ANTICANCER RESEARCH *25*: 2601-2608 (2005)

Review

Murine Polyomavirus Virus-like Particles (VLPs) as Vectors for Gene and Immune Therapy and Vaccines against Viral Infections and Cancer

KARIN TEGERSTEDT[1], ANDREA VLASTOS FRANZÉN[1], KALLE ANDREASSON[1], JEANNA JONEBERG[1], SHIRIN HEIDARI[2], TORBJÖRN RAMQVIST[1] and TINA DALIANIS[1]

[1]*Department of Oncology-Pathology, Karolinska Institutet, Cancer Center Karolinska, R8: 01, Karolinska University Hospital, 171 76 Stockholm;*
[2]*Department of Medicine, Center of Infectious Medicine, Karolinska Institutet, Karolinska University Hospital, 141 86 Stockholm, Sweden*

Abstract. *This review describes the use of murine polyomavirus "virus-like" particles (MPyV-VLPs), free from viral genes, as vectors for gene and immune therapy and as vaccines. For large-scale MPyV-VLP manufacture, VP1 is produced in a baculovirus insect cell system, E. coli or in yeast. MPyV-VLPs bind eukaryotic DNA and introduce this DNA into various cell types in vitro and in vivo. In normal and T-cell-deficient mice, this results in the production of anti-MPyV-VLP (and MPyV) antibodies. Furthermore, repeated MPyV-VLP vaccination has been shown to prevent primary MPyV infection in normal and T-cell-deficient mice, and the outgrowth of some MPyV-induced tumours in normal mice. Moreover, when inoculated with gene constructs encoding for HIV p24, MPyV-VLPs augment the antibody response to p24. In addition, MPyV-VLPs, containing fusion proteins between the VP2 or VP3 capsid protein and selected antigens, can be used as vaccines. Notably, one vaccination with MPyV-VLPs, containing a fusion protein between VP2 and the extracellular and transmembrane parts of the HER-2/neu oncogene, immunizes against outgrowth of a HER-2/neu-expressing tumour in Balb/c mice and also against the development of mammary carcinomas in BALB-neuT transgenic mice. Finally, a second polyoma VLP-vector based on murine pneumotropic virus (MPtV-VLP), which does not cross-react serologically with MPyV-VLP (and MPyV), has been developed and can be used to conduct prime boost gene and immune therapy and vaccination. In summary, MPyV-VLPs are useful vectors for gene therapy, immune therapy and as vaccines and, in combination with MPyV-VLPs, MPtV-VLPs are potentially useful as prime-boost vectors.*

Correspondence to: Prof. Tina Dalianis, Department of Oncology-Pathology, Karolinska Institutet, CancerCenterKarolinska R8:01, Karolinska University Hospital, 171 76 Stockholm, Sweden. Tel: +46 851776583, Fax: +46 851776630, e-mail: Tina.Dalianis@cck.ki.se

Key Words: Murine polyomavirus, VLPs, gene therapy, immune therapy, vaccines, review.

The identification of mutations in genes leading to genetic diseases and cancer has increased the number of therapeutic options for specifically tailored treatments, such as gene and immune therapy, as well as vaccines. To do so, several gene transfer systems based on viral vectors have been developed, of which the adeno- and retrovirus systems are the most widely used to date (1, 2). However, these systems have the drawback of retaining viral genes that may interact with host genes in an undesirable way (3). Furthermore, the efficiency of gene therapy, conducted through *e.g.* adenovirus vectors, can be reduced by pre-existing immunity to the virus (4, 5). To avoid some of these problems, other vehicles free of viral genes have been studied for use in gene and immune therapy. Some of the benefits of using one type of such vehicles, *i.e.* virus-like particles (VLPs), composed of the murine polyomavirus (MPyV) major capsid protein VP1, are described here. Moreover, an additional murine vehicle for prime-boost immune therapy is also presented.

Murine polyomavirus (MPyV)

Murine polyomavirus (MPyV) belongs to the polyomavirus family and induces a variety of tumours, hence its name "poly oma" from the Greek words "many tumours" [for reviews see Allison, 1980, Dalianis, 1990, Cole, 1996, Berke and Dalianis 2000 (6-9)]. MPyV infection causes tumour development in newborn mice with an immature immune system and in T-cell-deficient mice, but not in normal adult

