Delta baculo virus infects Culex mosquito larvae. The name baculovirus derives from the Latin word baculum, which refers to the rod-shaped morphology of their nucleocapsids. All baculo viruses have large, circular double stranded DNA genomes that range from 80–180 kbp and encode 90–180 genes. The name baculovirus derives from the Latin word baculum, which refers to the rod-shaped morphology of their nucleocapsids [1].

The majority of baculo viruses that infect larvae have narrow host ranges, that is, a specific baculo virus infects the larval stage of only one or a few host species. Baculoviruses form two different virion phenotypes: budded viruses (BVs) and occlusion-derived viruses (ODVs). BV consists of a single nucleocapsid surrounded by an envelope derived from the plasma membrane of a host cell. These BVs display a fusion protein to allow cell entry. ODVs in contrast are formed in the nucleus of infected cells and consist either of a single (SNPV) or multiple (MNPV) nucleocapsids. ODVs are surrounded by an envelope that is derived from the inner nuclear membrane and contains a number of proteins encoded by baculovirus genes. ODVs are also embedded in a crystalline matrix, forming occlusion bodies [2].

Bacculovirus only infect insects in nature and are non-pathogenic to vertebrates. In addition to insect cells, the virus 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, they have been utilized as gene delivery and vaccine vectors for a wide variety of applications [3]. Among the numerous baculoviruses, *Autographa californica* multiple nucleopolyhedron virus (AcMNPV) is the most widely studied and used in biotechnology.

AcMNPV genome encodes about 150 genes which are transcribed in a temporal fashion. Firstly, early genes are transcribed by the host RNA-polymerase II. Baculoviruses are extensively utilized as an excellent tool for production of recombinant protein in insect cells. Protein expression in insect cell culture is not the only application of baculoviruses. Baculovirus display consists of the expression of proteins or peptides on the surface of a virus. The surface displaying of antigenic epitopes make viruses efficient vaccine vehicles capable of mounting a strong specific immune response. Baculovirus based expression technology has matured considerably and is now commonly used to produce proteins of scientific interest and to manufacture commercial vaccines worldwide, both for human and veterinarian use. In addition, recombinant baculoviruses have found applications as gene delivery vectors for mammalian cells and as expression vectors for adeno-associated virus (AAV)-based gene therapy products [4].

Baculovirus

Baculoviruses are enveloped DNA viruses that infects insect mainly from the order Lepidoptera. Their genomes is circular double-stranded DNA molecules of about 80 to 180 kbp and are packed in rod-shaped nucleocapsids with a typical size of 40-50 nm in diameter and 200-400 nm in length. The genome of virus is replicated and transcribed in the nucleus of infected host cells where the large DNA is packaged into rod-shaped nucleocapsids [5]. Since the size of these nucleocapsids is flexible, recombinant baculovirus particles can accommodate large amounts of foreign DNA. *Autographa californica* multiple nucleo polyhedron virus (AcNPV) is the most extensively studied baculovirus strain. Its entire genome has been mapped and fully sequenced. The baculovirus genome is a large circularized DNA molecule with 130 kb of length and a maximum cloning capacity of at least 38 kb. This flexibility results particularly advantageous in contrast to retroviral and AAV vectors whose cloning capacities are limited [6].

Replication Cycle of Baculovirus

In the baculovirus, two distinct viral populations are formed in infected insect cells, occluded and budded virions. Infection cycle initiates when a budded virus (BV) interacts with the cell membrane and is endocytosed. Cell entry is mediated by a class-III viral glycoprotein located at the virion surface (Gp64) which interacts with cell receptor [7]. This interaction triggers clathrin-dependent endosomal internalization. This internalized vesicle becomes subsequently acidified. This causes a conformational change in Gp64 that result in the fusion of the viral envelope with the endosome membrane. Thus, the nucleocapsid is released in the cytoplasm and nucleocapsid is then transported to the nucleus where it transcribes its genes, replicates its DNA in the virogenic stroma where new nucleocapsids are assembled. Nucleocapsids then travel to the cytoplasmic membrane and budded through acquiring an envelope containing the surface protein Gp64.

