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Baculoviruses as Vectors in Mammalian Cells*

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Abstract: The *Baculoviridae* are a large family of enveloped DNA viruses exclusively pathogenic to arthropods. Baculoviruses have been extensively used in insect cell-based recombinant protein expression system and as biological pesticides. They have been deomostrated to be safe to mammals, birds and fish. Recently, baculoviruses has been shown to transduce different mammalian cells in spite of the fact that they cannot replicate in mammalian cells (11, 73, 76). This has resulted in the development of baculoviruses as mammalian expression systems and even as vestors for gene therapy.

Key words: Enveloped DNA viruses; Baculoviruses;

BACULOVIRUS TRANSDUCTION OF MAMMALIAN CELLS

Volkman and Goldsmith were the first to report that baculoviruses can be internalized by vertebrate cells (76). Later, two groups reported that a recombinant *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) containing a mammalian promoter driving a target gene cassette could be transduced in mammalian cells to express the reporter genes (10, 28). Efficient transduction was first observed in primary hepatocytes and hepatoma cells and mediated high expression of the target genes. Another study demonstrated that additional cell types could be successfully transduced including COS-7, HeLa and porcine kidney cells (64). In addition, baculoviruses were able to transduce nondividing cells such as human neural cells (62). Several recent reports have rapidly expanded the list of cells permissive to transduction by baculoviruses. The reported cell types amenable to transduction by baculoviruses have been reviewed (30, 36). These cells include cell lines originating from differrent mammals such as human, rodent, porcine, bovine. The cell range transduced by baculovirus was even extended to avian cells (66) and those derived from zebrafish (77).

The transduction efficiency is promoter-dependent. After transduction, transcripts of several immediate-early baculovirus genes accumulated at readily detectable levels in the transduced cells indicated that the baculovirus immediate-early promoters are active in mammalian cells, but baculovirus early promoters are not strong enough to efficiently drive the target genes expression in mammalian cell (35). Therefor, it is best to choose a mammalian promoter to drive a transgene. However, various mammalian promoters have different activities in mammalian cells. A recombinant

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baculovirus containing a β-gal cassette driven by a CAG promoter could transduce in less susceptible cells (10, 28). On the other hand, a recombinant baculovirus containing a CMV promoter and green fluorescent protein (GFP) gene transduced a large panel of cell lines and primary cultures (13). The addition of butyrate or Trichostatin A, a selective histone deacetylase inhibitor can enhance GFP production in the most cell lines (13). Therefore, the efficiency of transduction of mammalian cells is related to the activity of the mammalian promoter that is either directly or indirectly susceptible to silencing by histone modification.

One possibility to increase the transduction efficiency in certain cell lines is to insert a homologous region (*hr*) of AcMNPV upstream of the mammalian promoter (43). Interestingly, the *hr*1 can enhance promoter activity as an enhancer in mammalian cells when it presents in trans (75). Moreover, the insertion of *hr*1 can function to maintain the genetic stability of the recombinant baculoviruses because a spontaneous deletion of the heterologous gene (s) readily occurs in recombinant viruses (55, 56).

MECHANISMS OF BACULOVIRUS ENTRY INTO MAMMALIAN CELLS

Entry of the baculovirus budded virus phenotype into insect cells is thought to occur through an acid-pH-dependent endocytosis pathway (15). Likewise, baculoviruses are generally considered to enter mammalian cells in the same pathway, which can be inhibited by chloroquine that blocks endosomal maturation (10, 28). The viral envelope glycoprotein GP64 is shown to be essential for virus attachment and endosomal escape (27).

In support of the importance of GP64 is the finding that a monoclonal antibody specific for GP64 abolishes the capability of baculovirus vectors to transduce mammalian cells (23). When GP64 was over-expressed from an additional *gp*64 gene, the baculovirus exhibited a 10- to 100-fold increase in a reporter gene expression in a variety of mammalian cells in comparison to the parental virus (72). The function of GP64 in transduction by baculoviruses was further substantiated when a mutant virus lacking gp64 failed to transduce mammalian cells (1). However, a baculovirus with an F fusion protein, such as *Helicoverpa armigera* single nucleopolyhedrovirus (HearNPV), could not transduce mammalian cells. This apparent defect was rescued with the AcMNPV GP64 (39). Moreover, the range of mammalian cell types transduced by recombinant HearNPV expressing GP64 was consistent with those transduced by AcMNPV. Interestingly, a baculoviruses displaying vesicular stomatitis virus (VSV) G protein transduced human hepatoma and rat neuronal cells at efficiencies of 10-to 100-fold greater than baculoviruses lacking VSV-G (6).

