Review

Lessons from Immune Responses and Vaccines against *Murine Polyomavirus* Infection and Polyomavirus-induced Tumours Potentially Useful for Studies on Human Polyomaviruses

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Abstract. *During 2007-2008, three new human polyomaviruses, KI, WU and Merkel cell cancer polyomaviruses have been discovered, of which the latter has also been identified in a human tumour. This development revives the interest in both human and animal polyomaviruses and their potential role in tumour development and disease particularly in immune suppressed individuals. Murine polyomavirus (MPyV) has in the past been used for acquiring knowledge of transformation mechanisms in vitro, as well as in immunological studies with regard to virus-induced tumour development in the natural host of the virus. Here we summarize some of the accumulated knowledge achieved in the MPyV field in view of the balance between tumour virus, the immune system and tumour development, and discuss this in relation to infections with human polyomaviruses. We also present how virus-like particles (VLPs) and gluthatione-S-transferase VP1 can be used for vaccination against the same tumour virus not only in mice with a well-functioning immune system, but also in immune suppressed mice.*

During the past 12 years, the number of polyomaviruses has almost doubled by the use of molecular techniques for their identification and it is likely this trend will continue (1). This recent development includes the detection of three new human polyomaviruses, *KI-polyomavirus (KIPyV), WU polyomavirus (WUPyV)* and *Merkel cell cancer polyomavirus (MCPyV)* (2-4). The latter virus has been associated with Merkel cell cancer (3) a rare cancer in the elderly and immunosuppressed. To better understand and suggest how we should pursue studies on human polyomaviruses, we here present and comment on some accumulated data from studies with regard to immune responses, viral persistence and tumour development, mostly performed with *murine polyomavirus* in its natural host.

Murine polyomavirus (MPyV), the first well-described member of the polyomavirus family was detected in 1953, (5, 6), and named "poly oma" in Greek, due to its ability to induce many tumours when inoculated in newborn mice. Although the virus, similar to human polyomaviruses in man, is common in mice and potentially oncogenic, it does not normally induce tumours (7, 8). Nevertheless, MPyV has been very important and thoroughly used for molecular studies on transformation by tumour virus and for studies of virus persistence, virus-induced tumour development and studies of immune responses against a tumour virus in its natural host (9, 10). Thus, there is much accumulated knowledge within the polyomavirus field accumulated throughout five decades that may also be of use for studies on the recent new members of the family. This review will deal mainly with the interactions of MPyV with the immune system along with some words on the use of MPyV virus-like particles (VLPs) and glutathione-*S*-transferase(GST)-VP1 as vaccines against the virus and tumour development.

The Molecular Biology of *Murine Polyomavirus*

MPyV has, similar to all polyomaviruses, a circular double-stranded DNA genome of ~5 kbp and an icosahedral symmetric capsid with a diameter of 45 nm, for reviews see (11-13). Its genome is arbitrarily divided into an early, a

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late, and a regulatory region. The early region encodes the large T (LT), middle T (MT) and small T (ST) antigens, which share an initial 79 *N*-terminal amino acid sequence, while due to RNA splicing and frame shifts, they all have unique *C*-terminal ends. LT immortalizes cells by interacting with the Rb protein, but it is also necessary for lytic infection and is located in the nucleus, where it binds to the regulatory region and inhibits early transcription, and at the same time initiates late transcription as well as viral replication (12, 14, 15). MT is located in the cytoplasm and on the inside of the plasma membrane and indirectly inhibits the function of p53 (16). However, it is not present in most polyomaviruses, where instead LT binds not only to Rb, as mentioned above, but also to p53 and this way has a very strong growth promoting and transforming function. ST enhances the activities of MT and LT and is located in both the cytoplasm and the nucleus (13). The newly described KI, WU and MCPyV express only LT and ST (2-4, 17). The non-coding regulatory region is situated in between the early and late regions and contains the promotors and enhancers as well as the origin of replication (13). The late region encodes the major capsid protein VP1 and the minor capsid proteins VP2 and VP3. The viral capsid is formed predominantly by VP1 (360 molecules) distributed in 72 pentamers, and within the capsid around 12-20 VP2 and VP3 molecules bind to VP1. Purified VP1 can self-assemble into virus-like particles (VLPs) under certain conditions (18, 19), similar to that observed for human papillomavirus (HPV) L1, and this has been used for vaccine development.

