Minireview

Rafts, anchors and viruses — A role for glycosylphosphatidylinositol anchored proteins in the modification of enveloped viruses and viral vectors

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ABSTRACT

Lipid rafts have been proposed as sites for the assembly of a number of viruses and are considered to play a major role in pseudotyping events. As a consequence, host glycosylphosphatidylinositol (GPI) anchored proteins commonly associated with lipid rafts can be found being incorporated into viral lipid envelopes with beneficial consequences for viral replication. In this review we will look at the link between lipid rafts, GPI-anchored proteins and retroviral particles and how these relationships can be exploited for the modification of enveloped viruses.

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The lipid raft hypothesis

As late addendum to the fluid mosaic model of biological membranes, which describes cellular membranes as a sea of lipid molecules with floating protein entities, lipid rafts (LR) have caused quite some waves in the scientific community and the biological relevance and conditions of their existence remain highly disputed (Hancock, 2006; Hanzal-Bayer and Hancock, 2007; Michel and Bakovic, 2007; Shaw, 2006). LRs are membrane sub-structures or microdomains enriched for cholesterol and sphingolipids. According to the original LR hypothesis (Simons and Ikonen, 1997), formation of these microdomains occurs as a consequence of the different biophysical properties of lipids found in the plasma membrane which also have different propensities for association with one another, leading to "phase-separation" events. Supporting evidence for phase separation comes from the in vitro study of model membranes which indeed demonstrated that mixtures of cholesterol, sphingolipids and unsaturated phospholipids induce the formation of macroscopic domains of different lipid composition (Brown and London, 1998; Radhakrishnan and McConnell, 1999). Similar events are thought to occur in vivo, resulting in LR formation. However, application of advanced imaging technologies to the topic (Kenworthy,

Petranova, and Edidin, 2000; Kusumi et al., 2005; Mayor and Rao, 2004; Plowman et al., 2005; Sharma et al., 2004; Zacharias et al., 2002) showed that although they could not disprove the existence of lipid rafts, modifications of the original hypothesis were called for. In summary, these experiments suggested highly dynamic lipid rafts with short half lives and of very limited spatial dimensions, being strongly influenced by their protein content. LR size was estimated to be between 5 and 20 nm (Prior et al., 2003; Sharma et al., 2004), which would only have very limited space for associated proteins i.e. 3–5 molecules per LR and half lives were estimated in the 100 nm range (Kawasaki et al., 2001; Kusumi et al., 2004). Additionally, the association of different protein species depending on cell type will severely change the characteristics of LRs such as their spatial dimensions and half lives (Hancock, 2006). Two main roles have been proposed regarding the physiological functions of LRs: (i) the re-arrangement and trafficking of membranes and associated proteins (Hanzal-Bayer and Hancock, 2007) and (ii) facilitating signal transduction (Gupta and DeFranco, 2007; Jury et al., 2007; Patra, 2008). LR protein content is inherently linked to their functions and different types of proteins can be found to be enriched in lipid rafts, most prominently the glycosylphosphatidylinositol (GPI) anchored proteins (Brown, 1992; Legler et al., 2005; Paulick and Bertozzi, 2008).

Lipid rafts and GPI-anchored proteins

GPI-anchored proteins are membrane associated after post-translational modification and predominantly found on the external

