The ability of murine polyomavirus (MPyV)-VP1 virus-like particles (MPyV-VLPs) to immunize against MPyV tumour outgrowth was investigated. Non-immunized and mice immunized three times were challenged with MPyV or non-MPyV tumours and followed for tumour outgrowth. MPyV-VLP immunization abrogated outgrowth of some, but not all, tested MPyV tumours and delayed the outgrowth of a non-MPyV tumour to some extent. However, when mice were irradiated prior to tumour challenge to avoid an unspecific immune response, protection was MPyV-specific. In conclusion, VLP immunization for prevention of viral infection could also contribute to immune-protection against some tumours induced by the corresponding virus.

Murine polyomavirus (MPyV), a small DNA virus, encodes the regulatory proteins, large-T (LT), middle-T (MT) and small-T (ST) antigen and the capsid proteins VP1-3 (1). In newborn mice with an immature immune system, and in T-cell-deficient mice, MPyV causes persistent infection and tumours (2, 3). Specific antibodies prevent MPyV infection, while T-cell immunity is essential for prevention of tumour development and outgrowth (2-4). The target recognised by the cellular immune system, defined as the MPyV tumour specific transplantation antigen (TSTA) (5), has been shown to be associated to MPyV T-antigens (5,6). More specifically, short peptides, derived from T-antigens, were shown to be recognised in the context of determinants of the Major Histocompatibility Complex (5-7). Viral capsid proteins, unnecessary for transformation and regarded to be produced only during lytic infection, were not assumed to function as TSTAs (1,5). Moreover, LT, essential for late transcription, is frequently truncated in MPyV tumours, consequently abrogating late transcription and subsequently the production of capsid proteins (1). Nevertheless, some MPyV hair follicular tumours express VP1 (Benjamin, personal communication), suggesting that peptides derived from VP1 could potentially act as TSTAs. Furthermore, recently transcription of all MPyV proteins was shown early after MPyV infection, thus permitting VP1 production in non-lytically-infected cells (8). These data, and the fact that immunization with MPyV-VP1 virus-like particles (VLPs) protected against MPyV infection (4), prompted us to investigate if MPyV-VLPs could immunize against a potential VP1-TSTA and abrogate MPyV tumour outgrowth.

VLPs, of both MPyV and the related murine pneumotropic virus (MPtV) origin with 45% homology in their VP1 proteins (9), were produced (10) and used as immunogens. For comparison, MPyV (11) and a glutathione-S-transferase (GST) VP1 fusion protein (GST-VP1) (4) were also used as immunogens.

**Materials and Methods**

**Production and purification of VLPs and GST-VP1.** Baculovirus expressing MPyV-VLPs and MPtV-VLPs based on the VP1 protein were produced as described previously (10). The GST-VP1 fusion protein was expressed in *E. coli* BL21, as described previously (4).

**Tumour cells and estimation of viral presence in the tumour cells.** Three MPyV tumours, SECA, SEBB and SEBA, were used to test for MPyV-specific immunity, since they are rejected upon MPyV immunization (11). SECA is an ACA hair follicle tumour, SEBA a CBA sarcoma and SEBB a CBA fibrosarcoma, and all three tumours produce ST, MT and LT (11). As a non-MPyV control, the SC6 mammary tumour was used (12). Viral load was estimated by Taqman real-time quantitative PCR (Q-PCR) (13), the presence of MPyV-VP1 by Western blots and viral production by Southern blotting (6).
Mice and immunization procedures. Normal adult ACA and CBA mice bred at the animal facility of the Microbiology and Tumour Biology Centre, Karolinska Institute, Sweden, were used for these experiments. Groups of ACA or CBA mice were immunized weekly three consecutive times, each time both 10 µg subcutaneously (s.c.) and 10 µg intraperitoneally (i.p.) with either VLPs or GST-VP1, or with MPyV (11), or with irradiated (10,000 rads) non-MPyV SC6 tumour cells (12).

Tumour rejection tests. Rejection tests were performed one week after the last immunization in non-irradiated or irradiated (400 rad) mice, since irradiation has previously been shown to abolish non-specific boosting of natural resistance after MPyV inoculation (11). Mice were followed for tumour takes, which were estimated as mean tumour load (MTL) (7).

