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BACULOVIRUS: A VERSATILE VIRUS USED IN BIOLOGICAL CONTROL AND AS A VECTOR OF EXPRESSION OF HETEROLOGOUS PROTEINS APPLIED IN HUMAN HEALTH

BACULOVÍRUS: UM VÍRUS VERSÁTIL USADO NO CONTROLE BIOLÓGICO E COMO VETOR DE EXPRESSÃO DE PROTEÍNAS HETERÓLOGAS APLICADAS NA SAÚDE HUMANA

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ABSTRACT
Objective: reviewing the history, morphology, life cycle of baculovirus infection in the host insect, and finally as applications of baculovirus-mediated expression in pest control and gene therapy in mammalian cells. **Data source:** the databases used were: SciELO and PubMed, where there was a search for articles and books related to baculovirus focusing on its morphology, replication cycle, and biotechnological applications in health. It was adopted as initial criteria for selecting the keywords: "baculovirus", "baculovirus life cycle", "gene therapy", "baculovirus applications" and "biotechnological applications". **Results:** in recent decades, with the advancement of molecular biology, the baculovirus has evolved from a simple insect virus used only in biological control of agricultural pests, to versatile biotechnological tools that can be used to infect insect cells and also transducing mammalian cells for the expression of a wide variety of heterologous proteins. **Conclusion:** therefore, baculovirus has emerged as a new vector for gene therapy. In addition, the use of baculovirus-mediated gene transfer has been considerably expanded for drug screening, eukaryotic gene display, cancer therapy, tissue engineering, vaccines, and other applications.
Keywords: baculovirus application in biotechnology; gene therapy; viral vectors.

RESUMO
Objetivo: revisar a história, morfologia, o ciclo de vida de infecção do baculovírus no inseto hospedeiro e, finalmente, as aplicações de expressão mediada por baculovírus no controle de pragas e terapia genética em células de mamíferos. **Fonte de dados**: o desenvolvimento desse trabalho consistiu em uma revisão de literatura. As bases de dados utilizadas foram: SciELO e PubMed, onde foram feitas buscas por artigos e livros referentes ao baculovírus com enfoque na sua morfologia, ciclo de replicação e aplicações biotecnológicas na área de saúde. Adotou-se como critério inicial para seleção a utilização das palavras-chaves "*baculovirus*", "*baculovirus life cycle*", "*gene therapy*", "*baculovirus applications*" e "*aplicações biotecnológicas*". **Resultados:** nas últimas décadas, com o avanço da biologia molecular, os baculovírus evoluíram de um simples vírus de insetos usados até então apenas no controle biológico de pragas agrícolas, para ferramentas biotecnológicas versáteis que podem ser usadas para infectar células de insetos e também transduzir células de mamíferos para a expressão de uma grande variedade de proteínas heterólogas. **Conclusão:** o baculovírus surgiu como um novo vetor para a entrega de genes. Além disso, o uso da transferência de genes mediada por baculovírus foi consideravelmente expandida para triagem de medicamentos, exibição de genes eucarióticos, terapia de câncer, engenharia de tecidos, vacinas e outras aplicações.
Palavras-chave: aplicação de baculovírus na biotecnologia; terapia gênica; vetores virais.

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INTRODUCTION

The history of the discovery of baculoviruses dates from the development of the silk industry that occurred more than 5000 years ago in ancient China. Baculoviruses are infectious agents that are disseminated in various organisms within the classes of arthropods. Although there is a wide variety of baculovirus targets, the most studied are those that cause diseases in common pests (MARTIGNONI; IWAI, 1986).

Currently, there are more than 700 species of baculovirus so far isolated and cataloged (HERNIOU; JEHLE, 2007). However, only about 50 species had their genomes sequenced. The baculoviridae family is currently subdivided based on phylogenetic evidence

and molecular characteristics into four distinct genera: Alphabaculovirus, Betabaculovirus, Gammabaculovirus, and Deltabaculovirus (JEHLE *et al.*, 2006).

The baculovirus replication cycle within the infected insect cell is biphasic and involves the formation of two types of virions. Occlusion-derived virions (ODVs) are adapted to have stability in the environment external to the host insect and virions that sprout from the plasma membrane (BVs), which are not occluded, they are therefore responsible by the systemic spread within the host insect and consequent death of the insect (PASSARELLI; GUARINO, 2007).

Baculoviruses have a great diversity of biotechnological applications of economic interest, such as the expression of heterologous proteins. However, the use of baculovirus was initially considered as a biological insecticide in pest control. These viruses are considered highly virulent, selective (infect only certain groups of arthropods), and stable, and mainly with low environmental impact after their application (MOSCARDI, 2011).

Although baculoviruses have this versatility for numerous applications, their use as bioinsecticides was limited until recently because of their slow action of eliminating pests and technical difficulties for commercial production *in vitro*. Two approaches to the broader application of baculoviruses as biopesticides are expected to be implemented in the future. First, in countries where the use of genetically modified organisms is restricted, improvements will be mainly in the level of diagnostics, production *in vitro,* and changes in biopesticide formulations. In the second approach, the ability of baculoviruses to kill pests can be increased by genetic modifications of their genome by introducing genes from other natural pathogens (SZEWCZYK *et al.*, 2006).