Early Studies of *Murine Polyomavirus (MPyV)*-induced Tumour Development

Tumour development is not observed in colonies of MPyV in naturally infected mice (7), whereas it can occur in MPyV-infected newborn mice in colonies naïve to MPyV, or in immunosuppressed mice. In newborn mice naturally infected with MPyV, the presence of maternal neutralizing antibodies delays infection of the mice allowing the cellular immune system to mature and effectively deal with the infection (20, 21). Instead, if the newborn mice are thymectomized, the period of susceptibility to tumour development increases (22-24), again indicating that mature functional T-cells are responsible for resistance against tumour development. Moreover, passive transfer of antiviral antibodies within 24 hours of viral infection prevents tumour development in MPyV infected newborn mice lacking maternal antibodies, while depletion of immune T-lymphocytes by antiserum, abolishes protection (25). In summary, similar to other viral infections, also in humans, antibodies reduce the number of infectious particles, while T-cells are important for protection against tumour development (for reviews see (13, 26).

Definition of the MPyV Tumour-specific Transplantation Antigen (TSTA)

In the 1960s, it was demonstrated that adult mice and hamsters inoculated with MPyV instead of developing tumours, better resisted the outgrowth of a subsequent inoculum of an MPyV tumour, but not of a non-MPyV tumour (27, 28) Likewise, irradiated MPyV tumour cells could be used for immunization against MPyV tumours (29, 30) and a common antigen, the MPyV tumour-specific transplantation antigen (TSTA), was suggested to be present on all MPyV-induced tumours (31-33).

However, the molecular details of MPyV TSTA were not known then. Both T-antigens, not located on the cell surface and, since at the time processing and presentation of antigens was unknown, virally modified cellular surface proteins were suggested as TSTA, and several studies were performed to identify this antigen.

Various T-antigen mutants were all found to induced MPyV tumour rejection, and it was concluded that minor T-antigen deletions did not abolish TSTA activity (34). Similarly, cross-immunization between MPyV mouse and rat cell lines also occurred suggesting that TSTA, viral or cellular, was related between the species (35). Rats and mice were also successfully immunized with tumour cells, vaccinia vectors expressing either MT or LT, as well as recombinant purified ST or MT, and it was necessary to selectively abolish all T-antigens to abrogate TSTA activity (36-41). T-Antigens, were therefore assumed to be processed and presented as peptide "TSTAs" together with MHC determinants to the immune system (40, 42). In 1989, six peptides, together covering the entire MT, were produced and a significant MPyV tumour rejection response was obtained upon immunisation with a peptide corresponding to amino acids 162-176 of MT (43). This was, to our knowledge, also the first time a peptide antigen was used to vaccinate against a tumour *in vivo*. Later, other TSTA peptides were identified (44-46) and a LT peptide was eluted off an MPyV tumour (47), further strengthening the hypothesis of MPyV T-antigen peptide TSTAs.

Similar results (48, 49) were obtained in mice, with the primate simian virus 40 (SV40), demonstrating multiple antigenic sites of the SV40 TSTA by using cytotoxic T-lymphocyte clones (50). In humans, much less is known, but it is likely that the situation is similar, and recombinant T-antigens/peptide antigens from human polyomaviruses may be useful to boost immunity against these viruses.

Viral and Host Factors Influencing MPyV Persistence and Tumour Development

Various viral and host factors influence MPyV persistence and tumour development. Different MPyV mutants both structural and/or non-structural such as early region mutants, tsa (temperature sensitive), hrt (host range non-transforming),

and mlt (MT, LT) mutants, were shown to affect viral replication, transformation and/or host range (51-54). Others with mutations in the non-coding region, and in the coding regions of LT and VP1, had different capacities to induce tumours in specific mice, and differences were also observed in their tumour tropism (55-57).

Host factors, affecting the immune system were also identified, *e.g.* the expression of the MtV super antigen in one mouse strain, eliminated T-cells expressing Vb6, which increased the tumour incidence, indicating that mutations in one specific place could influence the outcome after MPyV infection (58-61). Different organs of the mouse also varied in their sensitivity to MPyV infection, with kidney and liver and lung being more sensitive to viral replication in newborns compared to adults (62). In addition, persistence and organ distribution varied also dependent on the route of inoculation (63, 64).