Fusion proteins

Entry of baculoviruses into host cells requires fusion of the viral envelope with the cytoplasmic membrane by the action of viral envelope fusion proteins. There are three classes of viral membrane fusion proteins have been identified. Class I which contain N-terminal hydrophobic fusion peptides, Class II, which fusion peptides are located in internal loops and Class III that exhibit distinct structural features in their architectures as well as in their membrane interacting fusion loops. Gp64 belongs to III group. The major envelope protein of the budded virions, Gp64, has been shown to mediate acid triggered membrane fusion both in virions and transfected cells. The native Gp64 is a phospho-glycoprotein. Gp64 is composed of five domains that result in a macromolecular structure [8]. The gp64 envelope glycoprotein of AcMNPV is an essential virion protein that is involved in both receptor binding and membrane fusion during viral entry into insect and mammalian cells [9]. The gp64 envelope glycoprotein consists of a signal peptide (SS) and a mature domain that includes the trans-membrane domain (TM) and cytoplasmic domain [10]. After expression in insect cells, gp64 protein is transported to the plasma membrane, directed by the SS of gp64, where gp64 is displayed on the surface of infected cells. It is a 64 kDa protein which forms trimmers and locates in the BV envelope. As Gp64 is a trans-membrane protein that exposes an outer domain, it can be used to display a selected protein on the BV surface.

A chimeric Gp64 can be constructed to contain the protein of interest allowing it to be incorporated in the BV structure upon infection of insect cells [11]. However, Gp64 conserves the typical characteristics of viral fusion proteins. It includes a fusion domain which mediates the fusion between the cell membrane and viral envelope; a transmembrane domain which anchors the protein in the lipidic bilayer and a multimerization domain that allows the protein to form trimmers. Gp64 also contains a seven residue C-terminal tail domain [7].

Baculovirus display

Eukaryotic systems represent a highly interesting model for the study of higher eukaryotic structures and interaction mechanisms because they provide posttranslational modifications and complex protein folding, in contrast to prokaryotic systems. Moreover, displaying a protein on the surface of a cell or a virus is a very successful strategy, for recreating and maturing binding properties such as antigenic recognition. Several strategies have been developed for displaying heterologous peptides or proteins on the baculovirus envelope by fusing the peptide or protein to gp64. In most instances the vector is designed with the aim of obtaining baculovirus particles that contain both wild-type gp64 and chimeric gp64 molecules [11].

Baculoviruses displaying proteins fused to Gp64 have proven to be very effective immunogens and they have been used successfully to generate antibody responses to a variety of displayed proteins. These viruses are able to mount a robust innate immune response by activating professional APCs, it is expected that baculovirus expressing a heterologous antigen on its surface could generate a specific response against this antigen. In fact, several works showed that baculoviruses expressing chimeric Gp64 on its surface were able to mount a very strong humoral response against the antigen displayed [13]. Furthermore, numerous studies used baculovirus display for the development of new generation vaccines. In this context, baculovirus surface display conferred protection and induced a strong humoral response against reovirus [14] and influenza [15].

Baculovirus display libraries

Surface display libraries represent a very useful methodology for selecting binding proteins out of defined pools of protein

variants. Although prokaryotic expression systems such as phage display technology or protein targeting to the cellular surface of *Escherichia coli* are widely used, they fail allowing the functional display of complex proteins such as eukaryotic glycoproteins which require a high degree of modification and processing. Eukaryotic expression libraries, in contrast, are a powerful tool for finding new ligands, identification of cellular interaction partners and affinity maturation of antibody and antibody fragments. Furthermore, baculovirus surface display libraries served to identify MHC class I and II mimotopes [11]. The expression of foreign proteins on the surface of insect cells, in occlusion bodies and on the baculovirus surface makes baculoviruses an important resource in biotechnology. Moreover, fusion proteins with the baculoviral envelope protein Gp64 as well as different foreign membrane proteins such as the influenza virus hemagglutinin or VSV-G protein have shown to be targeted to the surface of infected insect cells in several researches about baculovirus display. Then, it is possible take advantage of baculovirus display systems with the aim to generate a surface display library for high throughput screening. This procedure results in the construction of a baculovirus surface display library capable to allow the selection of the displayed peptide with optimal antigenicity. Baculovirus insect cell system consists in a highly useful tool for constructing and screening of surface display libraries, especially for the expression of eukaryotic complex proteins.