Another practical limitation regarding the use of baculovirus is that this virus has several disadvantages as vectors of gene therapy. One is that baculovirus induces a transient expression in mammalian cells. *In vivo*, the expression of the transgene typically decreases on the 7th day and disappears on the 14th day. The duration of the transgene expression *in vitro* using baculovirus is significantly lower than the expression mediated by retroviral, lentiviral, and AAV vectors (AIRENNE *et al.*, 2000; LEHTOLAINEN *et al.*, 2002).

However, biotechnology has advanced a lot in all areas of study and with different approaches, and it is increasingly observed the use of baculovirus in applications beyond the production of proteins in insect cells. These approaches include developing strategies for displaying peptides and heterologous proteins in virus particles and the insertion of mammalian expression cassettes in baculovirus to express genes in a highly efficient manner in a wide variety of mammalian cells. Baculoviruses also can transduce a wide variety of mammalian or non-human cell lineages (HU, 2008), with different purposes, such as expression of therapeutic genes to be used in cancer treatment (WANG *et al.*, 2006), ischemia and reperfusion (IRI) injury in transplanted organs (HITCHMAN *et al.*, 2011), tissue regeneration (WANG *et al.*, 2005) and as a vaccine vehicle (HU, 2008; MADHAN, 2014. PRABAKARAN; KWANG, 2010).

Thus, the use of baculovirus can be an alternative method of providing therapeutic genes to be used in various pathologies and mainly remaining confined to target organs. As BV genes that have these functions in insect cells are not expressed in mammalian cells, BV infection is unlikely to affect target cells. This is a crucial criterion for protocols that are employed in therapies that involve only the correction of a genetic defect, and do not lead to cell death of target cells (STANBRIDGE; DUSSUPT; MAITLAND, 2003).

METHODOLOGY

The development of this work consisted of a literature review with search in scientific sites such as SciELO and PubMed, for articles and books related to baculovirus focusing on its morphology, replication cycle, and biotechnological applications in the health area. The review of articles adopted as an initial criterion for selecting the use of keywords in English: "*Baculovirus*", "*baculovirus life cycle*", "*gene therapy*", "*baculovirus applications*" and in Portuguese "*biotechnological applications*". Articles published in English and Portuguese between 2005 and 2019 were used in the selection. Original studies not restricted to the pre-established selection interval were also used, due to their value as a reference. Ninety scientific articles were filtered, and, after a thorough review, 86 relevant articles were selected for the study.

THEORETICAL REFERENCE

A brief history and morphological properties of Baculoviruses

Baculoviruses are infectious agents that are disseminated in various types of organisms such as insects and shrimps. Although there is a wide variety of

baculovirus targets, the most studied are those that cause diseases in common pests such as insects (MARTIGNONI; IWAI, 1986).

The history of the discovery of baculoviruses dates from the development of the history of Ancient China silk industry, more than 5000 years ago. Since then, silkworm culture has spread throughout Asia, reaching Japan around 300 years AD and Europe around 550 years AD. As in any agricultural industry, many problems have caused a variety of diseases, compromising the local economy. With the emergence of new analysis tools, such as light microscopy, a typical characteristic of one of the types of diseases was identified. The presence of occlusion bodies (OB) was observed, which are highly refractory and symptomatic structures in insects affected by the infectious agent. These structures were in polyhedrical form and it was in the mid-19th century that these findings led to the nomenclature of diseases associated with these structures, then referred to as polyhedrosis (ROHRMANN, 2019).

Although the presence of infectious particles within occlusion bodies (OB) was suggested, only in the late 1940s the presence of a complete particle of a small rod-structured virus was definitively demonstrated by electron microscopy (BERGOLD, 1947). Consequently, years later, two polyhedra diseases were differentiated: those in which the occlusion bodies were in the cytoplasm of the host cell of the infected animal, called cytoplasmic polyhedrosis (CPVs), and those in which polyhedral structures developed in nuclei called nuclear polyhedra (NPVs) (XEROS, 1952).

NPVs are described as small rod-shaped, whose genetic material is DNA, while CPVs have capsids of icosahedral structure and have been inserted into the family Reoviridae (genus *Cypovirus*), in which viral particles are inserted whose genome consists of double strips of segmented RNA (VAN OERS; VLAK, 2007).

In the 1920s, the second category of baculovirus was described, whose characteristics are given by the presence of small and granular helical OBs (PAILLOT, 1926), so-called granulose virus (GV). The morphology observed in the OBs of these viruses was a crucial criterion for the division into two major groups of viruses: Nucleopolihedrovirus (NPV) and Granulovirus (GV) (Figure 1).

However, with advances in molecular biology techniques, other criteria for subdivision of different categories of viruses were used. The terminology for these viruses went through a series of names, including Borrelinavirus, Bergoldiavirus, Smithiavirus, Moratorvirus, and Vagoiavirus until, in the early 1970s, the nomenclature was altered and unified, giving rise to the Baculoviridae family (VAGO *et al.*, 1974). Baculoviruses are represented by a large and diverse group of pathogenic viruses for arthropods, mainly insects of the orders Lepidoptera, Hymenoptera, and Diptera. More than 700 different baculoviruses have been isolated from invertebrates and reported in the literature (HERNIOU; JEHLE, 2007).