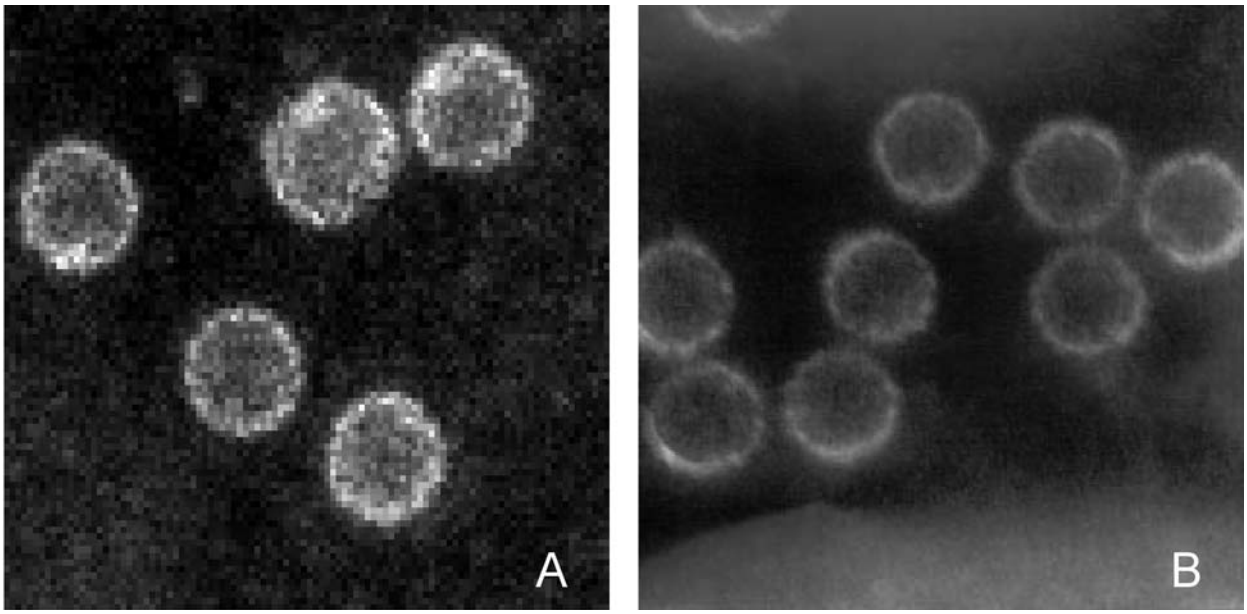

Figure 1. *MPyV (A) and MPtV (B) virus-like particles by electron microscopy in two different magnifications.*

mice, where the mice are also protected against a subsequent challenge with a MPyV tumour (6, 7). T-cell immunity is responsible for protection against MPyV tumour development and tumour rejection, while antibodies (including maternal antibodies) prevent virus infection (6). MPyV T-antigen-derived peptides, presented by Major Histocompatibility Complex (MHC) class I molecules on the tumour surface, are the targets of the cellular immune system (7, 9-12).

The MPyV genome is enclosed in a complex with cellular histones in a 45 nm virion. It consists of 5.3 kb double-stranded super-coiled circular DNA and is functionally divided into three regions (13). The *non-coding region* contains the origin of replication and the enhancer and promoter regions (13). The *early region* encodes three proteins named according to size; large T antigen (LT) 100 kD, middle T antigen (MT) 55 kD and small T antigen (ST) 22 kD, that contribute to the transforming and immortalising function of the virus (13). The *late region* encodes three capsid proteins, VP1-VP3, generated from a common pre-mRNA by alternative splicing (13). The major capsid protein VP1 (45 kD) accounts for 75% of the viral capsid proteins (13). Its N-terminus binds to MPyV DNA (14). VP1 recognizes sialyloligosaccharide residues on gangliosides on the cell surface, binds to most mammalian cells (13) and is crucial for viral uptake (15). VP2 (34 kD) contains the entire VP3 sequence and 115 additional N-terminal amino acids (a.a.) and is suggested to be important for un-coating, viral assembly and viral entry, while VP3 (23 kD) is probably active only in viral assembly (16). The viral capsid consists of 360 copies of VP1 arranged in 72 pentamers on an icosahedral lattice (17). Each pentamer associates with a copy of either VP2 or VP3 (16). The C-terminal "arms" of VP1 from each pentamer grasp neighbouring pentamers, tying the shell together (18). Calcium ions are required for virion stability (19). Elimination of either VP2 or VP3 substantially decreases viral growth (20). However, VP2 and VP3 are not necessary for the assembly of VLPs in insect or yeast cells (21).

MPyV has been an excellent source of knowledge about tumour development and immunity against tumours (6, 7) and for examining mechanisms of DNA replication and transcription, transformation and cell cycle regulation (13). Recently, MPyV has been used as a vector for gene and immune therapy and vaccines, as described below.

Murine pneumotropic virus – a second murine polyomavirus

Murine pneumotropic virus (MPtV) was the second murine polyomavirus to be identified, being isolated in 1953 by Kilham and Murphy and originally called Kilham virus or K-virus (22). MPtV causes interstitial pneumonia with high fatality in newborn mice, but in older animals it induces an unapparent but persistent infection (23). During primary infection, MPtV replicates in the vascular endothelial cells of the lung, spleen and liver, whereas later during the persistent phase, infected cells are found mainly in the kidney (24). One major difference between MPyV and MPtV is that inoculation of newborn mice with MPtV does not result in tumour

development (25). The genome of MPtV is a 4756 bp double-stranded circular DNA molecule that encodes two early proteins (LT and ST) and three late proteins (VP1-VP3) (26). Analysis of cloned DNA has established that MPtV belongs to the polyomavirus family, although it is not very closely related to MPyV. The functional domain of MPtV LT resembles the LT of Simian virus 40 (SV40) more than the LT of MPyV, and its overall amino acid constitution demonstrates a homology of 44% to SV40 and 36% to MPyV. Like SV40, MPtV also lacks MT. MPtV VP1 major capsid protein has an overall 45% homology (with some parts up to 80% homology) with MPyV VP1 (26). Since MPtV is very difficult to culture *in vitro* and is considered non-tumorigenic, this virus has not been studied as extensively as MPyV.

MPyV-VLPs

As early as 1978, chromatographically separated and purified MPyV VP1 was shown upon renaturation to form pentameric subunits, that were morphologically and biophysically similar to native virion capsomeres (27). Later, bacterially-expressed recombinant VP1 capsomeres, as well as VP1 produced in a baculovirus system and yeast, were demonstrated to form virus-like particles, with a size and morphology similar to that of native virions (Figure 1A) (28). It has been shown to self assemble into empty virus-like particles (VLPs). Today VLPs are usually expressed in a eukaryotic baculovirus insect cell system or in yeast (29-33). MPtV-VLPs, like MPyV-VLPs, are easily obtained after production of MPtV VP1 in a baculovirus system (Figure 1B), which will be discussed in more detail below (34).