In humans, it is also likely that the age of infection, as well as different polyomavirus strains, result in different profiles, with regard to viral replication and tissue distribution and tumour development. Moreover, the effectiveness of immune system and the MHC complex alleles may both be of importance, since Merkel cell carcinoma carrying integrated MCPyV and progressive multifocal leukoencephalopathy (PML) caused by the human polyomavirus JC are observed in few but not all elderly or immunosuppressed individuals (4, 65). In addition, it is known that MS patients treated with Natalizumab show decreases in T-cell efficiency (66).

Persistence and Tumour Development in Normal and Immunosuppressed Newborn and Adult Mice

MPyV infection of newborn mice results in a peak of viral replication 1 week after infection, which gradually declines, but remains persistent (21, 59, 61-64, 67). MPyV induced tumour development may occur 2-8 months post infection, and the tumour profile and its frequency is strain dependent and varies from 0-100%. MPyV infected newborn mice lacking functional T-cells *e.g.* nude mice or severe combined immunodeficiency (SCID) mice (which also lack functional B-cells) succumb to systemic infection within weeks (62, 68). In $CD4^{-/-}8^{-/-}$ double knockout newborn mice, with depressed T-cell function, one third of the mice died of MPyV infection, one third suffered from hind leg paralysis, while some remaining mice developed tumours (59). In MPyV-infected newborn $CD4^{-/-}$ or $CD8^{-/-}$ single knockout mice, with partially depressed T-cell function, tumour development was only slightly elevated in $CD8^{-/-}$, but not in $CD4^{-/-}$ mice, compared to that of controls (59,69). MPyV infections in newborn mice lacking functional B-cells such as in X-linked immunodeficiency (XID) (70) and $IgM^{-/-}$ mice, resulted in a poor control of MPyV infection, but the tumour incidence was similar to that of corresponding mouse strain (71, 72).

In MPyV infected adult mice, replication also peaks at 1 week, but infection is cleared more efficiently than in newborns, and persistence is not easily observed and tumours do not develop (21,73). Tumours develop depending on the strain, in 30-100% of nude and $CD4^{-/-}$, $CD8^{-/-}$ double knockout, but not in single knockout MPyV infected adult mice (21, 59, 60, 62, 69, 74). $IgM^{-/-}$ and XID adult mice with B-cell deficiencies have difficulties in limiting MPyV infection, but do not develop tumours (60, 72).

In summary, persistent MPyV infection is detected at a high level in newborn MPyV naïve mice, and in certain immune-deficient mice and tumour development may occur, while in adult mice with a mature functioning immune system, persistent infection is limited and tumour development not observed (for reviews see (10, 21)). Likewise, tumour development is never or very rarely observed in mice with B-cell deficiencies, despite viral dissemination (60, 75).

In humans, it is likely that infections with most human polyomaviruses are ubiquitous and that most newborn infants, including those born with different immunodeficiencies, have maternal antibodies at birth. However, still very little is known, and it is not impossible that some types of leukaemia, or childhood malignancies could be caused by a yet not known polyomavirus infection acquired very early on life. This brings us to the potential of the next chapter.

Vaccination Against MPyV Infection – Possible Applications in Humans

The MPyV system has also been utilized for studies on vaccination against persistent infection both in normal and immunodeficient mice. This type of studies, especially the latter type would be difficult to conduct in a clinical setting.

As early as in 1978, chromatographically purified MPyV VP1 were observed to reassemble into subunits similar to virion capsomeres (76). Later, VP1 produced in an insect cell/baculovirus system or in yeast were shown to form VLPs (77, 78). Moreover, large quantities of VLPs, both from yeast and insect cell/baculovirus systems, have been produced for related viruses *i.e.* for the production of VLP-based vaccines against HPV infection (79). Experimentally, MPyV VLPs have been shown to successfully immunize both normal and immune deficient $CD4^{-/-}8^{-/-}$ mice against MPyV infection (80, 81). The presence of IgG antibodies towards MPyV VLPs were detected in vaccinated immune deficient mice, although the antibody titres were slightly lower in $CD4^{-/-}8^{-/-}$ as compared to normal mice. This suggests the ability of MPyV-VLPs to induce both T-cell-dependent and-independent responses. An important reason for the induction of T-cell-independent B-cell responses may be the presentation of VP1 epitopes in a dense regular pattern on

