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Use of pseudotype replicons particle for the detection of neutralizing antibodies to highly pathogenic viruses (rabies viruses, avian influenza viruses)

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Key words
Highly pathogenic avian influenza virus, bat influenza A-like virus, rabies virus, neutralisation test, serology, pseudotype virus, zoonosis, biosafety, replicon particle, luciferase, green fluorescent protein

Aim of the study
The goal of this project was the development of a safe, sensitive and reliable serological test for the detection of neutralizing antibodies directed against rabies virus (RABV), avian influenza viruses (H5, H7, H9), and possible other enveloped, highly pathogenic or non-cultivable viruses.

Material and methods
The pseudotype virus neutralization (PVN) test is based on a recombinant vesicular stomatitis virus (VSV) which is devoid of the essential glycoprotein gene (VSVΔG). This virus was genetically engineered to express one or two of the reporter genes green fluorescent protein (GFP), firefly luciferase or secreted Nano luciferase. Pseudotyping of recombinant VSVΔG was achieved in two different ways, either by providing the foreign viral envelope protein "*in trans"* or by expression of the foreign viral glycoprotein from the VSVΔG genome ("*in cis"*).

Results and significance
The expression of the hemagglutinin (HA) and neuraminidase (NA) antigens of highly pathogenic avian influenza viruses (H5N1 or H7N1) from a modified VSVΔG genome resulted in propagation-competent pseudotype viruses that were classified into biosafety level 2. Fast and sensitive detection of infected cells was achieved by vector-mediated reporter gene expression. Pseudotype viruses expressing a mutant VSV matrix protein showed restricted spread in IFN-competent cells, thus contributing to biosafety. The pseudotype viruses containing HA from different H5 clades were specifically neutralized by immune sera directed against the corresponding clade (1). Moreover, the pseudotype viruses expressing secreted Nano luciferase were used to quantify the inhibitory activity of NA-specific antibodies (2). Interestingly, pseudotyping of VSV with the envelope glycoproteins of the highly pathogenic avian influenza virus A/chicken/Rostock/8/34 (H7N1) was possible only if the M2 ion channel protein was expressed along with HA and NA. The resulting pseudotype virus was found to be highly sensitive to amantadine, a classical inhibitor of the ion channel protein. Viral pseudotypes with the envelope glycoproteins of a low-pathogenic H9N2 virus have been successfully generated and are available for use in the PVN test.
Expression of the hemagglutinin-like protein H17 and H18 from the VSVΔG genome resulted in propagation-competent chimeric viruses which did not require co-expression of the neuraminidase-like proteins N10 or N11. With the help of these viruses permissive cell lines were identified that allowed for the first time generation and characterization of recombinant bat influenza A-like viruses H17N10 und H18N11 (3). For pseudotyping of VSVΔG with RABV glycoprotein, transgenic helper cell lines expressing the foreign viral glycoprotein in a regulated manner were generated. Taking advantage of these helper cell lines, propagation-incompetent replicon pseudotype particles were produced which allowed safe and sensitive detection of neutralizing antibodies. In addition, the system was expanded to other lyssaviruses such as Mokola virus and European lyssaviruses type 1 and 2. (4). Finally, propagation-competent VSVΔG expressing the glycoproteins of either Ebola virus, Sudan virus or Marburg virus have been generated which are currently used for the detection of neutralizing antibodies directed against these fatal hemorrhagic fever viruses under biosafety level 2 conditions.

In conclusion, this novel pseudotype virus system facilitates the detection of neutralizing antibodies directed against highly virulent or non-cultivable viral pathogens, circumventing the need for high-level biosafety containment. The use of reporter genes such as GFP, firefly luciferase and secreted Nano luciferase not only increases speed and sensitivity of detection but also offers the opportunity of automated and reliable read-outs.

Publications, posters and presentations

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