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side of the cell membrane, with significant amounts also located in intracellular membrane domains (Orlean and Menon, 2007; Paulick and Bertozzi, 2008). Proteins targeted for GPI anchoring contain a GPI signalling sequence (GSS) at the C-terminal end and addition of this feature to previously non-GPI-anchored proteins transforms them efficiently into GPI-anchored proteins (Brodsky et al., 1994; Brunschwig et al., 1999; Cordy et al., 2003; Jones and Geraghty, 2004; Kueng et al., 2007; Legler et al., 2005; Skountzou et al., 2007) (see Table 1), displaying properties typical of GPI-anchored proteins. The GSS is recognised in the endoplasmatic reticulum (ER) by the transamidase enzyme complex and is replaced by the preformed GPI anchor. GPI-anchored proteins share a common backbone structure, consisting of a phosphoethanolamine group next to the protein, followed by mannose residues, a non-acetylated glucosamine and a phosphoinositol moiety, which in turn is associated to the lipid residues responsible for membrane anchoring (Orlean and Menon, 2007). GPI-anchored proteins have a variety of different functions (Paulick and Bertozzi, 2008) from complement regulatory activity to signal transduction (see Table 1). GPI proteins can also be found in serum and other body fluids both with intact or absent GPI anchors (Landi et al., 2003). They are released from the plasma membrane to the surrounding milieu by a process termed shedding. Shedding can be induced by the activity of enzymes specific for cleavage of inositol phospholipids which remove parts of the GPI anchor (Lauc and Heffer-Lauc, 2006). Additionally, shedding can also be induced by the formation of membrane vesicles from intact cells (van Niel et al., 2001; Wolfers et al., 2001) and also by formation of small aggregates that may contain a small number of cellular lipid molecules in addition to few molecules of the GPI-anchored protein (Rooney et al., 1996). Processes that release GPI-linked proteins into the medium with intact GPI anchors are reversible and it has been shown in a variety of in vitro and in vivo systems that GPI-linked proteins can be re-inserted into cell membranes (Dunn et al., 1996; Kooyman et al., 1995; Rifkin and Landsberger, 1990; Rooney et al., 1993; Rooney et al., 1996; Vakeva et al., 1994). Therefore, GPI-anchored proteins can be considered to be "hypermobile". The physiological functions of GPI-anchored protein mobility are poorly defined, however shedding of GPI-anchored proteins could lead to a more systemic effect of the GPI-linked proteins or to activation events upon release.

Lipid rafts in virology

One interesting element of LRs is their apparent association with aspects of virus biology (for a summary see Table 2). They have been proposed as sites for entry and exit of a number of different virus species (Chazal and Gerlier, 2003; Suzuki and Suzuki, 2006; Wilflingseder and Stoiber, 2007). Involvement of LRs in the entry of virus particles to cells has been demonstrated for the non-enveloped SV40 (Papovaviridae) (Anderson et al., 1998; Gilbert et al., 2005; Norkin et al., 2002; Parton and Lindsay, 1999; Pelkmans et al., 2001; Stang et al., 1997; Tsai et al., 2003), rotavirus (Reoviridae) (Arias et al., 2002; Guerrero et al., 2002), rhinovirus (Picornaviridae) (Grassme et al., 2005), human adenovirus (Colin et al., 2005), entero- (Bergelson et al., 1994; Karnauchow et al., 1996; Stuart et al., 2002) and echovirus (Marjomaki et al., 2002) as well as the enveloped influenza virus (Orthomyxoviridae) (Lakadamyali et al., 2004; Sieczkarski and Whittaker, 2002), human immunodeficiency virus type 1 (HIV-1, Retroviridae) (Fantini et al., 1993; Hammache et al., 1998; Janes et al., 1999; Kozak et al., 2002; Puri et al., 1998) and members of the families herpesviridae (Bender et al., 2003; Cherukuri et al., 2004; Desplanques et al., 2008; Fingeroth et al., 1984; Lee, Church, and Wilson, 2003; Lyman et al., 2008; Tang et al., 2008), filoviridae (Aman et al., 2003; Bavari et al., 2002) and flaviviridae (Medigeshi et al., 2008). Most likely this is due to cellular receptors or co-receptors for virus entry being located in LRs, e.g. CD4 and CCR5 in the case of HIV-1 (Manes et al., 1999; Xavier et al., 1998). However, for other viruses which require LRs for entry, such as herpes simplex virus (HSV) cellular receptors are not primarily found in LRs (Bender et al., 2003).