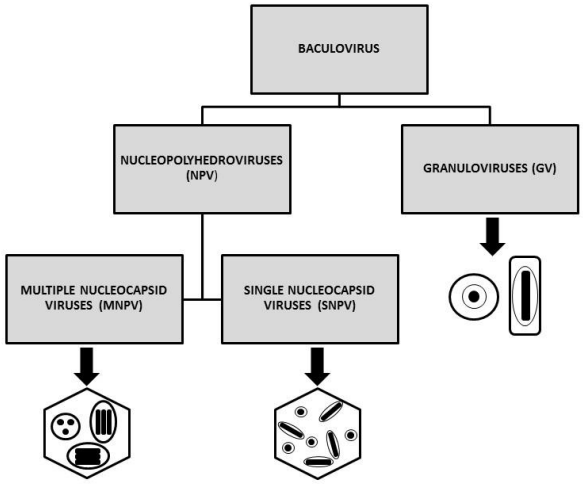

Figure 1. Schematic representation of the classification of baculoviruses in Nucleopolihedronvirus (NPV) with subdivisions in Multi Nucleocapsid Virus (MNPV) and Simple Nucleocapsid Virus (SNPV) and Granulovirus (GV).

Multiple NPV viruses are incorporated into OBs, which vary in their dimensions and can have sizes ranging from 0.15 µm to 15 µm in length, while granules (GV) are smaller, approximately 0.3 × 0.5 µm (width x length), and typically contain only a single virion, or rarely two or more virions (HERNIOU *et al.*, 2012). Baculovirus nucleocapsids have a diameter ranging from 30 to 60 nm and length from 250 to 300 nm (Figure 1).

These structures are inserted inside an envelope to form a virion (complete viral particle) (HERNIOU *et al.*, 2012). Two distinct forms of virions are produced during the infection process: an extracellular virus that sprouts from the plasma membrane of the infected cell to the extracellular environment, which has structures called peplomeres (containing, for example, the GLYCOPROTEIN GP64 and the F-protein), being enveloped individually (*budded virus*-BV or *extracellular virus*-ECV); and OB-derived virions (ODVs). Both have identical genotypes; however, they have distinct phenotypes (Figure 2).

Although BVs and ODVs have the same nucleocapsid, they differ from each other by the different origin of their envelopes (MCWILLIAM, 2007). BV envelopes are derived from plasma membranes modified by nucleocapsid budding, while ODVs envelopes are formed from membranes built within the nuclear region of the infected cell. BVs consist of only a single nucleocapsid surrounded by an envelope, while ODVs inserted within OBs that have single (S) or multiple (M) nucleocapsids are referred to as single NPVs (SNPVs) or multiple NPVs (MNPVs), respectively (IKEDA; HAMJIMA; KOBAYASHI, 2015) (Figure 2).

The names of the different species of baculovirus are given by adding the initials of the names of the insects from which they were initially isolated, such as *Autographa californica* MNPV (AcMNPV), *Mori Bombyx* NPV (BmNPV) and *Gemmatalis anticarsia* MNPV (AgMNPV), which were first isolated from the alfalfa looper (*A. californica*), silkworm (*B. Mori*) and gypsy moth (*L. shoot*), respectively (THEILMANN *et al.*, 2005; JEHLE *et al.*, 2006). The same is the case with representatives of the GVs class, such as *Segetum Agrotis* GV (AgseGV); *Choristoneura occidentalis* GV (ChocGV); *Cryptophlebia leucotreta* GV (CrleGV). However, this method of nomenclature occasionally creates confusion, as baculoviruses can have a great diversity of hosts or even when insects are permissive to infections by multiple species of baculovirus (JEHLE *et al*. 2006).

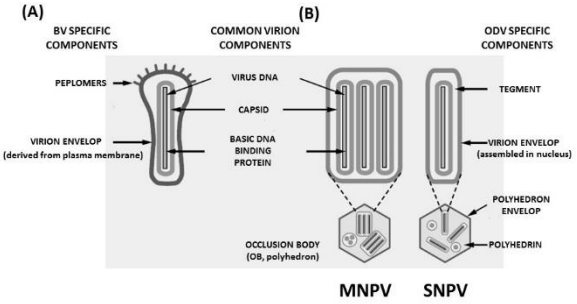

Figure 2. Representation of the two distinct NPV phenotypes produced during lepidopteran infection by NPV. (A) Sprouted virus (BV). (B) Occlusion-derived Virus (ODV). BVs and ODVs have identical genotypes, but different phenotypes due to distinct envelope origins. MNPV, with multiple nucleocapsids in one envelope; SNPV with a single nucleocapsid in an envelope. Adapted from Ikeda; Hamajima; Kobayashi (2015).

The viral replication process occurs in the nucleus of infected cells and has genomic material in the form of double and circular tape DNA, whose size can range from 80 to 180 Kpb in extension, encoding between 100 to 180 different proteins (THEILMANN *et al.*, 2005). About 50 species have had their genomes sequenced (NCBI databases). The baculoviridae family is currently subdivided based on phylogenetic evidence and molecular characteristics into four distinct genera: Alphabaculovirus (leptodper nucleopolihedronvirus), Betabaculovirus (granulovirus of lepidopterans), Gammabaculovirus (nucleopolihedrovirus of hymenethaps), and Deltabaculovirus (nucleopolihedrovirus of dipterans) (JEHLE et al. 2006). The virions of the genus Alphabaculovirus are designated as single (S) or multiple (M) depending on the number of nucleocapsids per ODV (viruses derived from occlusion), while those of the genera delta and gammabaculovirus usually contain a single nucleocapsid by ODV (VOLKMAN; SUMMERS, 1977).