Types of Baculovirus Display

There are different kinds of baculovirus display are available as follows

Baculovirus surface display using entire Gp64 (Chimeric proteins using the entire Gp64)

Gp64 can serve as a fusion partner that together with a chosen target protein gets incorporated into the cell membrane and into budded virions. In the first reports of virus display proteins were fused to the complete gp64. In these works the target proteins were cloned into a vector providing N-terminal fusion with the gp64 signal peptide and C-terminal fusion with the full length Gp64 coding region For the purpose of antigen display various epitopes were presented and shown to induce immune response. The advantages of this method reside in that all needed sequences for glycoprotein transport and maturation are present in the entire sequence of Gp64. Complete Gp64 fused antigens will be synthetized through the glycoprotein synthesis pathway and will be directed to plasmatic membrane and also budded virus envelope [11].

Baculovirus surface display using TM, MMD and CTD as a fusion partner for antigenic target

In order to facilitate the construction of a chimeric protein it was shown that is not necessary to conserve the complete structure of Gp64. The signal peptide (SP), the transmembrane (TM) and the cytoplasmic tail domain (CTD) were shown to be enough for the surface display, whereas the rest of the protein can be eliminated. This strategy avoids the need of dealing with large transfer vectors as well as permitting to increase the number of displayed proteins.

Baculovirus display using recombinant Gp64 expressing a small peptide (Peptide insertion on Gp64)

Another strategy consists in peptides directly engineered into the native Gp64 in order to increase the avidity of the displayed target. In this case a short peptide is inserted into the sequence of the wild type Gp64; being this protein the only variant expressed in the virion, in contrast to the previous approach where both wild type and the modified versions coexisted in the BV surface. It has been reported that this method resulted very efficient to mount a robust specific antibody response against the inserted peptide with a significantly increased avidity. Size of the peptides for insertion results in a limiting condition. Indeed, only small peptides have been inserted into the native gp64 with a maximum size of 23 amino acids [16].

Capsid display

Baculovirus surface display on the envelope, heterologous protein has been displayed on the capsid by fusion with the major capsid protein VP39 without any interference in the virus assembly. This finding suggested the possibility of performing insertions into the inner capsid of the BV particle. VP39 is the most abundant protein of the nucleocapsid and consist in a 39 KDa polypeptide with monomers arranged in stacked rings around the nucleoprotein core [17]. Antigen capsid display should be able to reach the cytosol and preferentially trigger MHC class I presentation pathway and mount a strong CD8+ T cell response. In this context Molinari *et al.*, 2011 developed a capsid display system and probed it fusing OVA with VP39 (BV-OVA) and showed that OVA could enter into the MHC class I pathway. Though the mechanism involved in OVA MHC class I presentation was not elucidated, all these data suggest that capsid display is more convenient over envelope surface display for CTL activation. Baculovirus are internalized by DCs and induce their maturation and the production of the pro-inflammatory cytokines IL-6 and IL-12 and are able to mount a type I IFN response. Finally, they were also examined the efficacy of the strong CTL and innate immune response elicited by baculovirus by the capacity of BV-OVA to confer protection against the classical MO5 melanoma tumor model. It was observed that inoculation with the BV-OVA protect against this tumor model.

In conclusion, capsid display results in a very attractive alternative for cells transduction and for triggering MHC class I presentation of antigenic peptides. In this way, capsid display showed to be strongly effective to mount a robust cellular response against heterologous proteins promoting both IFN secretion and cytotoxic CD8+ T cells activation.