Assembly and exit (or budding) of virus particles is also associated with LRs (Table 2). For non-enveloped viruses only rotavirus assembly has been shown to be associated with membrane microdomains (Delmas et al., 2004; Sapin et al., 2002). However, a range of enveloped viruses seems to use LRs as a platform for assembly and/or budding of viral particles, amongst them measles virus (Paramyxoviridae) (Manie

Table 1
Endogenous and recombinant GPI-anchored proteins

GPI anchored proteins		
Protein	**Accession (UniProt)**	**Function**
Endogenous		
Placental alkaline phosphatase (PLAP)	P05187	Hydrolase activity
Acetyl cholin esterase (AChE)	P22303	Cholin hydrolysis
Protectin (CD59)	P13987	Complement regulation
Decay accelerating factor (CD55)	P08174	Complement regulation
CD14	P08571	LPS/LBP complex receptor
F_C receptor gamma III (CD16b)	O75015	IgG binding
Folate receptor (hFR1)	P15328	Folic acid transport
TRAIL-R3	O14798	Decoy TRAIL receptor
Cellular prion protein (PrPc)	P04156	Unknown
Protein	**Reference**	**Function**
Recombinant		
Interleukin 2	Kueng et al., 2007	Cytokine
Interleukin 4	Kueng et al., 2007	Cytokine
Interleukin 7	Kueng et al., 2007	Cytokine
Interleukin 15	Kueng et al., 2007	Cytokine
GM-CSF	Kueng et al., 2007; Skountzou et al., 2007	Cytokine
Green fluorescent protein (GFP)	Legler et al., 2005	Fluorescence marker
B7.1	Brunschwig et al., 1999	Immune costimulatory
B7.2	Brunschwig et al., 1999	Immune costimulatory
ß-secretase (BACE)	Cordy et al., 2003	Protease
HSV gp	Jones and Geraghty, 2004	Viral glycoprotein
CD4	Brodsky et al.; 1994	HIV receptor

Endogenous GPI-anchored proteins serve a variety of different functions. In addition, non GPI-anchored proteins can be genetically engineered to yield recombinantly anchored proteins without losing the function of the endogenous protein (LPS, lipopolysaccharide; LPB, LPS binding protein; TRAIL-R3, tumor necrosis factor-related apoptosis-inducing ligand receptor 3; GM-CSF, granulocyte-macrophage colony stimulating factor).

Table 2
Lipid rafts in virology

|	Lipid rafts in viral life cycle				
	Virus	Family	Entry	Exit	Reference
Naked	Simian virus 40 (SV40)	*Papovaviridae*	X		Anderson et al., 1998; Gilbert et al., 2005; Norkin et al., 2002; Parton et al., 1999; Pelkmans et al., 2001; Stang et al., 1997; Tsai et al., 2003
	Rotavirus	*Reoviridae*	X	X	Arias et al., 2002; Delmas et al., 2004 Guerrero et al., 2002; Sapin et al., 2002
	Species C human adenovirus (HAdV)	*Adenoviridae*	X		Colin et al., 2005
	Enterovirus	*Picornaviridae*	X		Bergelson et al., 1994; Karnauchow et al., 1996; Stuart et al., 2002
	Echovirus type 1	*Picornaviridae*	X		Marjomaki et al., 2002
	Rhinovirus	*Picornaviridae*	X		Grassmé et al., 2005
Enveloped	Epstein-Barr virus (EBV)	*Herpesviridae*	X		Cherukuri et al., 2004; Fingeroth et al., 1994
	Herpes simplex virus type 1 (HSV-1)	*Herpesviridae*	X		Bender et al., 2003; Lee et al., 2003
	Human herpes virus 6 (HHV-6)	*Herpesviridae*	X		Tang et al., 2008
	Pseudorabies virus	*Herpesviridae*	X		Desplanques et al., 2008; Lyman et al. 2008
	West-Nile virus	*Flaviviridae*	X		Medigeshi et al., 2008
	Measles virus	*Paramyxoviridae*		X	Manie et al., 2000; Vincent et al., 2000
	Respiratory syncytial virus (RSV)	*Paramyxoviridae*		X	Marty et al., 2004; McDonald et al., 2004; Brown et al., 2002
	Sendai	*Paramyxoviridae*		X	Ali et al., 2000
	Marburg	*Filoviridae*	X	X	Aman et al., 2003; Bavari et al., 2002
	Ebola	*Filoviridae*	X	X	Aman et al., 2003; Bavari et al., 2002; Panchal et al., 2003
	Human immunodeficiency virus (HIV-1)	*Retroviridae*	X	X	Fantini et al., 1993; Hammache et al., 1998; Janes et al., 1999; Kozak et al., 2002; Lindwasser et al., 2002; Nguyen and Hildreth, 2000 Ono et al., 2001; Puri et al., 1998
	Murine leukemia virus (MLV)	*Retroviridae*		X	Pickl et al. 2001
	Influenza	*Orthomyxoviridae*	X	X	Barman et al., 2000; Lakadamyali et al., 2004; Scheiffele et al., 1997; Sieczkarski et al., 2002; Zhang et al., 2000
	Vesicular stomatitis virus (VSV)	*Rhabdoviridae*		X	Brown and Lyles, 2003