Viral replication cycle

The baculovirus replication cycle within the infected insect cell is characterized by being biphasic and involves the formation of two types of virions, which are produced in distinct phases of the infection cycle. ODVs are adapted to have stability and viability in the environment external to the host insect and the virions that sprout from the plasma membrane (BVs), which are not occluded and are therefore responsible by systemic dissemination from cell to cell within the insect. In addition, the virus replication cycle is divided into three consecutive phases according to the gene expression schedule (immediate-early/early; late and extremely late) (PASSARELLI; GUARINO, 2007).

The process of viral infection by baculovirus begins when insect larvae ingest the occlusion bodies present in the leaves of vegetables (KEDDIE; APONTE; VOLKMAN, 1989). ODVs are soaked in a protein matrix composed mainly of polyedrinprotein, which is expressed in the late phase of the infection cycle. When ODVs come into contact with the alkaline conditions present in the middle intestine of the insect, the occlusion body dissolves by the release of ODVs, and the polyedrin protein matrix is then degraded by proteinases present in the insect's intestine or by those associated with the visions (WANG; GRANADOS, 1997). Then, the ODVs stick to the microvilli of the brush surround the pillar's epithelial cells of the middle intestine of the insect.

ODVs have a set of envelope-specific proteinases called infectivity factors *per os* (*Pif*), which support the anchoring of the virus to receptors located on the surface of the epithelial cell membrane (HORTON; BURAND, 1993; KIKHNO *et al.*, 2002). Subsequently, the entry of the virus occurs through the

fusion of the viral envelope membrane with the microvilli of epithelial cells (non-endocytic way), followed by the release of nucleocapsids in the cytoplasmic region, decapsidation and release of naked viral DNA (OHKAWA; VOLKMAN; WELCH, 2010).

Once the nucleus is reached, the transcription process of immediate-early/early viral genes starts with the aid of host cell polymerase RNA (up to 6 h post-infection). These genes code mainly for transactivating proteins necessary for a subsequent expression of viral genes and to provoke a disturbance of normal host cell activity (PASSARELLI; MILLER, 1993).

The transition process from the early to the late phase of infection is marked by the beginning of viral DNA replication that occurs within 6:00 to 18:00 hours after the infection stage, along with the onset of the activity of a virus-encoded RNA polymerase (GRULA; BULLER; WEAVER, 1981). This last process will begin with an ordered cascade of gene expression necessary for the formation of viral components in the assembly stage of new nucleocapsids. The newly assembled nucleocapsids are then directed from the nucleus to the plasma membrane, where virion budding (BV) will occur (PASSARELLI, 2012).

BVs are part of the secondary infection stage, so once released by budding, they are transported throughout the hemolymph to infect new cells, leading to a systemic infection. After entering the target cells, the infection process is like what happens in primary infection, where nucleocapsides are directed to the nucleus for DNA replication and subsequent expression of viral proteins. The extremely late phase of infection (18 hours after infection) begins with the expression of proteins that constitute the matrix of ODVs (MONTEIRO *et al*., 2012).

The end of the secondary infection cycle occurs with infection of various tissues and cell lysis, culminating in the death and liquefaction of larvae, and finally the dissemination of ODVs to the environment, where they can remain stable and viable by various years until they are ingested by new larvae (VOLKMAN; SUMMERS, 1977). Therefore, ODVs embedded in OBs play a role in horizontal viral transmission, which occurs from insects to insects, while BVs are responsible by viral transmission from cell by cell within the larva of the infected insect. Unlike ODVs, where the entry of baculovirus occurs by membrane fusion, BVs enter cells through endocytosis (KATOU; IKEDA; KOBAYASH, 2006; Long *et al*., 2006).

Baculovirus applications

Use of baculoviruses as crop insecticides

Currently, granulose viruses and nuclear polyhedra viruses present several applications of economic interest as a model of the expression of heterologous proteins but were initially considered for use as biological insecticides in pest control. These viruses are considered highly virulent, selective and stable, and mainly with low environmental impact after their application, as they are highly specific to their host insects, that is, they do not affect other arthropods, including pest and parasitoid predators, vertebrates, plants and biosphere (MOSCARDI, 2011).

There is great rigor in several countries about the regulation of the manufacture, registration and use of pesticides, which results in higher costs and scarcity of these tools in some cases. Many of these insect species, which include some agricultural pests, have become difficult to control due to the development of resistance to chemical insecticides. Large-scale production and frequent use of insecticides have caused their accumulation in ecosystems, resulting in environmental contamination and toxicity for many different species, including humans. The spread of resistance to insecticides also threatens the efficacy of the insecticides currently used (FFRENCH-CONSTANT, 2013; Ranson *et al*., 2010; Rivero *et al*., 2010). Many studies have shown that baculoviruses, both GV and NPV, are promising and economically viable alternatives for chemicals in biological insect control (WHALON; MOTA-SANCHEZ; HOLLINGWORTH, 2008, STERNBERG; THOMAS, 2018).