Baculovirus as a vector

The baculovirus expression system (BVES) is a valuable tool for the production of vaccine. Many subunit vaccines have been expressed in this system. The first vaccines produced in insect cells for animal use are now available commercially. BVES is a fantastic tool for vaccine development and it has wonderful feature for future animal vaccine development. BEVS is a versatile system to produce high quality recombinant protein vaccine in insect cells. BEVS has been extensively used to express a large variety of proteins. Several researchers have proved that BEVS has the ability to produce significant amounts of the desired protein in a cellular environment, thereby enabling the correct folding, targeting and post-translational modification of the expressed protein [18].

Recombinant baculoviruses are attractive for high-level production of large eukaryotic proteins. Because of the flexibility of the AcNPV envelope, large DNA insertions can

be accommodated in its genome. Compared with others, baculovirus expression system also exhibits abundant yields with proper glycosylation and other modifications pivotal to immunogenic potential [19]. Thus, the baculovirus-insect cell expression system has been extensively utilized for the production of many recombinant proteins and commercial vaccines [20, 21]. The construction of recombinant baculovirus is performed in two steps. Foreign genes are first subcloned into a transfer vector propagated in *E. coli* and then inserted into the baculovirus genome by homologous recombination in insect cells producing recombinant progeny. AcMNPV has been developed as protein expression vectors.

The viral proteins p10 and polyhedrin are expressed abundantly in infected cells and are dispensable for virus replication, thus recombinant baculovirus can be constructed by placing the foreign gene under the control of p10 or polyhedrin promoter and utilized to infect insect cells for foreign gene expression. Baculovirus cloning capacity is as large as 38 kb [22]. Baculovirus neither replicates inside the transduced cells nor integrates its DNA into host chromosomes in the absence of selective pressure, hence easing the safety concerns [23]. Baculovirus's capability to transduce a wide variety of mammalian cells leads to the emergence of baculovirus as a novel vector for *in-vivo* and *in-vitro* gene delivery [24]. These genes are commonly under the control of polyhedrin and p10 promoters. In the late phase of infection, the virions are assembled and budded recombinant virions are released.

However, during the very late phase of infection, the inserted heterologous genes are placed under the transcriptional control of the strong AcNPV polyhedrin promoter. Thus, recombinant product is expressed in place of the naturally occurring polyhedrin protein. Usually, the recombinant proteins are processed, modified and targeted to the appropriate cellular locations.

There are several advantages of using baculovirus expression system over *E. coli*. Baculovirus expression vector system (BEVS) is one of the most powerful and versatile eukaryotic expression systems available. The BEVS is a helper-independent viral system which has been used to express heterologous genes from many different sources, including fungi, bacteria and viruses in insect cells. In this system several Baculovirus genes nonessential in the tissue culture life cycle may be replaced by heterologous genes. Since the baculovirus genome is generally too large to easily insert foreign genes, heterologous genes are cloned into transfer vectors.

Baculovirus as immunogens

The innate immune system provides the first line of host defense against infection. It is extremely important to mount a strong specific immune response by expressing co-stimulating factors necessary for the activation of adaptative immunity cells. It was shown that inoculation of a murine macrophage cell line with budded baculovirus induces the secretion of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-12 [25]. They induce pro-inflammatory cytokines secretion through a MyD88/TLR9-dependent signaling pathway. Toll-like receptors (TLRs) are a family of transmembrane proteins that recognize and bind endogenous and exogenous ligands. Signaling through TLR generally culminates in the production of pro-inflammatory cytokines resulting in modulation of several aspects of the innate immune response [26].

In the case of baculovirus, it has been reported that BVs could induce cytokine production through the TLR9 signaling pathway in mammals. TLR9 was shown to be responsible *in-vivo* for immune system stimulation by oligodeoxynucleotides. Baculoviruses triggers TLR-9 or TLR-3 dependent pathway for induction of innate immunity. BVs have strong adjuvant properties in mice, promoting potent humoral and CD8+ T cell adaptive responses. In addition, BVs induce the production of inflammatory cytokines by the *in-vivo* maturation of dendritic cells. Baculovirus administration on the innate immune response of chickens showed the upregulation of IFN-γ and IL-6 in the baculovirus treated chicken spleens and a decrease of the TGF-β gene expression. These facts indicated a strong proinflammatory immune response. The duration of the BV-induced response is very limited. The strong immune response induced by AcMNPV makes it a promising candidate for a novel adjuvant-containing vaccine vehicle against infectious diseases [27]. AcMNPV inoculation of mice induced NK cells activation. Inoculated animals there was up to fourfold increase in the number of NK cells in spleen, liver, bone marrow and thymus [28].

