Effect of virulence on immunogenicity of single and double vaccinia virus recombinants expressing differently immunogenic antigens: antibody-response inhibition induced by immunization with a mixture of recombinants differing in virulence

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It has been shown recently that the residual virulence of vaccinia virus (VV) is an important factor that influences the outcome of immunization with VV recombinants. This study focused on the correlation of the residual virulence of several VV recombinants with antibody responses against the strongly immunogenic extrinsic glycoprotein E of varicella-zoster virus and the weakly immunogenic extrinsic protein preS2–S of hepatitis B virus and against VV proteins, with mice used as a model organism. Furthermore, the effects of mixing different recombinants on the antibody response were studied. The results obtained indicated that: (i) the antibody response depended on the residual virulence of the recombinants, more so in the case of the weakly immunogenic protein; (ii) the residual virulence, the growth rate of the VV recombinants in extraneural tissues and the immunogenicity were associated features; (iii) immunization with mixtures of two differently virulent recombinants or with unequal amounts of two similarly virulent recombinants sometimes led to the suppression of antibody response. The appearance of this suppression was dependent on three factors: the residual virulence of the recombinants, the immunogenicity of the extrinsic proteins and the ratio of the recombinants in the mixtures. Thus, the data obtained demonstrate that there are various limitations to the use of replicating VV recombinants for immunization purposes.

Introduction
Numerous applications have demonstrated the utility of vaccinia virus (VV) as a eukaryotic expression vector. One of the most attractive is the use of genetically engineered VV as a live vaccine (for reviews see Mahr & Payne, 1992; Perkus et al., 1995; Moss, 1996). The large genome capacity of VV makes it possible to delivery multiple extrinsic proteins in the vaccine formula (e.g. Perkus et al., 1985; Wild et al., 1992; Fonseca et al., 1994; Tine et al., 1996).

Nevertheless, there are several questions associated with the preparation of safe and sufficiently immunogenic VV recombinants that will have to be answered first. It has been reported that the immunogenicity of recombinants based on efficiently replicating VV is dependent on the virulence of the virus used for the recombinant preparation (Morgan et al., 1988; Lee et al., 1992; Grigorieva et al., 1993; Kutinová et al., 1996). However, some proteins can apparently be sufficiently immunogenic even when presented by highly attenuated (e.g. Rodriguez et al., 1989; Giavedoni et al., 1991; Kutinová et al., 1996), inefficiently replicating or even non-replicating VV recombinants (Tartaglia et al., 1992; Taylor et al., 1992; Perkus et al., 1995).

The thymidine kinase (TK) gene of VV has been one of the major target genes used for construction of VV recombinants (for a review see Buller & Palumbo, 1992). The inactivation of the TK gene has usually resulted in a significant decrease of virulence of previously highly virulent VV (Buller et al., 1985). This effect has been much less marked when more attenuated...
VV is used for recombination (Buller & Palumbo, 1992; Kutinová et al., 1996). Similarly, the haemagglutinin (HA) gene has frequently been exploited as the insertion site for foreign genes (Buller & Palumbo, 1992). The inactivation of this gene can also affect the pathogenicity of some VV strains (Shida et al., 1988). Thus, multiple insertions of foreign genes in different loci of the VV genome could lower the immunogenicity of recombinants by excessive lowering of virulence. Consequently, the use of mixtures of single recombinants for immunization should be considered.

In this study, we describe the effect of a second foreign gene insertion on the antibody response to the weakly immunogenic preS2–S envelope proteins of hepatitis B virus (HBV) or the strongly immunogenic glycoprotein E (gE) of varicella-zoster virus (VZV), and the relationship between the antibody response and the change in virulence. Furthermore, we investigated how different doses of single recombinants can modify the antibody responses to certain antigens if administered in a mixture.

**Methods**

**Plasmid vectors.** The construction of recombination plasmids pM3 and pGS20-VZV gpl, which were used for insertion of the preS2–S gene of HBV or the gE (previously designated gpl) gene of VZV into the TK gene of VV, has been described previously (respectively by Němecfková et al., 1991; Ludvíková et al., 1991). Plasmids for insertion of foreign genes into the HA gene were constructed by using pVV-HA. This plasmid was prepared by cleaving the G fragment of a S11 genomic library of VV strain Praha with restriction enzymes HindIII and Sall and then cloning this 1796 bp fragment into pUC18. To prepare a plasmid for the insertion of VZV gE, plasmid pGS20-VZV gpl (Ludvíková et al., 1991) was digested with PstI and the fragment containing the VZV gE gene and the VV 7.5 promoter was ligated into pUC18. Consequently, the plasmid was cleaved with Smal and the 2.4 kb fragment was inserted into the NruI site of pVV-HA. The resulting plasmid was designated pGS20-VZV-HA.

