Scientific Institute
of Public Health

Division of Biosafety and Biotechnology

Vaccine production against Hepatitis B virus using a baculovirus vector

1. Aim of the project

Human hepatitis-B infection, caused by the hepatitis B virus (HBV), is a worldwide health problem. The disease is highly variable and can range from mild infections to chronic liver infections (chronic hepatitis, cirrhosis of liver and hepatocellular carcinoma (primary liver cancer)). Since no effective therapy exists against this disease, the prevention through vaccination remains the only method for its control. One possible vaccination strategy that can be used against viral infection is to produce artificial virus-like particles (VLPs) by overexpressing viral coat proteins, which will mimic the immunogenic epitopes located on intact virions. These VLPs, corresponding to empty virions, are therefore non-pathogenic and generally highly immunogenic, two characteristics required for the development of an effective vaccine.
In this example, in order to manufacture a vaccine against HBV, the gene encoding the small envelope protein HBsAg-S of this virus, which can self-assemble into well-organized and antigenic VLPs, is cloned into a baculovirus vector and is expressed in Sf-9 insect cells. Besides the construction of genetically modified cells, this activity involves the culturing of genetically modified organisms at a large scale, followed by extraction and purification processes of the antigenic preparation.

2. Vectors

The production of the final recombinant baculovirus expressing the gene of interest is a two-step process. First, the gene of interest is cloned into a baculovirus transfer vector. The second step consists in transfecting insect cells with the given baculovirus transfer vector and the *Autographa californica* nuclear polyhedrosis virus (AcNPV). Two sequences, which are homologous to AcNPV, are flanking the cloning region of the transfer vector in order to allow homologous recombination with the AcNPV DNA, transferring the target gene from the vector to the AcNPV and generating the recombinant baculovirus. This baculovirus expression vector system is generally used for high-level expression of cloned foreign genes.

1. The baculovirus transfer vector used in this example is the pVL1392 vector, which is commercially available. pVL1392 is a pUC8 vector containing a multiple cloning site (MCS) that allows the cloning of the gene of interest under the strong polyhedrin (polh) promoter of AcNPV.

2. The final recombinant vector corresponds to the *Autographa californica* nuclear polyhedrosis virus (AcNPV) expressing the gene of interest, in this case the gene coding for the HBsAg-S protein. AcNPV belongs to the *Baculoviridae* family and specifically infects lepidopterian insects. AcNPV belongs to the class of risk 2 for animals, but is not known to propagate in vertebrate hosts, and is therefore not considered as a threat for human health. Furthermore, AcNPV is the most thoroughly studied baculovirus and its DNA has been fully sequenced. It is important to note that the *polh* gene of the AcNPV is deleted from the recombinant vector. Polh protein is responsible for the formation of occlusions bodies protecting newly synthesized viral particles, facilitating therefore *in vivo* horizontal transfer. *polh*-negative recombinant viruses are still able to infect target cells, but due to their high sensitivity to degradation (in the environment and in the insect gut after ingestion), they have a poor survival time and a weakened pathogenicity.

3. Insert

The insert is the coding sequence for the HBsAg-S protein of HBV, corresponding to the small envelope protein of the virus. This insert is unlikely to alter neither the pathogenicity of the transfer vector, nor the pathogenicity of the recombinant baculovirus vector.
The *polh* promoter allows the expression of the protein of interest at a high level both in bacteria and in eukaryotic cells and it is known that HBsAg-S proteins can spontaneously assemble in empty virions, called virus-like

particles (VLPs). Nevertheless, VLPs are no effective viruses and can therefore be considered as non-harmful for the human health, animals, plants or the environment.

Note: HBV belongs to the class of risk 3 for human. However, in this example, only HBV DNA is handled (for PCR amplification before *E. coli* cloning) and wild type viruses are never manipulated.

4. Localization of inserted genetic material

1. Stable integration into the transfer vector.
2. Stable integration into the recombinant baculovirus genome.
3. Episomal in the insect cells.