MPyV-VLPs as vehicles for gene transfer

MPyV VP1 not only binds to the viral genome, but also binds indiscriminately to non-viral DNA (32). Hence, when purified MPyV-VLPs are incubated together with naked DNA they are able to form VLP/DNA complexes (29). Originally the DNA in these complexes was believed to be mainly inside the VLPs. However, later studies using electron microscopy demonstrated that most of the DNA was situated on the outside of the VLPs and that several VLPs can be associated with the same DNA plasmid (32, 36). In addition, DNase treatment of the VLP/DNA complexes showed that only DNA fragments of around 3 kb or less were protected inside the VLP (29). The ability of MPyV-VLPs to bind DNA of different origins has since been utilized for the transfer of exogenous DNA into different cells *in vitro* resulting in both transient and stable gene expression (29, 31, 35).

These *in vitro* studies were later followed by trials *in vivo*, where the efficiency and longevity of DNA delivered in association with MPyV-VLPs was compared to the delivery of naked DNA alone and to natural MPyV infection [for details see Heidari *et al.* (36)]. To evaluate the influence of the humoral immune system on this process, experiments were performed in parallel in B-cell-deficient (μMT), T-cell-deficient ($CD4^{-/-}8^{-/-}$) and normal C57BL/6 mice. An MPyV plasmid *dl1023-pBR322*, (9kb), defective in replication, was used as a reporter gene for later recognition by MPyV PCR. Mice were observed for 3-6 months and DNA introduced by MPyV-VLPs or MPyV was maintained throughout the whole observation period, while most mice receiving naked DNA alone cleared this DNA within a week.

Table I. *MPyV- but not MPtV-VLP immunization protects against MPyV infection in normal ACA mice.*

	ACA mice immunized with[1]:		
	MPyV-VLP	MPtV-VLP	Non-immunized
Parotid	0[1,2]	1	4
Lung	0	3	4
Bone	0	3	3
Heart	0	4	3
Spleen	0	2	3
Kidney	0	2	2
L.N.	0	4	4
Gonads	0	4	2
Total number of mice[3]	0/4	4/4	4/4

[1]Mice were immunized with 20 μg of VLPs once per week three consecutive times.
[2]Indicates number of mice with presence of MPyV DNA present in specific tissues/total number of mice.
[3]There were 4 mice in each immunization or control group.

To estimate relative DNA quantities introduced by MPyV-VLPs, and by DNA alone, in comparison to MPyV infection, two tissues where MPyV usually persists, heart and bone, were further evaluated by quantification of MPyV DNA using Q-PCR. Normal mice that received MPyV-VLPs/DNA complexes had 10-50 times higher DNA copy numbers/cell compared to mice receiving plasmid DNA alone, while in immune-deficient mice, the corresponding difference was 50-100 times. DNA transfer by MPyV-VLPs and MPyV in normal mice was similar, although there were variations in individual mice. The difference in DNA transfer in normal and T-cell-deficient mice may be due to the fact that the MPyV plasmid induced T antigen expression, and hence a T-cell response in normal mice, resulting in the elimination of plasmid carrying cells in normal mice (10, 37). The difference in DNA transfer in normal and B-cell-deficient mice may be because more viral particles enter the tissues in B-cell-deficient mice, since these mice are also devoid of background antibody activity (38).

Notably, hemagglutination inhibition (HAI)-inducing antibodies were detected in both normal and in T-cell-deficient mice inoculated with MPyV (1:6000) and MPyV-VLP/DNA

complexes (1:1600). Although 10^3-10^5 more VLPs than MPyV virions were introduced into the mice, the antibody response elicited by MPyV was significantly higher, which is probably because MPyV replicates *in vivo*. Mice inoculated with naked DNA did not develop any detectable antibodies and had antibody titres comparable with non-infected controls.

Other studies have also demonstrated the ability of MPyV-VLPs to act as carriers for the *in vivo* transduction of DNA to various tissues, followed by the expression of the introduced gene (35, 39). In one such study, the efficiency of using different routes (subcutaneous, intravenous, intraperitoneal or intranasal) for delivery of MPyV-VLP/DNA complexes was compared (35). The efficiency of delivery by different routes varied for different organs. In the same study, the β-galactosidase gene introduced together with MPyV-VLPs was still expressed 22 weeks after *in vivo* delivery. In another study, the β-galactosidase gene introduced together with MPyV-VLPs *in vivo* in mice gave rise to a T-cell response directed against the β-galactosidase protein (39).

In summary, MPyV-VLPs are useful gene therapy vectors and MPyV plasmid DNA was successfully transferred by MPyV-VLPs into a variety of cells *in vivo* and remained there for months, a longevity similar to that of natural MPyV infection. However, a humoral immune response was observed in both normal and T-cell immune-deficient mice inoculated with VLPs.