Although it is known that GP64 is the main protein for entry of the baculovirus particle, the molecule at the cell surface that interacts with the virus has not yet been fully elucidated in both insect and mammalian cells (37). Initially, Boyce and Bucher assumed that asialoglycoprotein could be involved in virus binding, since baculovirus transduction was liver specific (10, 28). However, Pk1 cells, which do not express asialoglycoprotein receptors can be successfully transduced, suggesting that asialoglycoprotein is not a key receptor (74). Duisit *et al.* demonstrated that electrostatic interactions may be involved in baculovirus binding to the mammalian cell surface since baculovirus binding can be inhibited by polybrene, a cationic compound

that neutralizes negatively charged epitopes on the cell membrane (17). These authors also suggested that heparan sulfate may be an important molecule for baculovirus binding. In addition, phospholipids may also be important in GP64 recognition (72). In spite of the fact that cell surface receptors have not yet been characterized, it is suggested that baculoviruses are internalized by endocytosis (13, 74). A recent report further confirmed that entry of baculoviruses into mammalian cells is primarily dependent on clathrin-mediated endocytosis (47), while cave olar endocytosis is somehow also involved in this process (44).

Although attachment and internalization are the important steps in the transduction of mammalian cells, the limited step may lie in cytoplasmic movement or nuclear entry of the nucleocapsids. Salminen *et al.* demonstrated that escape from endosomes was not an essential step (60). In permissive mammalian cells, the GFP-labeled viral nucleocapsid was observed in the nucleus at 4 hours post transduction. However, in nonpermissive cells, the nucleocapsid was not observed in the nucleus suggesting that nuclear transport may be the essential step for baculovirus transduction of certain cell lines (37). Nucleocapsids seem to induce the formation of actin filaments in the cytoplasm, which probably facilitates nucleocapsids entry into the nucleus (74). A more recent study demonstrated that the expression of host ß-actin was up regulated in the mammalian cells inoculated with AcMNPV (22). Additionally, it was suggested that intact microtubules constituted a barrier to baculovirus transport to the nucleus (60). Although some insights have been gained in recent year, the mechanisms of invasion, movement and nuclear entry of baculoviruses are still largely unknown.

BACULOVIRUS AS A DELIVERY VECTOR FOR GENE FUNCTION ANALYSIS

Baculovirus-mediated gene delivery has been developed as a tool to study gene function *in vitro* since they transduce a broad range mammalian cell types including primary cell types with high efficiency (30, 36). Ye et al. reported a baculovirus was used to study the function of herpes simplex virus 1 U (L) 34 gene (81). The same group has used baculovirus delivery system for elucidating several genes functions of herpes virus (49, 63, 81, 83, 84). Clay *et al.* reported that two recombinant baculoviruses containing an estrogen receptor or a reporter gene controlled by an estrogen receptor-responsive promoter were co-transduced into human osteosarcoma cells to study estrogen receptor function (12). In addition, baculovirus-mediated transient expression has been developed for high throughput screening. The assays have been applied for screening modulators of protein activity in ion channels (53), nuclear receptors (9, 12) and GPCRs (4). Jenkinson *et al*. have reported that a cell/cell fusion assay that mimics HIV viral/cell fusion process is amenable to a high-throughput screening format based on the baculovirus system (32). Another application is to screen antibodies destined for immunohistochemistry studies (69). Baculoviruses for high-throughput screening will be a promising application. Moreover, baculoviruses carrying a tumor-suppressor or suicide genes were also used in cancer therapy including osteosarcoma, human prostate cancer and glioma (67, 68, 78).

Although the replication of hepatitis B (HBV) and C (HCV) viruses has been substatially expounded, a major obstacle has been the lack of permissive cell lines. To overcome this problem, recombinant baculo-

viruses were constructed to carry and deliver the genomes of HBV (16) and HCV (21) into hepatoma cells lines. Then, replication of HBV DNA and RNA, and expression of viral genes were detected (16). This approach was successfully used to investigate the effect of anti-viral drugs on HBV infection, to detect the characteristics of anti-viral mutants of HBV and viral infection after anti-viral treatment. Baculoviurses have also been used to deliver HCV full-length and mini-genome under the tetracycline-inducible promoter into HepG2 cells. Although HCV RNA replication or virions were not detected, transcription and viral polypeptide processing were demonstrated (48). Baculoviruses have acted as novel tools for the analysis of HCV replication and host-cell interactions. Baculoviruses also provided a way to improve large-scale recombinant AAV vector production (65).

Following the development of RNA interference (RNAi), baculoviruses were developed to mediate RNAi to knock down target gene expression (50) or to inhibit HIV infection (34) in mammalian cells. Moreover, baculoviruses have been used to mediate RNAi *in vivo* by using a novel hybrid promoter consisting of the CMV enhancer and polymerase III H1 promoter. It was shown that RNAi delivered by a recombinant baculovirus suppressed expression of the target gene in rat brains by 82% (52).