Results

Immunization with MPyV-VLPs and MPt-VLPs do not prevent outgrowth of the non-MPyV tumour SC6. Immunization of ACA mice with MPyV-VLPs, MPt-VLPs, and GST-VP1 did not confer specific immunity to the non-MPyV tumour SC6 (Table I). Although delayed outgrowth of SC6 was observed in non-irradiated mice after MPyV-VLP and MPt-VLP immunization, this unspecific response was abolished completely in irradiated mice (Table I). As expected, SC6 grew in controls, but not in most SC6-immunized mice, irrespective of whether mice received irradiation or not before challenge (Table I).

Presence of VP1, viral load and viral production in the different tumour cell lines. MPyV tumours were compared with regard to VP1, viral load and viral production. Western blot showed a band equal in size to MPyV-VP1 in SEBA and in SEBB, but not in SECA or S6C (Figure 1C). Estimated by Q-PCR, SEBA had 120, SEBB 40 and SECA 0.5 copy numbers of MPyV DNA/cell, whereas S6C

Table I. 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, MPt-VLPs, GST-VP1, MPyV, or irradiated SC6 cells, as well as in non-immunized control mice.

| Tumour line | Exp no | Cell doses inoculated | Mouse strain | Whole body irradiation (400 rad) prior to tumour inoculation | Controls | MPyV-VLPs | MPt-VLPs | GST-VP1 | S6C | MPyV
|-------------|--------|-----------------------|--------------|---------------------------------------------------------------|----------|-----------|----------|----------|-----|--------
| SECAa       | 1      | 1 x 10⁴                | No           | 4/4                                                           | 0/4      | -         | -        | -        | -   | 0/4e   |
|             | 2      | 1 x 10³                | ACA          | 4/5                                                           | 0/5      | 0/5       | 0/5      | 1/5      | -   | -      |
|             | 3      | 1 x 10³                | Yes          | 5/5                                                           | 0/6      | -         | -        | -        | -   | -      |
| S6C³        | 1      | 1 x 10³                | No           | 5/5                                                           | 3/5 f    | 5/5 f     | -        | 0/5      | -   | -      |
|             | 2      | 1 x 10³                | ACA          | 5/5                                                           | 4/4      | 4/4       | -        | 2/4      | -   | -      |
|             | 3      | 1 x 10³                | Yes          | 4/4                                                           | -        | 4/4       | -        | 0/4      | -   | -      |
| SEBBb       | 1      | 1 x 10⁴                | CBA          | 3/5                                                           | 4/5 f    | -         | -        | -        | -   | -      |
| SEBB-TCc    | 2      | 1 x 10⁴                | Yes          | 6/6                                                           | 5/6 f    | -         | -        | -        | -   | -      |
| SEBA-TCc    | 1      | 5 x 10⁴                | CBA          | 4/4                                                           | 4/4      | -         | -        | -        | -   | -      |

a SECA is of ACA origin.
b SEBB and SEBA are of CBA origin.
c TC denotes short-term tissue cultured cells.
d (-) Indicates not done.
e 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 (7).
was negative (Figure 1D). Southern blotting on tumour DNA demonstrated high amounts of episomal MPyV DNA, indicative of viral production, only in SEBA (data not shown).

**Discussion**

The potential of VP1 to induce immunity against MPyV tumour outgrowth has now been demonstrated for the first time, since MPyV-VLPs, MPtV-VLPs and GST-VP1 all elicited MPyV tumour-specific immunity. This finding is in concordance with the findings of De Bruijn et al. (14), where the late protein L1 (presented as VLPs) of human papillomavirus (HPV) type 16 elicited an immune response too, and completely protected against outgrowth of an E6 and L1 transfected murine cell line. A cellular immune response against VP1-derived peptides on MHC molecules of MPyV tumours is most probably responsible for inhibition of tumour outgrowth, since both MPyV-VLPs and MPtV-VLPs induced protection, but are not sero-cross-reactive and do not prevent reciprocal viral infection (10). This hypothesis is supported in that MPyV and MPtV-VP1 homology is highest (up to 80%) within the internal domain of the capsids (9). The responsiveness of the tumours did, however, not correlate with overall expression of VP1, viral load or productive infection. The non-responsive of SEBA, in spite of VP1 expression and a high viral load, could be due to a minority of virus-producing cells in SEBA. In contrast, SECA and SEBB, the two better responders may, in spite of low VP1 expression and viral load, have a majority of tumour cells exposing VP1 peptides on their surface. Finally, the unspecific immunization of MPyV-VLPs and MPtV-VLPs obtained against S6C in non-irradiated mice was not unexpected and has been shown previously for MPyV (11).

In conclusion, VLP immunization, useful for prevention of viral infection, could not only be beneficial to inhibit viral infection itself, but may also contribute to protection against some tumours induced by the corresponding virus.
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