Pneumotropic murine polyomavirus (MPtV) VLPs as an additional vehicle for prime boost immune therapy and repeated gene therapy

To obtain a vehicle, to which there is no pre-existing human immunity, and which does not immune cross-react with MPyV, another murine polyomavirus, the murine pneumotropic virus (MPtV), was exploited [for further details see Tegerstedt *et al.* (34)]. MPtV VP1 expressed in a recombinant baculovirus system formed MPtV-VLPs readily and were shown to bind to and enter different cells, including dendritic cells (DC) and, moreover, internalization of MPtV-VLPs into cells was rapid. MPtV-VLP binding was, however, in contrast to MPyV and MPyV-VLP binding, neuroaminidase resistant and, unlike SV40 binding, independent of MHC class I expression. Furthermore, MPtV-VLPs did not immune cross-react serologically with MPyV-VLPs, or *vice versa* (when tested by ELISA) and immunization of mice with MPtV-VLPs did not protect mice from MPyV infection (Table I). These results suggested that MPtV-VLPs and MPyV-VLPs could potentially be interchanged as carriers of DNA in repeated gene therapy (34). Finally, MPtV-VLPs were shown to transduce foreign DNA *in vitro* and *in vivo*. In conclusion, there are now two murine polyomavirus vectors (MPyV-VLPs and MPtV-VLPs) available for conducting prime-boost gene and immune therapy (34).

MPyV-VLPs immunize both normal and T-cell-deficient mice against primary MPyV infection

The production of anti-MPyV-VLP antibodies, described above, in normal and T-cell-deficient mice is not favourable for repeated gene therapy. However, used appropriately, a vaccine based on VLPs able to protect individuals with a defective immune system against a persistent and potentially dangerous virus infection, is highly desirable. Hence, MPyV-VLPs were used to vaccinate against MPyV infection (40, 41).

MPyV-VLPs and, for comparison, modified MPyV-VLPs (M17-VLPs), that lack 7 amino acids on the DE loop of VP1, were used to immunise normal and T-cell-deficient ($CD4^{-/-}8^{-/-}$) C57BL/6 mice against MPyV infection [for further details see Heidari *et al.* (40)]. Mice were immunized four times intraperitoneally (*i.p.*) with either VLPs (1 μg), and then together with non-immunized controls inoculated with MPyV. MPyV-specific antibodies were observed in MPyV-VLP-immunized mice (ELISA and HAI) and antibody titres increased with each immunization. Sera from mice inoculated with M17-VLPs did not induce HAI and, when using an MPyV-VP1-specific ELISA, only low IgG titres were detected even after repeated vaccination. Slightly reduced antibody responses were generally observed in $CD4^{-/-}8^{-/-}$ mice in comparison to normal mice, which could be due to the fact that, although IgG switching occurs in the absence of T-cells, it is expected to be less effective (42).

Nonetheless, half of the MPyV-VLP- and M17-VLP-immunized mice were protected against MPyV infection, as judged by MPyV PCR, and the remaining mice exhibited MPyV DNA only in a few organs, while non-immunized mice showed a disseminated infection during the same time period (40). Notably, when evaluating normal and $CD4^{-/-}8^{-/-}$ mice separately, M17-VLP immunization was significantly less efficient than MPyV-VLP immunization for protection of T-cell-deficient mice against persistent MPyV infection. It was suggested that the DE-loop, absent in M17, is important in stimulation of T-cell-independent antibody responses.

To improve the immune response above, MPyV-VLPs were inoculated four times subcutaneously (*s.c.*) alone or with Freund's adjuvant into $CD4^{-/-}8^{-/-}$ C57BL/6 mice (41). For comparison, normal C57BL/6 mice were similarly immunized with VLPs alone [for details see Vlastos *et al.* (41)]. After completing immunization, immunized and control mice were challenged with MPyV. Three weeks later, all mice were sacrificed and tested for the presence of MPyV DNA by PCR. MPyV DNA was detected in control, but not in any of the immunized normal or $CD4^{-/-}8^{-/-}$ mice. MPyV-VLP-specific IgG antibodies (including IgG2a) were detected by ELISA in immunized but not control mice. In T-cell-deficient mice ($CD4^{-/-}8^{-/-}$) the best antibody titres were obtained after immunization with VLPs together with

Table II. *Specificity of MPyV-VLP immunity in ACA and CBA mice. Incidence of the MPyV-induced tumours SECA, SEBB, SEBA and the non-MPyV-induced tumour S6C in mice immunized with MPyV-VLPs, MPtV-VLPs, GST-VP1, MPyV, or irradiated S6C cells, as well as in non-immunized control mice. From Vlastos et al. (45), with permission from the publisher.*

Tumour line	Exp no	Cell doses inoculated		Whole body irradiation (400 rad) prior to tumour inoculation	Controls	Tumour takes in mice immunized with:				
						MPyV-VLPs	MPtV-VLPs	GST-VP1	S6C Irradiated 10000rad	MPyV
SECA[1]	1	1 x 10^4	ACA	No	4/4	0/4	-[4]	-	-	0/5[5]
	2	1 x 10^4		No	4/5	0/5	0/5	1/5	-	0/5[5]
	3	5 x 10^2		Yes	5/5	0/6	-	-	-	-
S6C[1]	1	1 x 10^3	ACA	No	5/5	3/5[6]	5/5[6]	-	0/5	-
	2	1 x 10^3		Yes	5/5	4/4	-	4/4	2[6]/4	-
	3	1 x 10^3		Yes	4/4	-	4/4	-	0/4	-
SEBB[2] SEBB-TC[3]	1	1 x 10^4	CBA	No	3/5	4/5[6]	-	-	-	-
	2	1 x 10^5		Yes	6/6	5/6[6]	-	-	-	-
SEBA-TC[3]	1	5 x 10^4	CBA	Yes	4/4	4/4	-	-	-	-

[1]SECA and S6C are of ACA origin.
[2]SEBB and SEBA are of CBA origin.
[3]TC denotes short-term tissue cultured cells.
[4](-) Indicates not done.
[5]All experiments where MPyV was used as immunogen were performed in non-irradiated mice, due to restrictions of the animal facilities.
[6]A delay in outgrowth, measured as mean tumour load, calculated by adding the individual tumour diameters and dividing the sum of the total number of mice, with or without tumours within each group, as described (10).

