



King, Barnabas and Daly, Janet M. (2014) Pseudotypes: your flexible friends. *Future Microbiology*, 9 (2). pp. 135-137. ISSN 1746-0913

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Pseudotypes: your flexible friends

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Pseudotype viruses: applications and troubleshooting, Cineworld, The O2, London, UK, 2 October 2013

Pseudotype viruses: applications and troubleshooting' (EuroSciCon 2013), a 1-day conference held at Cineworld: The O2 (London, UK) on 2 October 2013, focused on the technique of pseudotyping enveloped viruses (for a review of the technique, see [1]). The talks and posters covered the challenges and successes of pseudotyping viruses from a broad range of families (Retroviridae, Flaviviridae, Orthomyxoviridae, Bunyaviridae and Rhabdoviridae) for a variety of applications. The conference was chaired by Nigel Temperton, University of Kent (UK), who placed a strong emphasis on using this event to explore the technical challenges of pseudotyping viruses, especially during the poster and afternoon question and answer sessions.

Serological diagnosis of virus infection

The first talk, by Janet Daly, University of Nottingham (UK), provided an overview of current methods for serological diagnosis of viral infections. The plaque reduction neutralization test (PRNT) is typically used for viruses that cause transient or low-level viremia, which hampers diagnosis by molecular techniques. PRNT detects the presence of 'virus-neutralizing' antibodies (vNABs or NABs) in patient sera which, when mixed with infectious virus, bind the virus and prevent entry into cells. PRNT is considered the 'gold standard' diagnostic for a number of viruses, despite having significant limitations: it is low throughput, time consuming (virus-induced cytopathic effect can take 3–5 days to develop) and requires the use of infectious virus, which often requires a high level of containment. Pseudotype viruses (PVs) can be used as a surrogate for the wild-type virus used in PRNT as they are not infectious but express the targets of neutralizing antibodies. They are ideally suited to high-throughput assessment of sero-prevalence and -conversion using smaller sample volumes than conventional techniques.

The varied application of PVs for controlling influenza

Two talks from Simon Scott and Nigel Temperton, Viral Pseudotype Unit [2], University of Kent, described the versatility of influenza pseudotypes. In addition to the basic components, production of influenza PVs also requires the exogenous supply of a trypsin-like protease and recombinant neuraminidase for maturation of the hemagglutinin and release of the PVs from the cell surface, respectively. Simon Scott's group have developed a PV neutralization assay (PVNA) for equine influenza virus to improve the assessment of vaccine efficacy. In contrast to the traditional single radial

KEYWORDS

- enveloped viruses
- neutralizing viruses
- pseudotype viruses
- pseudotyping viruses

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hemolysis assay, the PVNA is high throughput and quantification is digital, not manual. Nigel Temperton's group have focused on the application of PV assays for pandemic preparedness by producing a comprehensive portfolio of PVs for different influenza virus hemagglutinin subtypes. By using commercial gene synthesis services, the group is able to analyze the potential threat of newly emerging viruses by generating PVs without needing to obtain live virus. These PVs can be used to determine whether current vaccines will provide protection against new strains and, by altering the target cell used in the PVNA, their zoonotic potential. The Viral Pseudotype Unit is also developing methods for freeze-drying PVs to facilitate distribution of PVs to other laboratories and multiplexing to improve sero-surveillance.

PVNA in the absence of an effective diagnostic

Molecular methods have surpassed serological techniques for clinical diagnosis of many diseases, particularly acute infections. However, Edward Wright, University of Westminster (London, UK), described the case of Serengeti National Park (Tanzania), where the highly unreliable power supply prevents detection of rabies virus infection by standard molecular methods. To overcome this challenge, Wright and colleagues have produced PVs of rabies challenge virus standard II on a lentivirus backbone [3]. Using a β -galactosidase reporter gene, for which low-cost colorimetric substrates are available, they were able to generate a PVNA highly suited for use in laboratories with limited resources and with comparable sensitivity with PVs using luciferase or GFP reporter genes. Later in the day, Brian Willett, University of Glasgow Centre for Virus Research (Scotland), highlighted how readily transferable the rabies PV system was; his group used plasmids provided by Wright to detect antibodies in lions.