Biological control can be a practice that has long-lasting effects due to the persistence of pathogens in the environment (FULLER; ELDERD; DWYER, 2012). Natural organisms and entomopathogens intentionally used in control can be established in the pest population and contribute to the protection of crops in the long term. Several pathogens, such as fungi, nematodes, bacteria, and viruses can be used to control pests in a very efficient way when applied artificially as insecticides (SAXENA, 2008; VASANTHARAJ, 2008).

The first report of the use of a baculovirus in the environment, in pest control, occurred in the 1930s accidentally when a parasitoid was imported from Europe to the USA and Canada to be used to control the mosquito of the species *Diprion Hercyniae*. It was observed that there was an effective inhibition of the

growth of this pest. In this case, the baculovirus accidentally introduced was a specific NPV for this insect. Since then, no control measure has been required against the *Diprion Hercyniae* (IBRD; ELGEE, 1957).

Two alternative pest management strategies are generally used: the first consists of spraying the areas infested with concentrated formulations of insecticides in an immediate attempt to control the pest or the areas are sprayed with low concentrations of baculovirus, leading to the permanence of the virus for several generations of insects (FUXA, 2004).

Although the practice of using baculovirus as an insecticide for the protection of agricultural crops has not been as extensive as expected, there are several reports of successful practices of the use of different species of this family in Latin America, such as *Anticarsia Gemmatalis* MNPV, *Autographa californica* MNPV^{+}, *Albula Spodoptera* NPV *Spodoptera Sunia* NPV *Pomonella Cydia* GV, among several others (HAASE; SCIOCCO-CAP; ROMANOWSKI, 2015).

Use of baculoviruses as pest insecticides that cause human health problems

Although in nature there is a great diversity of insect caterpillars, most of them do not pose a threat to human health. However, there are members of 12 lepidopteran families that can cause injuries in humans and are quite severe (DIAZ, 2005). In Brazil, it was found that larvae of the species *Obliqua lonomy* (Walker, 1855) (Lepidoptera: Saturniidae) cause several accidents (CARRIJO-CARVALHO; CHUDZINSKI-TAVASSI, 2007). This species can cause the death of people caused by LOPAP toxin (prothrombin activating protease of *Obliqua lonomy*), released by the cervids of the caterpillar on the skin of the victims at the time they come into contact, resulting in reactions ranging from local irritation to serious life-threatening conditions such as coagulopathy, acute renal failure and bleeding disorders (GAMBORGI; METCALF, METCALF, BARROS, 2006).

Due to a high rate of accidents with this caterpillar and a need to control the population of this insect, many studies have been focused on the search and identification of various pathogens and predators of this caterpillar, including the nematode *Hexamermis sp.* (MORAES, 2002) and a baculovirus: multiple nucleopolyhedrovirus of *Obliqua lonomy* (LoobMNPV), which was isolated from larvae of *L. Obliqua*. It was observed that the larvae infected with baculovirus presented all the common symptoms of an infection by this virus, although they did not present the characteristics of liquefaction and melanization (WOLFF *et al.*, 2002).

Baculoviruses are also commonly used as express vectors of a wide variety of recombinant proteins. Through genetic engineering of the species AcNPV, brought to improvement of baculoviruses already in use as biopesticides (SZEWCZYK *et al.*, 2006). This modification promoted a reduction in the time required for the virus to cause the death of the host insect. Therefore, the insecticide activity of wild type baculovirus can be improved by inserting heterologist genes. Many studies have already been published showing the expression in baculoviruses of genes encoding toxins specific to insects (e.g., toxins from scorpions, mites, spiders, sea anemones, and *Bacillus thurengiensis*) (LAPIED *et al.*, 2008; INCEOGLU *et al.*, 2006)

Use of baculovirus in therapeutic treatment

Gene therapy is a method that aims to transfer genetic material to a patient to treat a particular disease. Although this concept was defined many years ago, clinical research only began in the 1990s, when a study of an immunodeficiency disorder was conducted at the Health National Institute of the United States. Since then, about 2,500 clinical studies have been expanded and covering a wide variety of infectious pathologies, neurodegenerative diseases, and cancer (ANGUELA; HIGH, 2019).

Gene therapy aims at the expression of genes transferred at high levels in the long term, and enough to be considered therapeutic. The transferred gene usually consists of a normal copy of an altered gene. Another method of gene therapy is the suppression of the expression of a harmful gene, using methods of RNA interference (RNAi) or editing of the genome itself. This last tool to edit the genome and correct the altered gene is theoretically possible, however, there are no ongoing clinical trials (KOMOR *et al.*, 2016).

Although gene therapy is a very promising option, there is a need to use suitable vectors with low or no immunogenicity, which are more tissue-specific and can be produced more efficiently and relatively at low cost (RITTER; KUPIEC-WEGLINSKI, 2005).

Baculoviruses (BV) are models that meet all these requirements because they are highly specialized for host insect cells and have low immunogenicity in mammals, with no pre-existing immunological memory after the first administration.