Freund's adjuvant and the lowest antibody titres were obtained after immunization with MPyV-VLPs alone. Normal mice immunized with MPyV-VLPs alone had similar antibody titres to $CD4^{-/-}8^{-/-}$ mice immunized with MPyV-VLPs together with Freund's adjuvant.

In addition, to study the importance of the VLP conformation in the induction of immune protection against MPyV infection, immunization with MPyV-VLPs and with glutathione-S-transferase VP1 fusion protein was compared (41). GST-VP1 produced in large quantities in an E-coli system can self-assemble into dimers and pentamers but not into VLPs. T-cell-deficient ($CD4^{-/-}8^{-/-}$) and normal C57BL/6 mice were immunized repeatedly with either GST-VP1 or MPyV-VLPs and then, with controls, challenged with MPyV. All mice were sacrificed three weeks later and tested for the presence of MPyV DNA by PCR. MPyV DNA was detected in control and 40% of the GST-VP1-immunized $CD4^{-/-}8^{-/-}$ mice, but in no other immunized mice, indicating that MPyV-VLPs are more efficient than GST-VP1 for immunization against MPyV (41). It is likely that MPyV-VLPs, with repetitive structures and folding, are more potent as immunogens in comparison to the more linear GST-VP1 proteins, for triggering innate as well as adaptive immune responses.

As expected, both normal and $CD4^{-/-}8^{-/-}$ mice immunized with MPyV-VLPs exhibited specific antibody titres towards MPyV-VLPs [for further details see Vlastos *et al.* (41)]. However, in contrast to normal mice, $CD4^{-/-}8^{-/-}$ mice immunized with GST-VP1 had no antibodies to MPyV-VLPs. Furthermore, while GST-VP1-immunized normal mice readily exhibited antibodies to GST-VP1, in $CD4^{-/-}8^{-/-}$ mice the best response to GST-VP1 was induced after MPyV-VLP immunization. Hemagglutination of guinea pig erythrocytes was induced by MPyV-VLPs as expected, but not by GST-VP1. Hence, only immunization with MPyV-VLPs induced HAI in both normal and $CD4^{-/-}8^{-/-}$ mice. It has been suggested that the symmetrical structure of the viral capsid, with a regular pattern of exposed antigen epitopes, can induce a T-cell-independent antibody response. This suggestion is confirmed by the results of the present study. In conclusion, an efficient anti-MPyV-VLP antibody response protects normal and T-cell-deficient mice against viral infection and is best obtained by *s.c.* MPyV-VLP vaccination (41).

MPyV-VLPs as vaccine against MPyV tumours

As described above, T-antigen-derived peptides are suggested to be the most common targets for the T-cell responses that prevent tumour development and mediate tumour rejection (7, 10-12, 43). Viral capsids, mainly produced during lytic infection, were not primarily anticipated as targets for tumour immunity (7). However, a recent report (44) suggested that

early region transcription and late region transcription may occur in parallel, thus allowing the potential production of viral capsid proteins also in MPyV-induced tumour cells. In fact, the presence of the viral major capsid protein L1 is occasionally observed in human tumours induced by human papillomavirus, a virus related to MPyV (Hanna Dahlstrand, personal communication). These reports, in parallel to the observation of the very potent response against MPyV-VLPs in a T-cell-deficient context, prompted us to analyse whether MPyV-VLPs could, in fact, induce immunity against MPyV-induced tumours [for further details see Franzén *et al.* (45)].

MPyV-VLPs, MPtV-VLPs and GST-VP1, the latter two for comparison, were all used as immunogens to vaccinate against three MPyV tumours SECA, SEBA and SEBB and one tumour of non-polyoma origin, SC6 (45). ACA and CBA mice were immunized repeatedly, *i.p*. and *s.c*. in parallel, with MPyV-VLPs, MPtV-VLPs or GST-VP1 and, thereafter, challenged with tumour cells and followed for tumour outgrowth. MPyV, MPyV-VLP and MPtV-VLP immunization protected all and GST-VP1 immunization protected most ACA mice from outgrowth of SECA, whereas control mice developed tumours (Table II). Outgrowth of the non-MPyV tumour SC6 was prevented by immunization with irradiated SC6 cells, but was not prevented in VLP-immunized ACA mice (Table II). In similar experiments, a delay in outgrowth in MPyV-VLP-immunized mice was observed of the SEBB, but not of the SEBA polyoma tumour (Table II).

The fact that both MPyV-VLPs, as well as MPtV-VLPs, conferred immunity to SECA suggests, since MPyV and MPtV-VLPs do not cross-react serologically, that the induced immune response is T-cell-mediated (45). Most likely the immune response is directed against peptide antigens of VP1 presented by MHC class I molecules, and more specifically, against regions not exposed on the viral capsid surface, since it is there that the homology between MPyV and MPtV VP1 is the most pronounced (26). The fact that GST-VP1 immunization was not as efficient as MPyV-VLP immunization further emphasizes the fact that the specific structure of VLPs is of importance and may enhance both humoral and cellular immune responses.