A plasmid for the insertion of the preS2 and S regions of the HBV genome into the HA gene was constructed with the pM3 plasmid described previously (Němecfková et al., 1991). A 1.7 kb partial fragment was excised with EcoRI from plasmid pM3, blunt-ended with Klenow enzyme and then ligated into pVV-HA that had been cleaved with NruI. The resulting plasmid was denoted pM3-HA. Plasmid pkl for the construction of a VV recombinant expressing the VZV gL (gene 60) was prepared as described previously (Němecfková et al., 1996). In all recombinant plasmids, the HBV or VZV genes (except gl) were under the control of the 7.5 kD promoter. The gl gene was transcribed from the late 11K promoter.

**Preparation of recombinants.** Cloned VV lines P20 and P13, generated from smallpox Sevac VARIE vaccine (strain Praha) (Kutinová et al., 1995), were used as parental viruses for the preparation of recombinants. P20 was a highly attenuated virus (LD<sub>50</sub> > 10<sup>5</sup> p.f.u. in intracerebrally (i.c.) inoculated 3-week-old mice) and P13 was a less attenuated virus (LD<sub>50</sub> invariably ≤ 10<sup>5</sup> p.f.u. in repeated experiments in 3-week-old mice). The recombinants were constructed by the method of Perkus et al. (1986). TK– viruses were selected and plaque-purified as in previous experiments (Kutinová et al., 1990). Recombinant viruses with the foreign genes inserted into the HA gene were selected by staining with chicken erythrocytes (Shida & Matsumoto, 1983). Viruses from white plaques produced in CV-1 cells were plaque-purified three times and the production of the foreign antigen was confirmed before use. The double recombinants were obtained by insertion of a second foreign gene into the TK gene of recombinants containing either the preS2–S gene of HBV or the gE gene of VZV in the VV HA gene. A list of the recombinants used in this study is shown in Table 1. Depending on the parental virus, the foreign gene inserted and the insertion site,

### Table 1. Recombinants used and some of their properties

S and gE antigens were quantified by ELISA as described previously (Kutinová et al., 1994; Ludvíková et al., 1991); the absorbances for gE antigen from viruses 13 or 20 were subtracted as background from the absorbances of their recombinants.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Growth in CV-1 cells (log&lt;sub&gt;10&lt;/sub&gt; p.f.u. per 10&lt;sup&gt;6&lt;/sup&gt; cells)*</th>
<th>Production of S antigen (ng per 10&lt;sup&gt;6&lt;/sup&gt; cells)</th>
<th>Production of gE antigen (A&lt;sub&gt;492&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>6.5</td>
<td>&lt; 20</td>
<td>–</td>
</tr>
<tr>
<td>13Eh</td>
<td>6.2</td>
<td>&lt; 20</td>
<td>0.72</td>
</tr>
<tr>
<td>13Eh–St</td>
<td>6.2</td>
<td>719</td>
<td>0.48</td>
</tr>
<tr>
<td>13Sh</td>
<td>6.4</td>
<td>582</td>
<td>0.05</td>
</tr>
<tr>
<td>13Sh–Et</td>
<td>5.9</td>
<td>480</td>
<td>0.03</td>
</tr>
<tr>
<td>13E</td>
<td>6.0</td>
<td>522</td>
<td>0.79</td>
</tr>
<tr>
<td>13Ltt</td>
<td>6.3</td>
<td>&lt; 20</td>
<td>1.06</td>
</tr>
<tr>
<td>20</td>
<td>6.2</td>
<td>&lt; 20</td>
<td>–</td>
</tr>
<tr>
<td>20Sh</td>
<td>6.5</td>
<td>844</td>
<td>0.01</td>
</tr>
<tr>
<td>20Sh–Et</td>
<td>6.1</td>
<td>909</td>
<td>1.14</td>
</tr>
<tr>
<td>20Et</td>
<td>5.9</td>
<td>&lt; 20</td>
<td>0.79</td>
</tr>
<tr>
<td>20Eh</td>
<td>6.2</td>
<td>&lt; 20</td>
<td>0.76</td>
</tr>
<tr>
<td>20St</td>
<td>6.2</td>
<td>564</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Virus and antigen titres 48 h after infection at an m.o.i. of 0.1 p.f.u. per cell at 36 °C; virus titres at time 0 were 1–9–2.8 log<sub>10</sub> p.f.u. per 10<sup>6</sup> cells.