BACULOVIRUS TRANSDUCTION AS IMMUNIZING AGENTS

It was demonstrated for the first time by Gronowski *et al.* (23) that a wild-type baculovirus elicited the production of alpha and beta interferons (IFN) in transduced murine and human cell lines, which induced *in vivo* protection of mice from encephalomyocarditis virus infection. Baculovirus transduction also induced expression of cytokines such as TNF-a, IL-1a, and IL-1b in primary hepatocytes (7). In addition, baculovirus elicited a strong innate immune response, which protected mice from a lethal challenge with an influenza virus (2). It was also found that AcMNPV was capable of stimulating anti-VSV activity in mammalian cells, but the HearNPV and the *Spodoptera exigua* multiple NPV (SeMNPV) had no inhibitory effect on VSV infection (40). This protective immune response was induced via the TLR9/MyD88-dependent signaling pathway (1). The viral genome into TLR9-expressing cellular compartments was necessary for the induction of innate responses.

The ability of a baculovirus expressing antigens under control of mammalian cell-active promoter to induce an immune responses was first demonstrated by Aoki *et al* (5). These investigators found that a recombinant baculovirus expressing glycoprotein gB of pseudorabies virus induced antibodies against gB protein in mice. Intranasal inoculation with a baculovirus containing the hemagglutinin gene of the influenza virus, under the control of the chicken-actin promoter, elicited a strong immune response that protected mice from a lethal challenge with influenza virus (2). Similarly, a baculovirus that expressed hFIX elicited antibodies against hFIX. The antibody titers were directly related to the amount of protein produced by the virus (31). Antigen-specific immune responses were elicited with a virus expressing the HCV E2 protein and carcinoenbryonic antigen (20). Interestingly, a baculovirus displaying VSV-G appeared to be a more potent immunogen than the unmodified virus (20). A recent study showed that a VSV-G pseudotyped baculovirus vector-transduced

ribozyme to be a more potent inhibitor of HIV-1 replication in HeLa and $CD4^+$cells than a recombinant baculovirus vector-transduced ribozyme lacking VSV-G (34). This finding agrees with the previous statement that a baculovirus displaying VSV-G is a highly efficient transducer of mammalian cells.

Injection of a baculovirus containing carcinoembryonic antigen (CEA) induced a measurable anti CEA–specific $CD4^+$T cell response. Additionally, injection of another baculovirus expressing HCV E2 glycoprotein also induced an anti-E2 $CD8^+$T cell response as well as the innate immune response such as natural killer (NK) cell cytolytic activity (20). Moreover, when a baculovirus that contained E2 and displayed VSV-G on the envelope, the minimal dose required to elicit a measurable T cell response was tenfold lower than the unmodified baculovirus. The result suggested that the baculovirus displaying VSV-G was a more potent vaccine vector.

BACULOVIRUS DISPLAY SYSTEM

It has been quite recently established that the baculovirus AcMNPV display vector system enabled presentation of peptides or proteins on the viral surface glycoprotein, GP64. The tropism and transduction efficiency of a baculovirus displaying heterologous protein have also been reported. A baculovirus displaying HIV-1 envelope proteins can bind the CD4 receptor on T cells (8). It was observed that two viruses displaying either the synthetic IgG binding domains (ZZ) or the single chain antibody Fragments (scFv) specific for the carcinoembryonic antigen (CEA) improved binding. However,enhanced transgene expression was not observed (51). Similarly, a baculovirus displaying an integrin-specific motif RKK, as a part of two different loops of the green fluorescent protein (GFP) fused with the AcMNPV major envelope protein GP64, was able to bind a peptide representing the receptor binding site of an alpha2 integrin, the alpha2I-domain. However, the interaction was not strong enough to overcome binding of wild type gp64 to the unknown cellular receptor(s) on the surface of alpha2 integrin-expressing cells (CHO-alpha2beta1) or enhance viral uptake (59).

The avidin-biotin technology was used in baculovirus targeting that resulted in both enhanced and targeted transduction (58). A 5-fold increase in transduction efficiency in rat malignant glioma cells and a 26-fold increase in rabbit aortic smooth muscle cells were detected. The baculovirus displaying αVβ3-integrin-specific RGD motifs, derived from the C-terminus of coxsackievirus A9 or human parechovirus 1 VP protein, resulted in both improved binding and enhanced transduction of human lung carcinoma cells expressing αVβ3-integrins (19, 46). In addition to GP64 fusion, GFP was also fused to AcMNPV VP39 capsid protein. This new tool provided possibilities for specific intracellular and nuclear targeting of the viral capsids, and an enhanced baculovirus mediated gene delivery into mammalian cells (46). In our laboratory, we have also developed a new baculovirus display system, HearNPV F protein (envelop protein) fusion system (45), which provided targeting opportunities to transduce specific mammalian cell types. Normally, parental HearNPV is not capable to transduce mammalian cells but GP64 can reverse this phenomenon (39).