BV has no ability to replicate in mammalian cells and, although some BV genes are transcriptionally active, they are expressed at low levels (HITCHMAN, 2011).

Baculoviruses have the ability to transduce a wide variety of mammalian, human, or non-human cell lineages (HU, 2008), with different purposes, such as expression of therapeutic genes to be used in cancer treatment, such as in glioma maligns cells, PC3 prostate cancer cells (WANG *et al.*, 2006; STANBRIDGE; DUSSUPT; MAITLAND, 2003), in the synthesis of protective proteins to improve the effects of ischemia and reperfusion (IRI) injury on solid organs during transplant procedures (HITCHMAN *et al.*, 2011), transfer of genes to dorsal root ganglia for the regeneration of peripheral nerve cells (WANG *et al.*, 2005) and as a vaccine vehicle *delivery* (reviewed by MADHAN; PRABAKARAN; KWANG, 2010, HU 2008; Aoki *et al.*, 1999**)**.

Thus, the big question is whether the use of baculovirus an alternative method can be providing therapeutic genes to be used in various pathologies and mainly remaining confined to target organs. Mammalian cell viruses, specifically those used in gene therapies, rely on the host cell's cell machinery to complete its life cycle. One consequence is the possibility of viral proteins interacting with proteins and genetic material from host cells, often leading to an interruption of the cycle and the viability of the host cell. Because BV genes, which have these functions in insect cells, are not expressed in mammalian cells, BV infection is unlikely to affect target cells. This is a crucial criterion for the protocols employed in therapies that involve the correction of a genetic defect, rather than causing the cell death of target cells, such as those employed in cancer treatment therapies (STANBRIDGE; DUSSUPT; MAITLAND, 2003).

Treatment of tumours

One of the most common tumors in humans is glioma that tends to invade the brain in an extremely aggressive way (HOLLAND, 2000). Gliomas originate predominantly from astrocytes and are classified from 1 to 4, according to the degree of aggressiveness. Glioblastoma multiforme (level 4) represents almost half of all gliomas and is the most frequent type of primary brain tumor in adults and considered almost incurable. Although there are interventional surgeries (chemo- and radiotherapy), patients who have this type of tumor usually die within a very short period of time gene therapy can be a promising form for cancer treatment (KOST; CONDREAY; JARVIS, 2005).

WANG *et al.* (2006) developed a recombinant baculovirus vector that has the regulatory sequence of transcription of acid fibrillar glial protein (AFGP). This procedure was done in order to direct the expression of a diphtheria toxin gene in rat glioma cells in the sense of further minimizing the possible damage caused to neurons, even though baculoviruses appear to be more likely to infect glial cells than neurons in the brain (LI *et al.*, 2004).

This recombinant baculovirus significantly improved transduction in glioma cells, achieving up to 96% efficiency in rats. When used to produce the A-chain of the diphtheria toxin intracellularly in a rat glioma xenograft model, baculovirus was able to efficiently suppress tumor development. This baculovirus vector circumvents some of the inherent problems associated with mammalian viral vectors and provides an additional option for cancer gene therapy (WANG *et al.*, 2006). Studies using baculovirus as a vector of cancer therapy can be widely explored for other types of cancer, such as prostate cancer.

Thus, the virus appears to be a suitable vehicle for applications requiring short-term gene expression. Other advantages of baculovirus vectors include the ease of obtaining a recombinant viral vector and purification of large amounts of the virus with high tides, processes that could be extrapolated to pharmaceutical levels. However, although a good understanding of all these attractive characteristics of baculovirus is well understood, the use of gene therapy with this virus is still in its early stages, no application practically useful in cancer therapy has yet been produced in humans (KWANG; ZENG; WANG, 2016).

Regeneration of nerve ganglia

It is already well known that neurons in the spinal ganglion suffer degeneration in various types of neuropathies of the peripheral nervous system, such as, for example, after the transection of a peripheral nerve, where up to 40% of DRG neurons (dorsal root ganglia) die progressively while neurons that remain alive show a wide variety of pathological changes in the peril, from chromatolysis to positive regulation of cytokines, neuropeptides and transcription factors (GROVES *et al.*, 2003).

One way to prevent these damages in these therapies is to make use of the nerve growth factor (NGF), a neurotrophic polypetid (THORNE; FREY, 2001). However, these polypeptide factors used in

therapies are susceptible to proteolysis and, therefore, in this type of therapy would be necessary constant administrations, and in the long term. The delivery of genes of therapeutic interest to the DRG would be another way to prevent more neuronal deaths and nerve degeneration in peripheral neuropathies (GLORIOUS; MATA; FINK, 2003a). In previous studies using mice, it was observed that intramuscular or subcutaneous administration of viral vectors led to the capture of viruses and delivery of these genes. In particular, in these studies, herpes simplex virus (HSV) and poliovirus (poliovirus (poliomyelitis) were used, which are captured by nerve endings and then transported through axoplasma to the cell body of neurons in the DRG (JACKSON; MESSINGER; PALMER, 2003; GLORIOUS, GLORIOUS. MATA; FINK, 2003b).