† Synthesis of gl was confirmed by radioimmunoprecipitation assay (Němecfková et al., 1996).
recombinants were denoted ‘13’ (VV line P13) or ‘20’ (VV line P20); ‘S’ (preS2–S gene of HBV), ‘E’ (gE gene of VZV) or ‘L’ (gL gene of VZV); and ‘t’ (TK gene of VV) or ‘h’ (HA gene of VV).

Virulence tests. Residual virulence of the viruses was tested by i.c. inoculation into either 3-week-old (P13 virus and its derivatives) or 3-day-old (P20 virus and its derivatives) non-inbred ICR mice. Animals of two different ages were used because the test in the 3-week-old mice was not sensitive enough to detect the residual virulence of P20 virus and, on the other hand, the test in 3-day-old mice was too sensitive to differentiate between the derivatives of the less-attenuated P13 virus. The 3-week-old mice (seven per group) were inoculated i.c. with 0·05 ml of different virus dilutions. In each experiment, the parental P13 virus was tested in parallel in order to monitor any differences in mouse sensitivity. The 3-day-old mice were inoculated i.c. with 0·01 ml of different virus dilutions, with ten mice being used per dilution. In both tests, the animals were observed for 21 days. The LD50 was determined by the method of Reed & Muench (1938).

Immunization of mice. The viruses used for immunization were grown on chorioallantoic membranes of 11-1-day-old chicken embryos and partially purified according to Joklik (1962). Four-week-old outbred female mice, strain ICR, were injected intraperitoneally (i.p.) with different doses of virus (0·5 ml virus suspension per mouse). Ten mice were immunized in each group. Five weeks later, the mice were anaesthetized with halothane (Narcotan; Leuka, Prague) and bled and their sera were tested individually by ELISA for the presence of anti-VV, anti-S, anti-pre-S2 or anti-VZV antibodies, as described previously (Hamšíková et al., 1990; Ludvíková et al., 1991). Sera were diluted 1:10 and 8–16 antigen units were used (1 antigen unit was defined as the smallest amount of antigen still giving a positive reaction in the presence of excess antibody). Under these conditions, the absorbance correlated well with the antibody titre as determined by end-point titration (not shown). The significance of differences between the groups of mice was tested by the unpaired t-test at 95% confidence intervals.

Detection of the virus in mouse organs. Four-week-old mice were injected i.p. with 0·5 ml of the recombinant virus 13St or 13Eh (106 p.f.u. per mouse). Two mice from each group were anaesthetized and killed daily on days 1–7 after inoculation. Their spleens, kidneys, lungs and livers were removed and then washed repeatedly with PBS and frozen in PBS (1 ml per organ). The same organs from each pair of mice were combined and extracts were prepared by vigorous grinding with pulverized glass followed by centrifugation for 2 min at 12,000 r.p.m. in an Eppendorf centrifuge. The supernatants were tested for the presence of the virus by plaque assay in CV-1 cells.

Results

Virus growth and expression of S and gE antigens

In order to examine the in vitro properties of the recombinants used in the study, the viruses were assayed for growth and production of S or gE antigen in CV-1 cells (Table 1). There were no marked differences in growth rate in CV-1 cells between the different recombinants. The amounts of S or gE antigen produced were also similar and no dependence on parental virus or foreign gene-insertion site was apparent.