A variety of both naked and enveloped viruses is associated with lipid rafts or lipid raft components during viral exit or entry. The table provides a summary of these interactions.

et al., 2000; Vincent et al., 2000), respiratory syncytial virus (RSV, Paramyxoviridae) (Brown et al., 2002; Marty et al., 2004; McDonald et al., 2004), Sendai virus (Paramyxoviridae) (Ali and Nayak, 2000), Marburg and Ebola virus (Filoviridae) (Bavari et al., 2002; Panchal et al., 2003), vesicular stomatitis virus (VSV, Rhabdoviridae) (Brown and Lyles, 2003) and influenza virus (Orthomyxoviridae) (Barman and Nayak, 2000; Scheiffele et al., 1997; Zhang et al., 2000). In the family retroviridae, it was suggested that HIV assembles and buds through interactions with LRs (Chazal and Gerlier, 2003; Lindwasser and Resh, 2002; Nguyen and Hildreth, 2000; Ono and Freed, 2001; Wilflingseder and Stoiber, 2007). Additionally, LRs have also been implicated in assembly and budding of gammaretroviridae e.g. murine leukemia virus (MLV) and may be central to pseudotyping events e.g. the presentation of VSV-G proteins on the envelopes of MLV-derived viral particles (Briggs et al., 2003; Pickl et al., 2001). Pickl et al. demonstrated the co-localisation of viral proteins to LR markers and could identify these markers in viral membrane preparations. Additionally, proteins known to be associated to LRs (c-Src, c-Ras) were incorporated into virus particles, whereas proteins excluded from LRs were not found in viral particles (Pickl et al., 2001). They concluded that enveloped viruses employ membrane microdomains for particle assembly and budding, but more importantly, that the localisation of viral components to LRs is essential for pseudotyping events i.e. due to the propensity of different viral envelope glycoproteins for LRs they can be co-packaged into viral particles (Pickl et al., 2001). The finding that even phylogenetically distinct virus proteins like the VSV-G can be used for pseudotyping of retroviral particles may be explained by the fact that envelope proteins of different virus genera share the affinity for membrane microdomains. As well as the concept of LRs, their involvement in the life cycle of viruses is also controversial. One interesting approach to tackle this problem is comparative proteomics, extending the studies mentioned above by Pickl et al. Comparison of the protein content of cellular LR preparation and the protein content of viruses derived from these cells could help to find similarities and thus generate a link between LRs and viral budding. First efforts have been made to define the proteome of both LRs (Foster and Chan, 2007; Foster et al., 2003) and viral particles (Chung et al., 2006; Maxwell and Frappier, 2007; Saphire et al., 2006), however, extensive comparative information would be interesting.