Baculovirus as transducing agent

Baculovirus entry into mammalian cells represents an important goal for immune response induction and most recently for different gene therapies. It was initially suggested that baculovirus entry depended on electrostatic interactions, heparin sulfate and phospholipids [29]. Once inside the cells, baculovirus is transported to the endosome. Then, virions are released by the acid-triggered gp64 fusion [30] and subsequently transported into the nucleus [31] Reorganizing the actin cytoskeleton [32]. Inside the nucleus, baculoviral DNA could be recognized by the cellular transcription machinery and recombinant proteins could be expressed. Baculovirus-mediated transduction does not present any toxic effect against mammalian cells and does not disturb cell growth [33]. Baculoviruses do not replicate in transduced mammalian cells [34].

Advantage of baculoviruses as gene therapy vectors consists in its large cloning capacity. The baculovirus genome is a large circularized DNA molecule with 130 kb of length and a maximum cloning capacity of at least 38 kb. This flexibility results particularly advantageous in contrast to retroviral and AAV vectors whose cloning capacities are limited. In comparison with other viral vectors, baculoviruses are easy to produce. Baculovirus can be easily propagated by infecting insect cells in suspension culture or monolayer and harvesting the supernatant 3–4 days post infection. In addition, the construction, propagation and handling of baculoviruses can be performed in biosafety Level 1 laboratories without the need for specialized equipment. Finally, one of the most important advantages is that baculoviruses do not present preexisting immunity in mammalian. The use of baculovirus vectors in gene therapy, therefore, may avoid the problem of preexisting immunity [35].

Application

There are numerous applications of baculoviruses in modern microbiology and biotechnology. Baculovirus expression system has been extensively used as expression vectors in insect cell cultures to produced recombinant glycoprotein. This system is safe, easy to use and scale up system [36]. Baculovirus surface display based on the generation of Gp64

chimeric proteins result in a very efficient technology capable to induce a strong immune response against specific antigens [37]. Its adjuvant capacity makes baculoviruses in a promising alternative for the generation of immunological memory [27]. Baculoviruses were shown to be useful as gene therapy vectors which avoid the problem of preexisting immunity [35]. For all above reasons, baculoviruses represent a very useful tool in biotechnology as vaccination vectors.

Advantages

Since 1983, when B.E.V.S. technology was introduced, it has become one of the most versatile and powerful eukaryotic vector systems for recombinant protein expression. More than 600 recombinant genes have been expressed in baculoviruses to date. Since 1985, when the first protein (IL-2) was produced in large scale from a recombinant baculovirus, use of BEVS has increased dramatically [38]. Baculoviruses offer the following advantages over other expression vector systems

Safety: Baculoviruses are essentially nonpathogenic to mammals. They have a restricted host range, which often is limited to specific invertebrate species. Because the insect cell lines are not transformed by pathogenic or infectious viruses, they can be cared for under minimal containment conditions.

Ease of scale up: Baculoviruses have been reproducibly scaled up for the large-scale production of biologically active recombinant products.

High levels of recombinant gene expression: The recombinant proteins are soluble and easily recovered from infected cells.

Accuracy: Baculoviruses can be propagated in insect hosts which post-transnationally modify peptides in a manner similar to that of mammalian cells.

Conclusion

Bacculovirus only infect insects in nature and are non-pathogenic to vertebrates. Bacculoviruses are very useful tool in biotechnology which will be used as an expression vectors. In addition to insect cells, the virus 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, they have been utilized as gene delivery and vaccine vectors for a wide variety of applications.

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