Virulence of recombinants in mice

The residual virulence of parental viruses and their recombinants is shown in Table 2. Insertion of the gE gene into the HA gene of VV did not reduce the virulence of the parental P13 virus, as displayed by virus activity in mice. This recombinant, 13Eh, was used as the parental virus for the 13Eh–St double recombinant, where the preS2–S gene was inserted into the 13Eh TK gene. This second step resulted in a reduction of virulence. The virulence of the 13Eh–St double recombinant was similar to that of the single recombinant 13St (Table 2, expt no. 1). Surprisingly, the 13Sh recombinant was markedly more attenuated than the 13Eh recombinant. The insertion of the gE gene in the TK locus of the 13Sh recombinant resulted in a further decrease of virulence (recombinant 13Sh–Et) (Table 2, expt no. 2). Similarly, the insertion of either the gE or gL gene into TK of P13 virus produced markedly attenuated viruses (Table 2, expt no. 3). Differences between the recombinants derived from the highly attenuated VV clone P20 were relatively low and did not seem to be dependent on the foreign gene inserted or on the insertion site within VV (Table 2, expt no. 4).

Table 2. Virulence of VV recombinants in mice

Three-week-old mice were inoculated in experiments 1–3; 3-day-old mice were inoculated in experiment 4.

<table>
<thead>
<tr>
<th>Virus</th>
<th>LD50 (log10 p.f.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1·4</td>
</tr>
<tr>
<td>13Eh</td>
<td>0·9</td>
</tr>
<tr>
<td>13St</td>
<td>3·5</td>
</tr>
<tr>
<td>13Eh–St</td>
<td>3·4</td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2·1</td>
</tr>
<tr>
<td>13Sh</td>
<td>5·5</td>
</tr>
<tr>
<td>13Et</td>
<td>4·5</td>
</tr>
<tr>
<td>13Sh–Et</td>
<td>&gt; 6·5</td>
</tr>
<tr>
<td>Expt 3</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2·8</td>
</tr>
<tr>
<td>13Et</td>
<td>4·9</td>
</tr>
<tr>
<td>13Lt</td>
<td>5·4</td>
</tr>
<tr>
<td>Expt 4</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>3·9</td>
</tr>
<tr>
<td>20Sh</td>
<td>4·9</td>
</tr>
<tr>
<td>20St</td>
<td>5·3</td>
</tr>
<tr>
<td>20Et</td>
<td>5·7</td>
</tr>
<tr>
<td>20Sh–Et</td>
<td>5·4</td>
</tr>
</tbody>
</table>

Antibody responses to recombinant viruses

The ability of the recombinant viruses with differing virulence to induce an antibody response in mice is shown in Fig. 1. The comparison of the double recombinant 13Eh–St with single recombinants 13St and 13Eh (Fig. 1a) revealed similar levels of antibodies against both extrinsic proteins, the weakly immunogenic preS2–S and the strongly immunogenic gE. When mice were immunized...
with a mixture of the single recombinants, titres of antibodies against the S protein were significantly lower (\(P < 0.05\)) than after immunization with the 13Et recombinant alone. The difference in anti-preS2 antibodies was not significant (\(P = 0.115\)). The level of antibodies against gE remained unchanged.

The results of immunization with the less-virulent single and double P13 recombinants are shown in Fig. 1(b). The production of anti-S, anti-preS2 and anti-VV antibody was markedly less efficient after immunization with the highly attenuated double recombinant 13Sh–Et than with the parental single recombinant 13Sh (all differences were significant; \(P < 0.01\)). On the other hand, the level of anti-gE antibodies was similar to that in the mice immunized with recombinant 13Et. In contrast to the result shown in Fig. 1(a), a mixture of the single recombinants (13Sh + 13Et) induced antibody levels comparable to those seen with the single recombinants (13Et or 13Sh) inoculated alone.

The antibody response after administration of recombinants based on the low-virulence virus line P20 was limited to anti-VV and anti-gE antibodies (Fig. 1c). Similar to the results outlined above, the double recombinant 20Sh–Et and the single recombinant 20Et induced comparable levels of anti-gE antibody, but these levels seemed to be lower than in mice inoculated with the P13-derived viruses. Surprisingly, when single recombinants 20Sh and 20Et were inoculated as a mixture, the production of anti-gE was somewhat lower (the difference was not significant; \(P = 0.085\)) than after immunization with the 20Et single recombinant alone. The antibody response to VV was very low in all instances. The experiment shown in Fig. 1 was repeated, with almost identical results.