Modification of retroviral vectors using GPI-anchored proteins

The first suggestion to use GPI-anchored proteins for the modification of retroviral vectors may have well come from the findings that different retroviral species, most prominently HIV-1, incorporate GPI-anchored proteins, like the complement regulatory factors CD55 and CD59 during budding. Viral particles benefit from these incorporated host factors as they show increased resistance to complement activity (Saifuddin et al., 1997). Both primary HIV-1 isolates and virus isolates derived from virus producing cell lines were shown to activate the complement cascade, however, levels of complement-mediated virolysis were low (Banki et al., 2005; Stoiber et al., 2008). When virus was produced from cell lines significantly lower levels of virolysis were seen after over-expression of CD59 and CD55 (Saifuddin et al., 1997) indicating the complement-protective activity of the acquired proteins. The transfer of this phenomenon to MLV-derived viral particles by over-expression of CD59 in virus producer cell lines (Breun et al., 1999) constitutes a first attempt to use GPI-anchored proteins for the modification of retroviral vectors (RVs). Expression of GPI-anchored proteins in virus producing cells and subsequent insertion into budding viral particles (Breun et al., 1999; Kueng et al., 2007; Skountzou et al., 2007) – potentially fuelled by the co-localisation of GPI-anchored proteins with sites of virus assembly and/or budding in LRs (see Fig. 1, left) – is the first of two ways to modify enveloped retroviral vectors with GPI-anchored proteins. Additionally, purified GPI-anchored proteins can be re-inserted into viral envelopes in a process termed "viral painting" post-budding (Metzner et al., 2008) (see Fig. 1, right) without the need for genetic modification of virus producing cells and independent of any
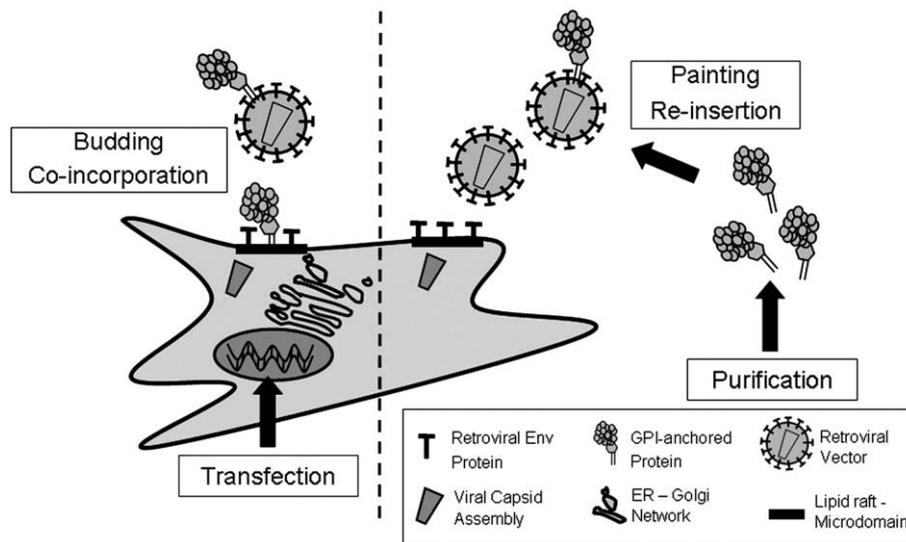
Fig. 1. Modification of retroviral vectors with GPI-anchored proteins. GPI-anchored proteins can be used to modify retroviral vectors using two different routes. Classically, transfection of retroviral producer cell lines leads to the production of modified virions (left). Co-localisation of GPI-anchored proteins with sites of viral budding – i.e. at lipid rafts – leads to the incorporation of the GPI protein into viral particles. Additionally, re-insertion of purified GPI-anchored proteins ("viral painting") to viral proteins exploits the affinity of the lipophilic GPI anchor to the viral envelope.

association of viral budding and LRs. Taken together with the fact that non GPI-anchored proteins can be genetically engineered to become GPI-anchored proteins, this makes them interesting targets for modification of retroviral and other enveloped virus vectors.

Modification of retroviral vectors by transfection of virus producer cell lines

Co-transfection of plasmid vectors carrying genes for the production of RVs with constructs expressing the GPI-anchored proteins (Kueng et al., 2007; Skountzou et al., 2007) or super-transfection of pre-existing virus producing cell lines (Breun et al., 1990) leads to the formation of viral particles presenting GPI-anchored molecules on their envelopes. These particles acquire novel properties as a consequence of the incorporation of the GPI-anchored protein.

In 1999, Breun et al. demonstrated that super-transfection of the retroviral producer cell line PALSG/S, which is based on the murine NIH3T3 cells, with the human GPI-anchored protein CD59, yields viral particles that are resistant to the activity of complement in serum (Breun et al., 1999). Incorporation of CD59 in viral particles was shown and infectious titers of CD59 protected virus were increased up to 100-fold. These results suggested for the first time that incorporation of recombinantly expressed GPI proteins into the envelopes of viral particles is possible and that these modifications can be useful for gene therapy approaches. Interestingly, even viral vectors produced from human cell lines are partially susceptible to complement activity, due to the fact that viral proteins themselves interact with proteins of the complement system and can initiate complement activity. Therefore, production of RV from human cells does not completely circumvent the need for complement protection.