David Griffiths, Moredun Research Institute, introduced Jaagsiekte sheep retrovirus (JSRV), the causative agent of ovine pulmonary adenocarcinoma. Current diagnosis of JSRV infection relies upon clinical presentation. The apparent lack of an acquired immune response to the virus has hampered development of serological assays and vaccines. However, using PVs with target cells that stably express the JSRV host cell receptor ovine hyaluronidase-2, Griffiths and colleagues have provided the first evidence that

sheep naturally infected with JSRV are capable of producing antibodies to the virus. Their work is now focused on developing the PV system as a diagnostic.

Pseudotyping as a research tool

Katherine Sutherland, Colindale Public Health England (London, UK), described a fascinating use of HIV particles pseudotyped with the VSV-g to analyze resistance to antiviral compounds. Standard screening processes for protease inhibitors focus on the development of mutations in the protease itself. However, by cloning the *gag/pol* gene sequences from patients into the HIV PV backbone, Sutherland and colleagues are uniquely able to analyze the effect of cleavage site mutations within the Gag/Pol polyprotein. Interestingly, they found that cleavage site escape mutants are largely responsible for the development of resistance to protease inhibitors [4].

Brian Willett and his colleagues are using PVs to study a range of viruses for a variety of applications. We were informed that pseudotyping can be relatively straightforward when the virus of interest is closely related to the backbone virus; for example, feline immunodeficiency virus (FIV) envelope (Env) on a HIV backbone, whereas pseudotyping Envs from unrelated viruses can be more problematic. FIV alters its tropism throughout infection, going from an 'early infection' form, characterized by a dependence on the CRD2 of the FIV entry receptor CD134, to a CRD2-independent 'late infection' form [5]. This shift in tropism can be monitored using the PV system and target cells differentially expressing the wild-type or mutant CD134.

Pseudotyping Rift Valley fever virus (RVFV) was initially unsuccessful until high levels of expression of the RVFV Env proteins Gn and Gc were achieved; even then PV titers were relatively low. It is thought this is because RVFV buds into the Golgi while the HIV particles, which form the backbone of the PVs, assemble at the plasma membrane. Therefore, it was only when levels of RVFV glycoprotein saturated the normal route of maturation that sufficient amounts reached sites of assembly, enabling PV formation.

Canine distemper virus (CDV) brought a different challenge as high levels of cytopathic effect in PV producer cells caused by the CDV glycoproteins prevented accumulation of high titers of infectious PVs. But, by drawing from

previous studies on measles virus [6], a combination of truncations of the CDV glycoproteins H and F was found that produced usable titers of infectious PVs.

HCV is a highly variable virus, with much of the diversity within the Env glycoprotein genes, E1 and E2. Alex Tarr, University of Nottingham, and colleagues have isolated, cloned and sequenced the Env genes from over 600 patients and analyzed their infectivity using a luciferase-based PV system [7]. Approximately 25% of the isolates produced infectious PVs and a selection of the most infectious PVs was used to screen patient samples to identify broadly neutralizing sera. The patient sera tested fell into three distinct groups: those that broadly neutralized; those that neutralized some isolates but enhanced the infectivity of others and those that were broadly enhancing (the majority). vNAbs isolated from the broadly neutralizing sera mapped to the binding site of one of the HCV receptors, CD81. The group is investigating the use of a pool of vAbs as a therapy for prevention of rapid HCV infection of transplanted livers.

Ask an expert

In the afternoon there was a vibrant question and answer session on the production and analysis of PVs and the reagents used. Notably, the panel recommended the use of a visual output reporter, such as GFP or β -galactosidase in conjunction with X-gal, rather than luciferase, to enable discrimination between wide-spread low-level reporter expression and isolated cells, with high levels of reporter expression to facilitate optimization of the PV production protocol.

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Several speakers described the application of a NanoSight (Amesbury, UK) nanoparticle analyzer to quantify the size and number of nanoparticles per preparation. Comparison of wild-type and PV preparations using this technology could be useful for evaluating the relative efficiency of particle production. There was also a consensus of opinion that using secreted alkaline phosphatase as a reporter in combination with a chemiluminescent substrate would offer the greatest benefit in terms of cost, throughput and sensitivity.

Conclusion

The fascinating array of talks from experienced researchers working on a wide range of viruses provided attendees with an excellent overview of the varied applications and challenges of pseudotyping. Unlike other virus-specific conferences, where competition between groups can limit frankness, this event was buzzing with a strong sense of openness, with participants willing to exchange both experimental details and reagents. Such was the success of the event that a further meeting in a year's time is already planned.

Financial & competing interests disclosure

The meeting was organised by EuroSciCon [8]. B King is a Horserace Betting Levy Board-funded Research Fellow. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.