the surface of the virus/VLPs (82). This finding, presents some hope in the potential ability to vaccinate against polyomavirus infections in immunosuppressed humans. However, there have been difficulties in producing VLPs from the new human polyomaviruses so far, and to obtain large quantities of VP1 for vaccination, other techniques may be of use. For this purpose, it is of interest to compare also other ways of presenting VP1 to the immune defence than in the context of VLPs.

Experimentally, vaccination against MPyV infection has also been performed using a GST VP1 fusion protein (80). Production of GST-VP1 in E-coli does not result in VLP formation, but in VP1 dimers and pentamers, which can be used for vaccination. Vaccination of normal and $CD4^{-/-}8^{-/-}$ double knockout, mice with GST-VP1 resulted in 100% protection of normal and 60% protection of $CD4^{-/-}8^{-/-}$ mice against MPyV infection and the antibody response in the knockout mice was much weaker, suggesting that MPyV-VLPs, presenting the epitopes in a repetitive structure are more potent than GST-VP1 for vaccination in the T-cell deficient context (80). Nevertheless, GST-VP1 vaccination does show potential even in an immunodeficient setting and it is possible that one could with the help of different adjuvants improve this potential.

In summary, MPyV-VLP and GST-VP1 immunization can protect against primary MPyV infection, although immunosuppressed individuals may require the addition of adjuvants in order to achieve complete protection with the latter. This suggests that if needed, it should be possible to vaccinate or boost immunity against human polyomaviruses that are possibly oncogenic or that have pathogenic effects in immunosuppressed patients (80).

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References

1 Dalianis T and Garcea RL: Welcome to the *Polyomaviridae*. Semin Cancer Biol *19*: 209-210, 2009.

2 Allander T, Andreasson K, Gupta S *et al*: Identification of a third human polyomavirus. J Virol *81*: 4130-4136, 2007.

3 Feng H, Shuda M, Chang Y *et al*: Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science *319*: 1096-1100, 2008.

4 Gaynor AM, Nissen MD, Whiley DM *et al*: Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog *3*: e64, 2007.

5 Gross L: A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. Proc Soc Exp Biol Med *83*: 414-421, 1953.

6 Stewart SE: Leukemia in mice produced by a filterable agent present in AKR leukemic tissues, with notes on a sarcoma produced by the same agent. Anatomical Record *117*: 532, 1953.

7 Rowe WP, Hartley JW, Brodsky I *et al*: Observations on the spread of mouse polyoma virus infection. Nature *182*: 1617-1619, 1958.

8 zur Hausen H: Other virus infections possibly involved in human cancers. *In*: zur Hausen H. Infections Causing Human Cancer. Weinheim: Wiley-Vch Verlag Gmbh & Co, pp. 366-385, 2006.

9 Atkin SJ, Griffin BE and Dilworth SM: Polyomavirus and simian virus 40 as cancer models: history and perspectives. Semin Cancer Biol *19*: 211-217, 2009.

10 Ramqvist T and Dalianis T: Murine polyomavirus tumour specific transplantation antigens and viral persistence in relation to the immune response, and tumour development. Semin Cancer Biol *19*: 236-243, 2009.

11 Griffin BE, Soeda E, Barell BG *et al*: Sequence and analysis of polyoma DNA. *In*: Tooze J. DNA Tumour Viruses. Cold Spring Harbor, New York: Cold Spring Harbor Lab, pp. 843-910, 1980.

12 Ito Y: Organization and expression of the genome of polyoma virus. *In*: Klein G. Viral Oncology. New York: Raven Press, pp. 447-480, 1980.

13 Cole CN: Polyomavirinae: The viruses and their replication. *In*: Fields BN, Knipe DM and Howley PM. Fields Virology, 3rd Ed. Philadelphia, PA: Lippincott-Raven, pp. 1997-2026, 1996.