More recently, two studies have used co-transfection approaches to produce virus-like particles (VLPs) displaying GPI-anchored molecules in mammalian (Kueng et al., 2007) and insect cells (Skountzou et al., 2007). In both cases, recombinant GPI-anchored proteins were generated from different cytokine species and tested for their functional properties, compared to their soluble counterparts, in order to achieve different goals. Whereas Kueng et al. could demonstrate that the GPI-anchored cytokines can elicit cellular responses such as differentiation and proliferation with similar efficiency as the soluble cytokines, Skountzou et al. showed that GPI-anchored cytokines engineered onto VLPs based on simian

immunodeficiency virus (SIV) can be used to enhance immunogenicity of the VLPs in immunisation studies.

Kueng et al. (2007) used the GSS of the human low affinity $F_c\gamma$III receptor CD16b to construct GPI-anchored forms of interleukins (IL) such as IL2, IL4, IL7, IL15 and granulocyte-macrophage colony stimulating factor (GM-CSF) (see Table 1) and found that the cytokines genetically engineered for GPI anchoring are preferentially incorporated into VLPs based on expression of Moloney MLV (MoMLV) *gagpol* constructs in the human embryonal kidney cell line, HEK293. Additionally, they could demonstrate that incorporation into the VLP is dependent on the type of membrane association displayed by the cytokines i.e. GPI-anchored cytokines where incorporated, whereas cytokines fused to *trans*-membrane domains were not. This is evidence for the co-localisation of viral budding and GPI-anchored proteins and also indicates that incorporation of GPI-anchored is not exclusively due to their over-expression in the virus producing cell lines. GPI-anchored IL4 and GM-CSF displayed on VLPs were used to successfully differentiate monocytes into dendritic cells in co-cultivation experiments. Moreover, morphological and biochemical (i.e. cytokine fingerprints) hallmarks of differentiation were comparable to results achieved with soluble cytokines. VLPs functionalised with GPI-anchored IL2 were used for co-stimulation of peripheral blood mononuclear cells (PBMCs) and increased proliferation in a dose-dependent manner was detected, again similar to results obtained with the soluble cytokine (Kueng et al., 2007).

Skountzou et al. (2007) used a similar approach to incorporate immune-stimulatory molecules e.g. GM-CSF into SIV-based VLPs. The rationale was to develop VLPs suitable for vaccination purposes. HIV vaccines usually produce a significant cytotoxic T cell response, however, generation of neutralizing antibodies is usually weak. Subsequently, vaccines prolong survival in primates but protective immunity, i.e. prevention of infection, is not achieved. Incorporation of immune-stimulatory factors into viral antigen carrying VLPs is one strategy to generate vaccines with increased efficacy. VLPs displaying GPI-anchored GM-CSF were produced in insect cells and used to test immunological response *in vitro* and *in vivo*. Chimeric VLPs did indeed increase activation of B cells and generally induced enhanced humoral immune responses. Interestingly, incorporation of fusion proteins of GM-CSF containing the *trans*-membrane domain of the HIV gp160 (instead of a GPI anchor) into VLPs did not increase immune responses in mice, although localisation of the fusion protein to the cell

membrane was observed and the fusion protein was functional. Incorporation of the fusion protein was achieved, however, with less efficiency than the GPI-anchored form, indicating that incorporation is not solely a consequence of over-expression. The incorporation levels of recombinant GPI-anchored GM-CSF were somewhat similar to the levels of Env proteins found in viral particles. Taken together, these results suggest that recombinant GPI-anchored proteins can be associated with viral particles with high efficiency.