The results shown in Fig. 1(a) suggested that the presence of a less-attenuated recombinant in a mixture with a more-attenuated recombinant suppressed the immunogenicity of the latter virus. In order to examine this phenomenon further, we immunized mice with four mixtures of these viruses, in which the ratio of the two recombinants varied. Each mixture contained the same amount of the less-attenuated recombinant, 13Eh, and a different amount of the more-attenuated recombinant, 13Et. Simultaneously, both recombinants were inoculated separately at the same concentrations as used in the mixtures. The levels of antibodies against the VV, gE, S and preS2 antigens are presented in Fig. 2(a–d). It can be seen that in mice inoculated with \(1 \times 10^6\) p.f.u. of single recombinant, the anti-VV antibody response was significantly higher (\(P < 0.05\)) after the administration of the more-virulent 13Eh virus than after the administration of the less-virulent 13Et virus. When the dose of the 13Et recombinant was increased, whether alone or in a mixture, the anti-VV antibody response tended to be higher. The levels of anti-gE antibodies were similar in all groups, thus indicating that the different doses of
Fig. 2. Antibody responses in mice immunized with differently virulent VV recombinants. Mice were immunized with four mixtures of recombinants containing $5 \times 10^5$ p.f.u. of the relatively virulent recombinant 13Eh (Eh) and increasing amounts of the less virulent recombinant 13St (St); numerals 1–8 adjoined to virus designations indicate multiples of the lowest virus dose (St1, $5 \times 10^5$; St2, $1 \times 10^6$; St4, $2 \times 10^6$; St8, $4 \times 10^6$ p.f.u.). Simultaneously, both recombinants were inoculated separately, in the same concentrations as used in the mixtures. Antibodies against VV (a), gE (b), S (c) and preS2 (d) were detected by ELISA. Bars indicate 95% confidence intervals. High SD in case of antibodies against the weakly immunogenic S and preS2 antigens are usually seen in immunization of outbred mice.

Fig. 3. Antibody responses in mice immunized with mixtures of similarly virulent recombinants. Mixtures of recombinants contained $1 \times 10^6$ p.f.u. of recombinant 13Et (Et) and increasing doses of a similarly weakly virulent recombinant 13Lt (Lt) (Lt1, $1 \times 10^6$; Lt3, $3 \times 10^6$; Lt10, $1 \times 10^7$ p.f.u. per dose). In parallel, both recombinants were inoculated separately, 13Lt only at the highest concentration used in the mixtures. Anti-gE (hatched bars) and anti-VV (filled bars) antibodies were detected.

13St recombinant had no marked influence (differences between antibodies of the Eh1 and St8 + Eh1 groups were not significant; $P = 0.434$). In contrast, the production of both anti-S and anti-preS2 antibodies was sometimes inhibited when the 13Eh recombinant was present in the virus mixture. This inhibitory effect of 13Eh was significant (anti-S antibody $P < 0.01$; anti-preS2 antibody $P < 0.05$) in the group where $1 \times 10^6$ p.f.u. of 13St recombinant was used. No inhibition appeared after the use of higher doses of the 13St virus.

Fig. 3 presents the results of an experiment in which mice were immunized with the attenuated recombinant 13Et in mixtures with increasing doses of a recombinant of comparable virulence, 13Lt. An equal amount of 13Lt virus in the mixture did not change the anti-gE antibody response in comparison with the levels produced by the 13Et recombinant administered alone. When threefold or tenfold greater amounts of recombinant 13Lt were used, a significant decline (threefold, $P < 0.01$; tenfold, $P < 0.0001$) in anti-gE antibody formation was apparent. The rate of suppression was dependent on the concentration of the 13Lt recombinant in the mixture.

To test whether the close association between the degree of residual neurovirulence and the immunogenicity of the different recombinants was due to less efficient growth of the more-attenuated viruses in extraneural tissues, mice were inoculated i.p. with $10^6$ p.f.u. of the relatively highly virulent
recombinant 13Eh or the less-virulent recombinant 13St. The virus titre in various organs was then followed for the next 8 days. As shown in Table 3, the growth rate of viruses in mouse organs correlated well with their residual virulence. While the more-virulent 13Eh virus was already detected on the first day in spleen and liver and on subsequent days also in kidneys and lungs, the 13St virus was detected later, at lower titres and only in spleen and liver. As might be expected, higher levels of anti-VV antibodies were detected in mice inoculated with the more-virulent virus, although the kinetics of antibody formation seemed to be comparable in both instances (Fig. 4).

**Discussion**

The results presented here and in previous papers (Kutinová et al., 1996, 1999) were obtained with recombinant VV of different neurovirulence expressing either highly or weakly immunogenic extrinsic proteins. The collection of recombinants at our disposal allowed the present, more systematic study on the effect of the virus virulence and foreign-protein immunogenicity on antibody responses to VV recombinants administered in different combinations.