14 Cowie A and Kamen R: Multiple binding sites for polyomavirus large T antigen within regulatory sequences of polyomavirus DNA. J Virol *52*: 750-760, 1984.

15 Land H, Parada LF and Weinberg RA: Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature *304*: 596-602, 1983.

16 Qian W and Wiman KG: Polyoma virus middle T and small t antigens cooperate to antagonize p53-induced cell cycle arrest and apoptosis. Cell Growth Differ *11*: 31-39, 2000.

17 Dalianis T, Ramqvist T, Andreasson K *et al*: KI, WU and Merkel cell polyomaviruses: A new era for human polyomavirus research. Semin Cancer Biol *19*: 270-275, 2009.

18 Brady JN, Kendall JD and Consigli RA: *In vitro* reassembly of infectious polyoma virions. J Virol *32*: 640-647, 1979.

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

20 Allison AC and Law LW: Effects of antilymphocyte serum on virus oncogenesis. Proc Soc Exp Biol Med *127*: 207-212, 1968.

21 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.

22 Miller JF: Influence of thymectomy on tumor induction by polyoma virus in C57bl mice. Proc Soc Exp Biol Med *116*: 323-327, 1964.

23 Allison AC and Taylor RB: Observations on thymectomy and carcinogenesis. Cancer Res *27*: 703-707, 1967.

24 Vandeputte M, Denys P Jr, Leyten R *et al*: The oncogenic activity of the polyoma virus in thymectomized rats. Life Sci *7*: 475-478, 1963.

25 Allison AC: Interactions of antibodies, complement components and various cell types in immunity against viruses and pyogenic bacteria. Transplant Rev *19*: 3-55, 1974.

26 Swanson PA, 2nd, Lukacher AE and Szomolanyi-Tsuda E: Immunity to polyomavirus infection: the polyomavirus-mouse model. Semin Cancer Biol *19*: 244-251, 2009.

27 Sjogren HO, Hellstrom I and Klein G: Resistance of polyoma virus immunized mice against transplantation of established polyoma tumors. Exp Cell Res *23*: 204-208, 1961.

28 Habel K: Resistance of polyoma virus immune animals to transplanted polyoma tumors. Proc Soc Exp Biol Med *106*: 722-725, 1961.

29 Sjogren HO: Further studies on the induced resistance against isotransplantation of polyoma tumors. Virology *15*: 214-219, 1961.

30 Sjogren HO: Studies on specific transplantation resistance to polyoma virus-induced tumors. II. Mechanism of reistance induced by polyoma virus infection. J Natl Cancer Inst *33*: 375-394, 1964.

31 Habel K: Polyoma tumor antigen in cells transformed *in vitro* by polyoma virus. Virology *18*: 553-558, 1962.

32 Habel K: Virus tumor antigens: specific fingerprints? Cancer Res *26*: 2018-2024, 1966.

33 Sjögren H: Studies on specific transplantation resistance to polyoma virus-induced tumors. I. Transplantation resistance induced by polyoma virus infection. J Nat Cancer Inst *32*: 361-374, 1964.

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

35 Ramqvist T and Dalianis T: Polyoma virus-induced tumor-specific transplantation antigen (TSTA) is a mouse and rat cross-species-reacting antigen. Eur J Cancer Clin Oncol *20*: 1557-1560, 1984.

36 Treisman R, Novak U, Favaloro J *et al*: Transformation of rat cells by an altered polyoma virus genome expressing only the middle-T protein. Nature *292*: 595-600, 1981.

37 Lania L, Griffiths M, Cooke B *et al*: Untransformed rat cells containing free and integrated DNA of a polyoma nontransforming (Hr-t) mutant. Cell *18*: 793-802, 1979.

38 Dalianis T, Ramqvist T and Klein G: Studies on the polyoma-virus-induced tumor-specific transplantation antigen (TSTA) – does middle or large T-antigen play a role? Int J Cancer *34*: 403-406, 1984.

39 Lathe R, Kieny MP, Gerlinger P *et al*: Tumour prevention and rejection with recombinant vaccinia. Nature *326*: 878-880, 1987.

40 Ramqvist T, Pallas DO, DeAnda J *et al*: Immunization against the polyoma tumor-specific transplantation antigen (TSTA) with polyoma T-antigens. Int J Cancer *42*: 123-128, 1988.