Surprisingly, the efficiency of MPyV-VLP immunization was not correlated to VP1 expression, a high viral load or viral production, since SEBA was shown to have more VP1 and a higher viral load compared to SECA [for details see Franzén *et al.* (45)]. However, one must bear in mind that the methods used for detection of VP1 proteins and genes (Western and Southern blots) only measure the mean content of VP1 protein or DNA/cell line. In a virus-producing tumour, only a minority of the population of the cells may be responsible for virus production. It is likely, therefore, that a rejection response was directed towards the majority of SECA tumour cells and only to a minority of SEBA virus-producing cells. The differences between the MPyV tumours after VLP immunization cannot be attributed to differences in immune sensitivity, since it has previously been shown that all three tumours are rejected upon MPyV immunization (46). In conclusion, MPyV-VLP immunization may be useful in abrogating the outgrowth of some MPyV tumours.

Genetic vaccination is augmented by MPyV-VLPs

The fact that MPyV-VLPs can be used to vaccinate against MPyV infection in a T-cell-deficient context suggested that they may have immune stimulatory qualities. They were, therefore, utilized to stimulate DNA gene gun vaccination, together with a plasmid encoding HIV p24 [for details see Rollman *et al.* (47)]. T-cell (ELISPOT) and B-cell responses (ELISA) were compared in individual mice, vaccinated with 20 μg HIV p24 DNA alone, or together with 2 μg, 20 μg or 100 μg of MPyV-VLPs. Using 20 μg of HIV p24 DNA for vaccination generally gives a threshold effect. The addition of 2 μg MPyV-VLPs to DNA vaccination did not influence the immune response. The addition of 20 μg or 100 μg MPyV-VLPs to DNA vaccination did, however, result in enhanced B-cell responses in a proportion of immunized mice compared to immunization with HIV p24 DNA alone. The results were suggested to be due to the fact that MPyV-VLPs might have a DNA carrier or an adjuvant effect (47).

MPyV-VLPs as carrier vehicles for proteins – a novel approach

The presence of viral DNA or any DNA in vectors for vaccination poses the threat that this DNA may be potentially dangerous (3). Adding viral DNA may lead to the integration of oncogenic sequences into the host DNA, or the addition of any DNA could be dangerous if inserted in sensitive positions and thus creating mutations or activating genes that should not be activated. To avoid the insertion of any DNA, a novel vaccine approach, that incorporates part of a protein into MPyV-VLPs, has also been tested (48).

Introduction of chimeric VP2 fusion proteins on the inside of MPyV-VLPs. An alternative approach to the one described above is to utilize the ability of the minor capsid proteins to bind to the inside of VP1 pentamers in MPyV-VLPs [for details see Tegerstedt *et al.* (48)]. These can theoretically harbour approximately 30 VP2 and VP3 molecules. By producing both VP1 and VP2 in the same baculovirus, it is possible, during VLP assembly, to introduce a number of VP2 molecules within these VLPs. In this way, a fusion protein, between a foreign protein and VP2 or VP3, can be produced, introduced into cells by VLPs and processed and presented to the immune system, potentially eliciting an

immune response against cells carrying the foreign protein. This approach was tested using the HER-*2*/*neu* proto-oncogene, which is frequently overexpressed in breast, gastric, ovarian, lung and pancreatic cancer and which has been used previously in vaccination studies as a target protein (49).

MPyV-VLPs, containing a fusion protein between MPyV VP2 and the extracellular and transmembrane domain of HER-*2*/*neu* (HER-2_{1-683}PyVLPs), were produced in a baculovirus insect cell system. They retained their shape and harboured approximately 3-4 $HER2_{1-683}$VP2 fusion proteins per MPyV-VLP and were tested for their ability to vaccinate against HER-*2*/*neu*-expressing tumours in two different *in vivo* models. Protection was assessed both against a lethal challenge with a BALB/c mammary carcinoma transfected with human HER-2 (D2F2/E2) and against the outgrowth of autochthonous mammary carcinomas in BALB-*neu*T mice, transgenic for the activated rat HER-*2*/*neu* oncogene (50). A single injection of HER-2_{1-683}PyVLPs prior to tumour inoculation induced a complete rejection of D2F2/E2 tumour cells in BALB/c mice. Similarly, a single injection of HER-2_{1-683}PyVLPs at six weeks of age protected BALB-*neu*T mice with atypical hyperplasia from a later outgrowth of mammary carcinomas, whereas all controls developed palpable tumours in all mammary glands. VLPs containing only VP1 and VP2 did not induce protection. The protection elicited by HER-2_{1-683}PyVLPs vaccination was most probably due to a cellular immune response, since a HER-2-specific response was demonstrated in an ELISPOT assay, whereas antibodies against HER-2 were not detected in any of the two models. The results demonstrate the feasibility of using MPyV-VLPs carrying HER-2/*neu* fusion proteins as safe and efficient vaccines against HER-2-expressing tumours (49).

Conclusion

The possibility of using a vehicle that does not require viral genes for gene therapy represents a great step forward in that the potential danger of activating viral oncogenes is avoided. Vehicles that can transduce DC can be very useful for immune therapy and the possibility of repeating gene and immune therapy is of importance, since one treatment is not always sufficient. Obtaining knowledge of how MPyV-VLPs can improve DNA vaccination and protect against viral infection and some viral-induced tumours can be one of the initial steps towards protection against viral infection and cancer.

Acknowledgements

This work was supported in by the Swedish Cancer Foundation, the Swedish Research Council, the Stockholm Cancer Society, King Gustav V's Jubilium Foundation, the Stockholm City Council and the Karolinska Institutet, Sweden.