Baculoviruses displaying proteins fused to GP64 have been proven to be very effective immunogens. AcMNPV displaying the human nuclear receptors LXRβ and FXR was first used to raise monoclonal

antibodies. This study illustrated that a baculovirus display is a versatile tool applicable in antigen presentation and for rapid production of functional monoclonal antibodies (41). In a similar manner, antibodies against a variety of proteins have been generated, including human peroxisome proliferator activated receptors (PPARs) (70), *Plasmodium berghei* circumsporozoite protein (82), hemagglutinin protein of Rinderpest virus (57), *Theila parva* sporozoite surface antigen P67 and foot-and-mouth disease virus proteins (33) .

In addition, the baculovirus display technology has been used to construct and screen a eukaryotic epitope library (18). HIV-1 gp41 epitope (ELDKWA), specific for the neutralizing human mAb 2F5, was inserted into the antigenic site B of influenza virus A hemagglutinin, and expressed on the surface of baculovirus-infected insect cells. The library consisted of 8 000 variants out of which one clone showed an increased specific binding capacity when screened by fluorescence activated cell sorting (18). Similarly, baculovirus-infected insect cells were used as a display platform for class II major histocompatibility complex (MHC II) molecules covalently bound to a library of potential peptide mimotopes (14). Later, the same group successfully used this baculovirus-based display system to identify antigen mimotopes of MHC class I-specific T cells (80).

IN VIVO GENE DELIVERY AND THERAPY

Owing to the highly efficient gene delivery into many mammalian cells, baculoviruses have exhibited the potential ability to be an ideal vector for gene therapy and other in vivo applications. Sandig *et al.* (61) first reported that transgene expression was undetectable when a baculovirus vector was introduced into mice and rats by a variety of methods, including direct injection into liver parenchyma. Shortly after that the same group found that baculoviruses activate the classical complement system pathway and become rapidly inactivated (27). On the positive side, baculoviruses can effectively transduce hepatocytes of complement-deficient mice (27). In addition, a recent study further showed that naturally occurring IgM antibodies with high affinity to baculoviruses may be partially responsible for the inactivation (25). However, baculovirus successfully transduce immune-privileged tissues/organs such as brain (38, 79, 78), testis (71), eye (24) and rabbit intervertebral discs (42). Even though the complement system appears to be a significant barrier, it has been reported that baculovirus can deliver genes *in vivo* into other mammalian tissues including rabbit carotid artery (3), rat liver (31) and mouse skeletal muscle and liver (54). The reason for this is that may be the complement system did not completely inactivate all baculoviruses.

A number of methods have been developed to overcome complement inactivation and to facilitate genes delivery *in vivo* (30), including incubation of the virus with serum deficient in various complement factors (29), protection of virus from inactivation by soluble complement receptor type 1 (sCR1) (26), anti-C5 antibody, or a cobra venom factor (CVF) (29). Baculoviruses specifically transduced the epithelium of the choroids plexus in ventricles with a transduction efficiency as high as 76 % (38). A more recent study showed that the baculovirus effectively suppressed tumor development, when used to produce the A-chain of diphtheria toxin intracellularly in a rat C6 glioma xenograft model (52). Hence, baculovirus

vectors could be very useful in gene therapy in brains.

It was observed that a regulator of complement activity (sCR1) protected baculoviruses from serum inactivation (26) and the complement resistant virus was engineered by fusing another regulator, the decay accelerating factor (DAF), with GP64 (31). Such a complement-resistant baculovirus vector was used to transiently deliver the human factor IX gene to neonatal rats by an intrahepatic injection. Additionally, pseudotyped baculoviruses displaying VSV-G protein are more resistant to complement than unmodified virus. In fact, the pseudotyped viruses are more resistance to inactivation by human, rabbit, guinea pig, hamster and mouse serum, but are sensitive to rat serum (71). The pseudotyped viruses can transduce hepatocytes (6), skeletal muscle (54), cerebral cortex and testis (71) of mice.

CONCLUDING REMARKS AND PROSPECTS

Baculoviruses have revealed a powerful ability to deliver genes into mammalian cells. Recombinant baculoviruses possess several advantages in gene therapy including the broad range of susceptible mammalian cells, little or no observable cytopathic effect, ease of production and large packaging. Moreover, the viruses are inherently unable to replicate in mammalian cells, avoiding any risk of outbreak of the replication-competent virus (58). Moreover, baculoviruses are a good tool for functional analysis of genes and good vectors to counter act viral infections or as immunizing agents. Although baculoviruses have shown a prospect in gene therapy, the mechanisms involved in viral transduction are still unclear. Knowledge of the baculovirus-cell interactions will help to design high effective or targeting vectors.

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