41 Ramqvist T, Reinholdsson G, Szigeti R *et al*: Studies on the polyoma tumor-specific transplantation antigen (TSTA): selection and characterization of TSTA-negative segregants from somatic hybrids. Int J Cancer *40*: 74-80, 1987.

42 Reinholdsson G, Ramqvist T, Brandberg J *et al*: A polyomavirus tumor-specific transplantation antigen (TSTA) epitope is situated within the *N*-terminal amino acid sequence common to middle and small T-antigens. Virology *166*: 616-619, 1988.

43 Ramqvist T, Reinholdsson G, Carlquist M *et al*: 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.

44 Reinholdsson-Ljunggren G and Dalianis T: Modifications of an immunodominant peptide antigen induce different anti-polyoma tumor responses in two separate mouse strains. Int J Cancer *51*: 968-972, 1992.

45 Reinholdsson-Ljunggren G, Franksson L, Dalianis T *et al*: Identification of H-2Kb-, Db- and Dd-binding peptides derived from amino acid sequences of polyoma virus T antigens. Int J Cancer *54*: 992-995, 1993.

46 Reinholdsson-Ljunggren G, Ramqvist T, Ahrlund-Richter L *et al*: Immunization against polyoma tumors with synthetic peptides derived from the sequences of middle- and large-T antigens. Int J Cancer *50*: 142-146, 1992.

47 Berke Z, Palmer S, Bergman T *et al*: 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.

48 Chang C, Martin RG, Livingston DM *et al*: Relationship between T-antigen and tumor-specific transplantation antigen in simian virus 40-transformed cells. J Virol *29*: 69-75, 1979.

49 Flyer DC, Pretell J, Campbell AE *et al*: Biology of simian virus 40 (SV40) transplantation antigen (TrAg). X. Tumorigenic potential of mouse cells transformed by SV40 in high responder C57BL/6 mice and correlation with the persistence of SV40 TrAg, early proteins and sequences. Virology *131*: 207-220, 1983.

50 Campbell AE, Foley FL and Tevethia SS: Demonstration of multiple antigenic sites of the SV40 transplantation rejection antigen by using cytotoxic T lymphocyte clones. J Immunol *130*: 490-492, 1983.

51 Benjamin TL: Host range mutants of polyoma virus. Proc Natl Acad Sci USA *67*: 394-399, 1970.

52 Stoker M and Dulbecco R: Abortive transformation by the Tsa mutant of polyoma virus. Nature *223*: 397-398, 1969.

53 Smolar N and Griffin BE: DNA sequences of polyoma virus early deletion mutants. J Virol *38*: 958-967, 1981.

54 Carmichael GG, Schaffhausen BS, Dorsky DI *et al*: Carboxy terminus of polyoma middle-sized tumor antigen is required for attachment to membranes, associated protein kinase activities, and cell transformation. Proc Natl Acad Sci USA *79*: 3579-3583, 1982.

55 Freund R, Dawe CJ and Benjamin TL: The middle T proteins of high and low tumor strains of polyomavirus function equivalently in tumor induction. Virology *167*: 657-659, 1988.

56 Dawe CJ, Freund R, Mandel G *et al*: Variations in polyoma virus genotype in relation to tumor induction in mice. Characterization of wild type strains with widely differing tumor profiles. Am J Pathol *127*: 243-261, 1987.

57 Freund R, Mandel G, Carmichael GG *et al*: Polyomavirus tumor induction in mice: influences of viral coding and noncoding sequences on tumor profiles. J Virol *61*: 2232-2239, 1987.

58 Lukacher AE, Freund R, Carroll JP *et al*: Pyvs: a dominantly acting gene in C3H/BiDa mice conferring susceptibility to tumor induction by polyoma virus. Virology *196*: 241-248, 1993.

59 Berke Z, Wen T, Klein G *et al*: Polyoma tumor development in neonatally polyoma-virus-infected $CD4^{-/-}$ and $CD8^{-/-}$ single knockout and $CD4^{-/-}8^{-/-}$ double knockout mice. Int J Cancer *67*: 405-408, 1996.