In agreement with some earlier reports (Morgan et al., 1988; Lee et al., 1992; Grigorieva et al., 1993; Kutinová et al., 1996), the virulence of parental VV appeared to be the most important factor that influenced the result of immunization. The varying immunogenicity of recombinants that differed in residual virulence could be due to different rates of replication in vivo. Our data seem to indicate that there was a close link between neurovirulence and the capability of the virus to grow in extraneural tissues. Apparently, the site of foreign-gene insertion influenced the immunogenicity of the extrinsic protein only if the recombination was associated with a decrease in residual virulence. The effect of the decreased virulence was significantly more apparent in the case of a weakly immunogenic protein than a strongly immunogenic protein. This and similar observations made in the past represent a definite problem for any future attempts to introduce VV-based vaccines.

The mixed administration of equal amounts of two attenuated recombinants, one of them expressing a weakly immunogenic extrinsic protein and the other a strongly immunogenic extrinsic protein, did not change the antibody response to either of the proteins. However, the mixed administration of a more-virulent recombinant expressing the strongly immunogenic protein and an attenuated recombinant expressing the weakly immunogenic protein resulted in inefficient priming by the latter. This lack of priming was not observed if the proportion of the less-virulent recombinant in the mixture was increased. The restoration of the antibody response to the weakly immunogenic protein was then directly proportional to the concentration of the less-virulent virus expressing it.

The antibody response to the strongly immunogenic protein was not influenced in animals inoculated with the mixtures mentioned above, irrespective of the proportion of the recombinants. However, if the more-virulent recombinant
was replaced in the mixture by a less-virulent virus expressing the strongly immunogenic protein, the antibody response to this protein was also repressed if the amount of the other, less-virulent recombinant in the mixture was increased.

The nature and the mechanisms of this suppression could be related to phenomena observed in vitro in the past. For example, Moss et al. (1971) described the exclusion of a superinfecting rifampicin-resistant VV from cells infected with wild-type VV. We also observed a decrease in extrinsic protein synthesis in CV-1 cells infected with a VV recombinant inoculated simultaneously with another VV recombinant; this decrease was proportional to the increasing concentration of the latter recombinant (L. Kutinová & Š. Němečková, unpublished). It is difficult to imagine that a similar phenomenon could occur in vivo after introduction of a mixture of viruses containing only millions of p.f.u. into the peritoneal cavity. However, it has been shown in vivo that the number of infectious particles in VV stocks may be much higher than that determined by titrations in tissue culture (Kutinová et al., 1995, 1996). Moreover, Christen et al. (1990) have demonstrated that the expression of early genes of VV in infected cells is required for the exclusion of a superinfecting virus. One might therefore expect that defective virus particles, present in the virus suspensions and/or formed in the course of virus replication, that are capable of initiating an abortive virus growth cycle might also act as inducers of exclusion phenomena. In addition, interferon-α and possibly other cytokines that are produced by virus-infected cells could play an important role through their ability to decrease the susceptibility of still-uninfected cells to subsequent infection. Furthermore, a more-virulent and better-replicating virus will spread at a higher rate to lymphoid tissue and other sites in the animal body and this may handicap the other virus. Since superinfection of already-infected cells or cells resistant to infection will be inefficient and the immunity developing against VV will interfere with replication of the virus, this effect should be of a higher significance for the slower-growing partner virus. Deficient priming by this virus is then the consequence. If this reasoning is correct, it becomes understandable why an increase in the amount of the ‘weaker’ virus in the mixture can overcome this blockage or why an increase in the amount of a similarly virulent virus will suppress priming even by a highly immunogenic protein. However, the complexity of the mechanisms operating in the interference phenomena described is not fully understood and further investigations are needed.

In conclusion, the present data seem to suggest how some of the problems associated with the use of differently virulent and differently immunogenic recombinants and their mixtures for immunization could be overcome. At the same time, however, they also demonstrate the limitations of these approaches.

We should very much like to acknowledge the contribution of the late Dr V. Simonová to this study. The present work was supported by grants IGA 1936-3, IGA 4409-3 and IGA 4414-3 obtained from the Czech Ministry of Health.

References


Received 24 June 1999; Accepted 7 July 1999