5. Receptor organisms

1. The *E. coli I* K-12 strain is used to clone the gene of interest into the transfer vector pVL1392. *E. coli* K-12 can be considered as a non-pathogenic strain belonging to the class of risk 1. For more information about this strain, see the risk assessment sheet "Cloning of promoters found in human tumors into *Escherichia coli*" at http://www.biosafety.be/CU/RA_Fiches/Intro_and_menu.html

2. In this example, the commercially available insect cell line Sf-9 is transfected with the transfer vector and the AcNPV described previously. Those cells were derived from pupal ovarian tissue of the fall armyworm *Spodoptera frugiperda*. Sf-9 is highly sensitive to baculovirus infection and is therefore commonly used to propagate recombinant baculoviral vectors and to produce recombinant proteins. This cell line belongs to the class of risk 1.

6. Biological hazards and/or considerations related to the manipulation of the resulting GMMs

Three types of genetically modified micro-organisms are discussed in this example:

1. GM *E. coli:* Transformation of the *E. coli* strain with the given transfer vector does not confer the host organism with any potential harmful effect to humans, animals, plant or environment. The resulting GMM remains non pathogenic.

2. GM baculovirus: The baculovirus vectors do not represent any threat for human health or for the environment since:
   - the recombinant vectors have a very narrow viability in the environment and in the insect gut (no propagation of the virus by the insect) due to the removal of the *polh* gene. Accidental release of GM baculoviruses presents a negligible hazard.
   - baculoviruses only propagate in insect cells. It has been demonstrated that even if virions are able to enter certain cell lines derived from vertebrate species, they are naturally unable to replicate in those cells.
   - no cytotoxic effects mediated by this virus in mammalian cells have been reported so far.

3. GM insect cells: In this example, insect cell lines (Sf-9), belonging to the class of risk 1, are deliberately infected with a recombinant baculovirus vector expressing the HBsAg-S protein of the human hepatitis B virus. Only HBsAg-S proteins are expressed and no infectious virus can therefore be produced. The GM insect cells do not represent a threat for human health, animals, plants or the environment.
   However, the risk related to the use of these insect cells is that they can become inadvertently contaminated with pathogens before or during manipulations. Even if the cells manipulated in this example are insect cells and the risk of causing harm to human health is considered to be negligible, **good microbiological practices** are necessary to avoid all potential accidental contamination.

7. Class of risk of the resulting GMMs

All three GMMs described can be considered as **class of risk 1**.

8. Class of risk of the activity

The present activity (production of a vaccine against the human hepatitis B virus) corresponds to a large-scale industrial application. This implies a considerable increase of the volume of cultivation and the quantity of biomass produced compared to standard laboratory-scale activities. However, taking into account the nature of the modified organisms, the nature of the products and the nature of the industrial process, the activity still remains of **class of risk 1**.

9. Recommended containment measures

Since this is a production process, involving high volumes of biomass compared to standard laboratory-scale activities, a “biosafety level 1 – Large-Scale” facility (BSL1-LS) is recommended, where practices, equipments and control measures are adapted to large-scale productions.

Note: HBV is a virus belonging to class of risk 3 for humans. In case of wild type HBV manipulation during the production activity, working in a biosafety level 3 – large scale facility (BSL3-LS) implemented with a biosafety cabinet type II is therefore required.

10. References and further readings

Lanford RE, Luckow V, Kennedy RC, Dreesman GR, Notvall L, Summers MD. (1989). Expression and characterization of hepatitis B virus surface antigen polypeptides in insect cells with a baculovirus expression system. J Virol. 63 (4): 1549-57.

Price PM, Reichelderfer CF, Johansson BE, Kilbourne ED, Acs G. (1989). Complementation of recombinant baculoviruses by coinfection with wild-type virus facilitates production in insect larvae of antigenic proteins of hepatitis B virus and influenza virus. Proc Natl Acad Sci U S A. 86 (5): 1453-6.

Woo WP, Doan T, Herd KA, Netter HJ, Tindle RW. (2006). Hepatitis B surface antigen vector delivers protective cytotoxic T-lymphocyte responses to disease-relevant foreign epitopes. J Virol. 80 (8):3975-84.