References

1 Mulligan RC: The basic science of gene therapy. Science *260*: 926-932, 1993.
2 Breyer B, Jiang W, Cheng H, Zhou L, Paul R, Feng T and He TC: Adenoviral vector-mediated gene transfer for human gene therapy. Curr Gene Ther *1*: 149-162, 2001.
3 Verma IM and Somia N: Gene therapy – promises, problems and prospects. Nature *389*: 239-242, 1997.
4 Robbins PD, Tahara H and Ghivizzani SC: Viral vectors for gene therapy. Trends Biotechnol *16*: 35-40, 1998.
5 Mountain A: Gene therapy: the first decade. Trends Biotechnol *18*: 119-128, 2000.
6 Allison AC: Immune response to polyoma virus and polyoma virus-induced tumors. *In:* Klein G: Viral Oncology, Raven Press, New York, pp. 481-487, 1980.
7 Dalianis T: Studies on the polyoma virus tumor-specific transplantation antigen (TSTA). Adv Cancer Res *55*: 57-85, 1990.
8 Cole CN: Polyomavirinae. *In:* Fields: Virology, pp. 1997-2026, 1996.
9 Berke Z and Dalianis T: Studies on polyomavirus persistence and polyomavirus-induced tumor development in relation to the immune system. Adv Cancer Res *79*: 249-276, 2000.
10 Berke Z, Palmer S, Bergman T, Wester D, Svedmyr J, Linder S, Jornvall H and Dalianis T: A short peptide eluted from the H-2Kb molecule of a polyomavirus-positive tumor corresponds to polyomavirus large T antigen peptide at amino acids 578 to 585 and induces polyomavirus-specific immunity. J Virol *70*: 3093-3097, 1996.
11 Ramqvist T, Reinholdsson G, Carlquist M, Bergman T and Dalianis T: A single peptide derived from the sequence common to polyoma small and middle T-antigen induces immunity against polyoma tumors. Virology *172*: 359-362, 1989.
12 Wilson CS, Moser JM, Altman JD, Jensen PE and Lukacher AE: Cross-recognition of two middle T protein epitopes by immunodominant polyoma virus-specific CTL. J Immunol *162*: 3933-3941, 1999.
13 Cole CN and Conzen SD: Polyomavirinae: the viruses and their replication. *In:* Fields: Virology, pp. 2121-2174, 2001.
14 Moreland RB, Montross L and Garcea RL: Characterization of the DNA-binding properties of the polyomavirus capsid protein VP1. J Virol *65*: 1168-1176, 1991.
15 Tsai B, Gilbert JM, Stehle T, Lencer W, Benjamin TL and Rapoport TA: Gangliosides are receptors for murine polyoma virus and SV40. EMBO J *22*: 4346-4355, 2003.
16 Barouch DH and Harrison SC: Interactions among the major and minor coat proteins of polyomavirus. J Virol *68*: 3982-3989, 1994.
17 Rayment I, Baker TS, Caspar DL and Murakami WT: Polyoma virus capsid structure at 22.5-A resolution. Nature *295*: 110-115, 1982.
18 Liddington RC, Yan Y, Moulai J, Sahli R, Benjamin TL and Harrison SC: Structure of simian virus 40 at 3.8-A resolution. Nature *354*: 278-284, 1991.
19 Brady JN, Winston VD and Consigli RA: Dissociation of polyoma virus by the chelation of calcium ions found associated with purified virions. J Virol *23*: 717-724, 1977.
20 Mannova P, Liebl D, Krauzewicz N, Fejtova A, Stokrova J, Palkova Z, Griffin BE and Forstova J: Analysis of mouse polyomavirus mutants with lesions in the minor capsid proteins. J Gen Virol *83*: 2309-2319, 2002.

21 Montross L, Watkins S, Moreland RB, Mamon H, Caspar DL and Garcea RL: Nuclear assembly of polyomavirus capsids in insect cells expressing the major capsid protein VP1. J Virol *65*: 4991-4998, 1991.

22 Kilham L and Murphy HW: A pneumotropic virus isolated from C3H mice carrying the Bittner Milk Agent. Proc Soc Exp Biol Med *82*: 133-137, 1953.

23 Greenlee JE: Effect of host age on experimental K virus infection in mice. Infect Immun *33*: 297-303, 1981.

24 Greenlee JE, Clawson SH, Phelps RC and Stroop WG: Distribution of K-papovavirus in infected newborn mice. J Comp Pathol *111*: 259-268, 1994.

25 Parsons DF: Morphology of K virus and its relation to the papova group of viruses. Virology *20*: 385-388, 1963.

26 Mayer M and Dorries K: Nucleotide sequence and genome organization of the murine polyomavirus, Kilham strain. Virology *181*: 469-480, 1991.

27 Brady JN and Consigli RA: Chromatographic separation of the polyoma virus proteins and renaturation of the isolated VP1 major capsid protein. J Virol *27*: 436-442, 1978.

28 Salunke DM, Caspar DL and Garcea RL: Self-assembly of purified polyomavirus capsid protein VP1. Cell *46*: 895-904, 1986.

29 Forstova J, Krauzewicz N, Sandig V, Elliott J, Palkova Z, Strauss M and Griffin BE: Polyoma virus pseudocapsids as efficient carriers of heterologous DNA into mammalian cells. Hum Gene Ther *6*: 297-306, 1995.

30 Gillock ET, Rottinghaus S, Chang D, Cai X, Smiley SA, An K and Consigli RA: Polyomavirus major capsid protein VP1 is capable of packaging cellular DNA when expressed in the baculovirus system. J Virol *71*: 2857-2865, 1997.