60 Heidari S, Berke Z, Berglof A *et al*: Susceptibility to polyoma virus tumorigenesis in X-linked immunodeficient (XID) and B-cell-deficient (microMT) mice is not increased. In Vivo *13*: 439-444, 1999.

61 Wirth JJ, Martin LG and Fluck MM: Oncogenesis of mammary glands, skin, and bones by polyomavirus correlates with viral persistence and prolonged genome replication potential. J Virol *71*: 1072-1078, 1997.

62 Wirth JJ, Amalfitano A, Gross R *et al*: Organ- and age-specific replication of polyomavirus in mice. J Virol *66*: 3278-3286, 1992.
63 Dubensky TW and Villarreal LP: The primary site of replication alters the eventual site of persistent infection by polyomavirus in mice. J Virol *50*: 541-546, 1984.
64 Dubensky TW, Freund R, Dawe CJ *et al*: Polyomavirus replication in mice: influences of VP1 type and route of inoculation. J Virol *65*: 342-349, 1991.
65 Major EO, Amemiya K, Tornatore CS *et al*: Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev *5*: 49-73, 1992.
66 Khalili K, White MK, Lublin F *et al*: Reactivation of JC virus and development of PML in patients with multiple sclerosis. Neurology *68*: 985-990, 2007.
67 Dubensky TW, Murphy FA and Villarreal LP: Detection of DNA and RNA virus genomes in organ systems of whole mice: patterns of mouse organ infection by polyomavirus. J Virol *50*: 779-783, 1984.
68 Berke Z and Dalianis T: Persistence of polyomavirus in mice infected as adults differs from that observed in mice infected as newborns. J Virol *67*: 4369-4371, 1993.
69 Berke Z, Wen T, Jin S *et al*: Polyomavirus persists in CD4/8 double-knockout, but not in CD4 or CD8 single-knockout mice. Virology *212*: 268-271, 1995.
70 Scher I: The CBA/N mouse strain: an experimental model illustrating the influence of the X-chromosome on immunity. Adv Immunol *33*: 1-71, 1982.
71 Kitamura D, Roes J, Kuhn R *et al*: A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. Nature *350*: 423-426, 1991.
72 Berke Z, Mellin H, Heidari S *et al*: Adult X-linked immunodeficiency (XID) mice, $IGM^{-/-}$ single knockout and $IGM^{-/-}$ $CD8^{-/-}$ double knockout mice do not clear polyomavirus infection. In Vivo *12*: 143-148, 1998.
73 Berke Z, Dalianis T, Feinstein R *et al*: Persistence of polyomavirus in adult SCID C.B-17 mice. In Vivo *8*: 339-342, 1994.
74 Demengeot J, Jacquemier J, Torrente M *et al*: Pattern of polyomavirus replication from infection until tumor formation in the organs of athymic nu/nu mice. J Virol *64*: 5633-5639, 1990.
75 Berke Z, Mellin H, Heidari S *et al*: Adult X-linked immunodeficiency (XID) mice, $IGM^{-/-}$ single knockout and $IGM^{-/-}$ $CD8^{-/-}$ double knockout mice do not clear polyomavirus infection. In Vivo *12*: 143-148, 1998.
76 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.
77 Montross L, Watkins S, Moreland RB *et al*: Nuclear assembly of polyomavirus capsids in insect cells expressing the major capsid protein VP1. J Virol *65*: 4991-4998, 1991.
78 Palkova Z, Adamec T, Liebl D *et al*: Production of polyomavirus structural protein VP1 in yeast cells and its interaction with cell structures. FEBS Lett *478*: 281-289, 2000.
79 Ramqvist T, Andreasson K and Dalianis T: Vaccination, immune and gene therapy based on virus-like particles against viral infections and cancer. Expert Opin Biol Ther *7*: 997-1007, 2007.
80 Vlastos A, Andreasson K, Tegerstedt K *et al*: 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.
81 Heidari S, Vlastos A, Ramqvist T *et al*: Immunization of T-cell-deficient mice against polyomavirus infection using viral pseudocapsids or temperature sensitive mutants. Vaccine *20*: 1571-1578, 2002.
82 Bachmann MF, Rohrer UH, Kundig TM *et al*: The influence of antigen organization on B-cell responsiveness. Science *262*: 1448-1451, 1993.

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