Protein engineering of retroviral vectors using GPI-anchored proteins

Transfection of producer cell lines is not the only possibility to use GPI-anchored proteins for modification of RVs. As mentioned previously one interesting feature is their "hypermobility" — the potential to shift from cell-bound to different forms of vesicular bodies or aggregates and *vice versa*. Interestingly, when the proteins are extracted and purified from the cells they are produced in and incubated with other cells, re-insertion takes place and localisation is once again efficiently conferred to the plasma membrane. This process is known as cellular "painting" (Legler et al., 2005; Medof et al., 1996). Moreover, these proteins retain their function throughout the process. It has been suggested that painting of cells might be of therapeutic use, for example in correcting paroxysmal nocturnal hemoglobinuria (PNH) — a disease caused by acquired mutations in PIGA, a component of the GPI-anchor biosynthesis pathway, leading to complement-mediated hemolysis due to loss of complement regulators CD59 and CD55 by providing these factors (Sloand et al., 2004) or for the treatment of cancer by introducing co-stimulatory proteins to tumor cells and thereby increasing immunogenicity (McHugh et al., 1999).

We have recently described the extension of this method for the insertion of GPI-linked proteins into viral envelopes ("viral painting", see Fig. 1).

We could demonstrate association of the GPI-linked protein CD59his to viral vectors based on MLV and HIV-1 (Metzner et al., 2008). Viral particles and affinity-purified protein were incubated for at least 3 h at 37 °C to facilitate insertion of the GPI-anchored proteins. After removal of non-associated and/or endogenous protein, signals for CD59 could only be detected when both purified GPI-linked protein and viral particles were present. The association is specific and the painted virus particles remain infectious after insertion of the GPI-linked protein, albeit at reduced efficiencies caused predominantly by the duration of the painting process, rather than the actual incorporation of GPI-anchored molecules into the viral envelope. Preliminary results suggested that the amount of GPI-anchored proteins associated with virus particles is dependent on the concentration of purified GPI protein during incubation, allowing for insertion of different amounts of GPI-anchored proteins into viral envelopes. Estimates of the number of GPI-anchored proteins painted onto retroviral particles were in the range of the numbers observed for Env molecules per virion and is thus similar to that achieved after incorporation of hybrid proteins produced in co-transfection experiments (Skountzou et al., 2007).

The potential for modification of enveloped viral vectors with GPI-anchored proteins

Regardless of their involvement in the controversial lipid rafts, GPI-anchored proteins appear to be exceptionally useful for the modification of retroviral vectors. Two profoundly different strategies can be employed, each with distinct advantages: (i) transfection of viral producer cell lines and (ii) direct protein engineering of viral particles by painting. Stable transfection of retroviral producer cell lines co- or super-transfected with endogenous or recombinant GPI-anchored proteins will provide a long-term, reliable source of modified viral particles with high reproducibility. Recent studies using transfection methods to express GPI-anchored proteins in virus producing cell lines

have demonstrated the usefulness of this approach to increase immunogenicity of VLPs and in eliciting specific responses like differentiation and proliferation from target cells upon co-cultivation. The strength of viral painting lies in different areas than for co-transfection approaches: The association is quick — 3 h, probably less are sufficient for maximum painting (work still in progress). The method is very flexible since different types of RV, regardless of the transfer vectors included, can be modified. In theory, also other enveloped viruses like hepatitis B or influenza virus can be modified, due to the fact that affinity of GPI-anchored proteins to viral envelopes is based on the shared lipophilicity only. Moreover, no genetic modification of producer cell lines is necessary. Finally, preliminary experiments suggest that the amount of GPI-modified proteins on the virus is controllable. Viral painting may be the method of choice for modification of enveloped viral particles in all circumstances where (i) flexibility is required, e.g., in response to genetic heterogeneity in gene therapy patients or in response to quickly changing antigen presentation for vaccination, and (ii) genetic modification of virus producing cell lines is difficult e.g. when using cytotoxic proteins or when handling genetically and/or biochemically poorly defined virus species or variants.

Using GPI-anchored proteins for modification of enveloped viral particles may profit virology researchers in two ways. On the basic science side, they can be helpful in elucidating the links between membrane microdomains and virus biology and enable a better understanding of the interactions between the virus and the host (cell) membranes during virus entry and exit to cells. Secondly, this type of modification promises great potential for more applied fields, e.g. viral gene therapy or vaccine development and may lead to development into clinical solutions. Therefore research in this area should be encouraged.

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