31 Soeda E, Krauzewicz N, Cox C, Stokrova J, Forstova J and Griffin BE: Enhancement by polylysine of transient, but not stable, expression of genes carried into cells by polyoma VP1 pseudocapsids. Gene Ther *5*: 1410-1419, 1998.

32 Stokrova J, Palkova Z, Fischer L, Richterova Z, Korb J, Griffin BE and Forstova J: Interactions of heterologous DNA with polyomavirus major structural protein, VP1. FEBS Lett *445*: 119-125, 1999.

33 Palkova Z, Adamec T, Liebl D, Stokrova J and Forstova J: Production of polyomavirus structural protein VP1 in yeast cells and its interaction with cell structures. FEBS Lett *478*: 281-289, 2000.

34 Tegerstedt K, Andreasson K, Vlastos A, Hedlund KO, Dalianis T and Ramqvist T: Murine pneumotropic virus VP1 virus-like particles (VLPs) bind to several cell types independent of sialic acid residues and do not serologically cross react with murine polyomavirus VP1 VLPs. J Gen Virol *84*: 3443-3452, 2003.

35 Krauzewicz N, Cox C, Soeda E, Clark B, Rayner S and Griffin BE: Sustained *ex vivo* and *in vivo* transfer of a reporter gene using polyoma virus pseudocapsids. Gene Ther *7*: 1094-1102, 2000.

36 Heidari S, Krauzewicz N, Kalantari M, Vlastos A, Griffin BE and Dalianis T: Persistence and tissue distribution of DNA in normal and immunodeficient mice inoculated with polyomavirus VP1 pseudocapsid complexes or polyomavirus. J Virol *74*: 11963-11965, 2000.

37 Luthman H, Nilsson MG and Magnusson G: Non-contiguous segments of the polyoma genome required in cis for DNA replication. J Mol Biol *161*: 533-550, 1982.

38 Berke Z, Mellin H, Svedmyr J, Wen T and Dalianis T: Persistence of polyomavirus in antibody deficient mice, X-linked immunodeficiency (XID) mice, IgM-/- single knockout and CD8-/-, IgM-/- double knockout mice. In Vivo *12*: 143-148, 1998.

39 Clark B, Caparros-Wanderley W, Musselwhite G, Kotecha M and Griffin BE: Immunity against both polyomavirus VP1 and a transgene product induced following intranasal delivery of VP1 pseudocapsid-DNA complexes. J Gen Virol *82*: 2791-2797, 2001.

40 Heidari S, Vlastos A, Ramqvist T, Clark B, Griffin BE, Garcia MI, Perez M, Amati P and Dalianis T: Immunization of T-cell deficient mice against polyomavirus infection using viral pseudocapsids or temperature sensitive mutants. Vaccine *20*: 1571-1578, 2002.

41 Vlastos A, Andreasson K, Tegerstedt K, Hollanderova D, Heidari S, Forstova J, Ramqvist T and Dalianis T: VP1 pseudocapsids, but not a glutathione-S-transferase VP1 fusion protein, prevent polyomavirus infection in a T-cell immune deficient experimental mouse model. J Med Virol *70*: 293-300, 2003.

42 Bachmann MF and Zinkernagel RM: Neutralizing antiviral B cell responses. Annu Rev Immunol *15*: 235-270, 1997.

43 Liunggren G, Liunggren HG and Dalianis T: T cell subsets involved in immunity against polyoma virus-induced tumors. Virology *198*: 714-716, 1994.

44 Chen L and Fluck M: Kinetic analysis of the steps of the polyomavirus lytic cycle. J Virol *75*: 8368-8379, 2001.

45 Franzén AV, Tegerstedt K, Holländerova D, Forstova J, Ramqvist T and Dalianis T: Murine polyomavirus VP1 virus-like particles immunize against some polyomvirus induced tumors. In Vivo *19(2)*: 323-326, 2005.

46 Dalianis T, Magnusson G, Ito Y and Klein G: Immunization against the polyoma virus-induced tumor-specific transplantation antigen by early region mutants of the virus. J Virol: 43 772-777, 1982.

47 Rollman E, Ramqvist T, Zuber B, Tegerstedt K, Kjerrstrom Zuber A, Klingstrom J, Eriksson L, Ljungberg K, Hinkula J, Wahren B and Dalianis T: Genetic immunization is augmented by murine polyomavirus VP1 pseudocapsids. Vaccine *21*: 2263-2267, 2003.

48 Tegerstedt K, Lindencrona J-A, Curico C, Andreasson K, Forni G, Tullus C, Dalianis T, Kiessling R and Ramqvist T: A single vaccination with polyomavirus VP1/VP2HER-2 virus-like particles (VLPs) prevents outgrowth of HER-*2*/*neu* expressing tumors. Cancer Res. In press, 2005.

49 Kiessling R, Wei WZ, Herrmann F, Lindencrona JA, Choudhury A, Kono K and Seliger B: Cellular immunity to the HER-2/*neu* protooncogene. Adv Cancer Res *85*: 101-144, 2002.

50 Di Carlo E, Diodoro MG, Boggio K, Modesti A, Modesti M, Nanni P, Forni G and Musiani P: Analysis of mammary carcinoma onset and progression in HER-*2*/*neu* oncogene transgenic mice reveals a lobular origin. Lab Invest *79*: 1261-1269, 1999.

Received April 4, 2005
Accepted May 4, 2005