WANG *et al*. (2005) analyzed the effects by lumbar intrathecal injection in rats of viral vectors such as adenovirus and baculovirus and observed that this method was also shown to be quite effective for administration for transfer *in vivo* genes for DRG. This is a simple and relatively noninvasive approach that may be applicable to repeated delivery of genes to the peripheral nervous system. This method can stimulate the growth of nerves that have been injured, and also has great potential for other clinical applications in the peripheral nervous system, such as treatment of diabetic neuropathy, protection against the degeneration of neurons in the DRG caused by genetic mutations, and relief of pain caused by neuropathies, neural injury, inflammation or tumor invasion. Therefore, these authors observed that baculovirus could also migrate by axonal transport to neuronal cell bodies after being internalized in nerve terminals. These findings corroborate the hypothesis that baculoviruses are capable of infecting adult neurons in DRG.

Treatment against ischemia and reperfusion (IR) injury

Ischemia is characterized by a lack of blood supply to an organ, which results in tissue damage or dysfunction due to a lack of oxygen, glucose, and other nutrients transported through the bloodstream. This causes the tissue to become hypoxic or, without any oxygen anoxia situation, and can result in the accumulation of metabolic residues. However, restoration of blood flow after a period of ischemia maybe even more harmful than initial ischemia and is the main cause of the ischemia (IRI) (DEVARAJAN, 2006). IRI can significantly reduce the chances of recovery in all types of solid organ transplantation and is a common clinical problem with increasing incidence and serious consequences (DEVARAJAN, 2006).

However, cells have a large number of genes with a protective activity whose expression is increased in situations where the cell is threatened by harmful stimuli and protects them by inhibiting apoptosis and inflammatory responses, such as superoxide dismutase (SOD) and genes that are members of the B2 cell family of lymphoma (BcL2). For example, the antioxidant enzyme SOD degrades superoxide radicals that are toxic and therefore protects cells against high levels of reactive oxygen species (VALDIVIA *et al.*, 2009) and bcl-2 proteins that play a central role in blocking apoptosis (LOPEZ-FOG; TOLEDO, TOLEDO, TOLEDO-PEREYRA, 2005). The AcMNPV virus can transduce a wide diversity of mammalian cells. This suggests that the virus may also be useful for the delivery of protective genes to improve the effects of II on solid organs during transplant procedures (HITCHMAN, 2011).

Use with vaccine

Vaccination is an efficient way to prevent the occurrence of many infectious diseases in humans. Due to the advance in infectious diseases and the improvement and development of more effective vaccines, it has made possible better control of the spread of these diseases. However, there is a possible imminent danger or reemerging pathogens, which inserts a serious problem and threatens public health. Among the types of vaccines available on the market, inactivated vaccines are generally considered safer and with considerable stability. Due to their inactive nature, these vaccines are administered in people with weakened immune systems without having a more serious complication due to an opportunistic infection. However, the immune responses induced by this type of vaccine are mainly humorous, and repeated immunizations are required to optimize immunity to the target disease (BAXTER, 2007).

Another strategy is the use of live attenuated vaccines, which are more effective in inducing the immune response. The main risks of these vaccines are a possible reversal and recombination with circulating strains of pathogens, as well as an incompatibility of the vaccine with immunocompromised individuals, elderly, chronically ill, and pregnant women (AMANNA; SLIFKA, 2009).s recombinant subunit vaccines, on the other hand, can usually be used

regardless of health status, but have other disadvantages, such as the need for the use of adjuvants to improve immunogenicity beyond the difficulty of purification due to the hydrophobic nature of antigens. This is the last factor that reduces the cost-benefit of vaccine production (KUMBHANI *et al.*, 2007).

Viral vaccine vectors emerged from the 1980s and have a more favorable safety profile in relation to vaccines derived from live attenuated infectious agents and have a better immunogenicity capacity compared to inactivated vaccines. Viral vaccine vectors can present the desired antigens in their native conformation, leading to a stronger immunogenic response with consequent maintenance of higher levels of gene expression compared to DNA vaccines (DRAPER *et al.*, 2008).

Although the types of vaccines mentioned above have a number of advantages, there are some considerations for the use of these vaccines in humans such as a pre-existing immunity of the vector that can have a serious impact on the efficacy of the vaccine and also transgenes with very large sizes can cause genetic instability thus compromising yield. To circumvent the immunity problems of the preexisting vector, vectors were developed from recombinant viruses of non-human origin. Thus, it is possible to avoid neutralization of the viral vector by preexisting antibodies. In recent years, an increasing number of studies of baculovirus has been observed as a vehicle for the production and delivery of vaccines. These studies indicate that the use of baculoviruses appears to be very promising (PREMANAND; Wee; PRABAKARAN, 2018).

Among the various baculoviruses, AcMNPV is the most widely studied. Aoki *et al.* (1999) demonstrated that a recombinant baculovirus that expresses the gB glycoprotein of the pseudo-rabies virus induces antibodies against this protein in mice. Abe *et al.* (2003) later, proved that immunization of mice with a baculovirus that expresses influenza virus hemagglutinin (HA) via intranasal administration provoked innate immune responses and provided rats with a high level of protection against an influenza virus. Baculovirus-mediated immune responses can also be triggered by the antigen displayed on the surface of the viral envelope. Using the surface display techniques of the circumsporozoite protein of *Plasmodium Berghei* (PbCSP), a malaria-causing parasite in certain rodents and a sebsehot immunization of rodents by the modified baculovirus induced high levels of antibodies and IFN-γ secretory cells against PbCSP and protected about 60% of mice (YOSHIDA *et al.*, 2003). In a recent work developed by IYORI *et al.* (2017), a baculovirus optimized to produce malaria-causing sporogoid vaccine was developed in mice with very promising results. Therefore, there has been an increasingly significant increase in vaccine development using baculovirus as a vehicle for antigen expression, which leads to the belief that it can be considered as an excellent platform of rehenouement expression directed to vaccination (PREMANAND; WEE; PRABAKARAN, 2018).

Limitations of the use of Baculovirus as a system of rehelogous expression

A major limitation of the use of BEVS for heterologous expression is that infection of insect cells by baculovirus results in death and cell lysis, which limits the expression of baculoviral proteins in a window of time between the onset of late viral gene expression and the time of cell death, which greatly compromises the yield of protein production of biotechnological interest (BLISSARD; ROHRMANN, 1990).

Thus, protein expression is typically restricted to three days after infection of insect cells. In addition, the protein secretion pathway of insect cells is compromised during the phases after infection with baculovirus, which limits the degree of secretion of recombinant proteins. Secretory pathway compromise is caused, at least to certain levels, by the accumulation of large amounts of chitinase and catepsin enzymes (a protease) that are encoded by the virus in the secretory pathway (THOMAS et al., 1998). After lysis, viral cathepsin is released in the culture overn and can degrade recombinant proteins after being activated by treatment with caotropic reagents such as SDS or low pH. To minimize the negative impacts of chitinase and baculovirus cathepsin on the yield and integrity of secretory pathways proteins, some vectors of baculovirus without chitinase and cathepsin have been developed and optimized in recent years (KABA *et al.*, 2004).

Lately, several recombinant vaccines have been produced in BEVS (ZHANG; Murhammer; LINHARDT, 2002), but this platform is not used to produce therapeutic glycoprotein, as most of them require complex human-typical glycoylate patterns that insect cell lines used in BEVS are unable to provide (STEELE *et al.*, 2017). Another problem is that some insect cell systems produce N-glycans with immunogenic

epitopes. In the last 20 years, these problems have been addressed to obtain glycoenengineering platforms capable of producing non-immunogenic N-glycans (GEILER; MABASHI-ASAZUMA; JARVIS, 2015).

Baculoviruses also have disadvantages as vectors of gene therapy since they have a transient expression in mammalian cells. *In vivo*, the transgene expression is completely null after 14 days of transfection. The duration of the transgene expression *in vitro* using baculovirus is significantly lower than the expression mediated by retroviral, lentiviral, and AAV vectors (AIRENNE *et al*., 2000; LEHTOLAINEN *et al*., 2002).

Baculoviral vectors differ mainly from other viral vectors at a time when the transported genes may persist in the host nucleus. In the case of retroviral, lentiviral, and adenoviral vectors, viral DNA can remain in the nucleus, both integratedly and episomal, for a longer period. In fact, previous studies have shown that baculoviral DNA persists in the nuclei of mammalian cells transduced for a maximum of 48 h, therefore, is considered a very short period for an optimized expression (TJIA *et al*., 1983).

Another disadvantage of the use of baculovirus as a vector of gene therapy is inactivation by the complement system. Contact between baculovirus and the seeric complement system results in rapid inactivation of budding virions. There are several modifications necessary to reduce the negative effect of the complement on baculovirus-mediated transduction. However, the add-on system is not a problem just for baculovirus. It is also a powerful barrier to *in vivo* other gene delivery systems, such as liposomes, murine retroviruses, and various synthetic DNA complexes (HU, 2008).

FINAL CONSIDERATIONS

The baculovirus expression system (BV) in cells has proven to be an extremely valuable tool for the production of recombinant proteins. Continuous improvements in vector design and simplification of recombinant virus isolation techniques, combined with the relative ease of small and large cell cultures, resulted in the widespread use of this system. Many laboratories are beginning to automate the production of a large number of viruses and protein production schemes using advanced methods of cloning, robotic liquid handling, and protein purification instruments.

Although there are several limitations and technical problems with the use of baculoviruses, biotechnology has advanced a lot in all areas of study and with different approaches, and the use of baculovirus in applications beyond the production of proteins in insect and larva cells is increasingly observed. These approaches include the development of strategies for the display of peptides and heterologas proteins on the surface of the baculovirus as well as the insertion of expression cassettes in baculoviruses for the production and release of different proteins in a highly efficient manner in a wide variety of mammalian cells. This relatively new approach offers several advantages, including the virus's inability to replicate in mammalian cells; absence of cytotoxicity, technical simplicity, and safety compared to viral vectors derived from mammalian cells. Although the use of baculovirus has not yet reached the clinical stage, preclinical trials in animals and *ex live* using human cells demonstrated the considerable viability of gene transfer mediated by these viruses in various applications of gene therapies, among them: vaccination, tissue regeneration, and cancer therapy.

In conclusion, with the obvious advantages of baculoviruses for various types of therapies, integrated efforts have been increasing to establish quality control methods and standards for products derived from these viruses and the performance of gene therapy in clinics in the coming years, and then a plausible expectation.

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