Induction of neutralizing antibodies by varicella-zoster virus gpII glycoprotein expressed from recombinant vaccinia virus

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The gpII glycoprotein of varicella-zoster virus (VZV) was produced in CV1 cells via vaccinia virus recombinants. Two different DNA constructs were expressed: the first one encodes the complete gpII protein (gpII s+a+) and the second a truncated species lacking the membrane anchorage domain (gpII s+a-). To achieve expression both coding sequences had to be engineered at the 5’ end by substituting the unusually short (24 bp) natural signal sequence by a more conventional one encoding 29 amino acids. Recombinant gpII proteins were detected in vaccinia virus-infected cells by ELISA and immunoprecipitation. Both forms of recombinant gpII proteins were produced as glycosylated single-chain molecules of respectively 110K and 90K. Upon reduction these were only partially converted into subunits. A rabbit infected with the vaccinia virus recombinant expressing the complete gpII produced antibodies which recognized VZV antigens and neutralized VZV infectivity in vitro, independent of complement.

Varicella-zoster virus (VZV) induces two diseases in humans, varicella, which results from the primary infection with the virus, and herpes zoster which occurs upon reactivation of latent VZV infection.

The complete nucleotide sequence of the virus genome has been determined (Davison & Scott, 1986) and was shown to contain five glycoprotein (gp) open reading frames (ORFs). Of these, ORF 31, which is located in the Ul region of the genome, encodes gpII, the second most abundant and immunogenic surface glycoprotein (Davison et al., 1986). The corresponding DNA sequence specifies a polypeptide of 868 amino acid residues, comprising an eight amino acid signal peptide, the main body of the protein and hydrophobic anchor region (amino acids 699 to 743) followed by a positively charged C-terminal domain (Keller et al., 1986). The protein isolated from the virus consists mainly of a 120K to 140K heterodimer (Grose et al., 1984) which, upon reduction, dissociates into polypeptides of similar M, (approx. 65K). Monoclonal antibodies and polyclonal (MAbs) antibodies derived from mice immunized with gpII purified from the virus have the capacity to neutralize viral infectivity in vitro (Vafai et al., 1984; Edson et al., 1985a).

VZV gpII shares a large degree of homology with the glycoprotein gB of herpes simplex type 1 (HSV-1) (Edson et al., 1985b) which was shown to stimulate protective immunity in mice when administered as protein (Manservigi et al., 1990) or through expression by a recombinant vaccinia virus (Willey et al., 1988). HSV-1 gB was shown to be involved in virus penetration into cells and cell fusion during infection (Sarmiento et al., 1979).

In the present study, we attempted to express the VZV gpII protein via vaccinia virus recombinants in order to evaluate the immunogenic properties of the protein in laboratory animals. This kind of information is particularly important in view of the potential use of VZV gpII as a component of a future subunit vaccine for human use.

In the initial experiments, we constructed a recombinant vaccinia virus carrying the complete natural sequence coding for VZV gpII under the control of the vaccinia virus P7.5 promoter, following the procedure described by Mackett & Smith (1986) and using the transfer vector pULB5213, a derivative of pSC11 (Chakrabarti et al., 1985). In this construct, pNIV2030 (Fig. 1a), the sequence encoding the signal peptide of gpII consists of 24 bp (eight amino acids) including the ATG initiation codon. Although care had been taken to generate an appropriate initiation consensus sequence (Kozak, 1986) in the vicinity of the ATG codon, no expression was observed (data not shown). On this basis and in view of the unusually short signal peptide in the natural sequence, we constructed a gpII coding sequence starting 63 bp upstream from the natural ATG, maintaining the optimal initiation context. Although the sequence extension is not naturally translated, it encodes hydrophobic amino acids typical of a signal peptide. In

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Fig. 1. GplI coding sequences from pNIV2030, pNIV2050 and pNIV2051. (a) Structure of gplI coding cassettes. (b) Signal peptide of the gpII protein as encoded by VV2030 and VV2050. The transfer plasmid pNIV2030 was constructed to direct the expression of the natural gpII pro-protein, pNIV2050 was engineered to encode an extended signal peptide, and pNIV2051 for a protein devoid of an anchor sequence. ATGs preceded by the translation initiating ACC consensus sequence (Kozak, 1986) are underlined. HIII, HindIII; HII, HincII; aa, amino acids.

Fig. 2. Immunoprecipitation of crude extracts from RAT-2 (a) or CV1 (b) cells infected with recombinant vaccinia viruses. (a) Lanes 1 to 3, independent VV2050 recombinants; lane 4, vaccinia virus recombinant expressing β-galactosidase; lanes 5 and 6, independent VV2051 recombinants. (b) Lanes 1 and 5, VV2050 and VV2051, in the presence of tunicamycin; lanes 2 and 6, VV2050 and VV2051, controls; lanes 3 and 7, VV2050 and VV2051 treated with glycanase F; lane 4, Mr standards.

The final construct, pNIV2050 (Fig. 1a and b), the original ATG encodes an internal methionine and the signal peptide consists of 29 amino acid residues. A derivative of pNIV2050, pNIV2051, was also generated; it carries the same extended signal sequence but now has the anchor domain deleted by the insertion of a stop
viruses were tested for the expression of the gpII protein. Recombinant transfer plasmids pNIV2050 and pNIV2051 were then used to construct vaccinia virus recombinants VV2050 and VV2051 as described above.

CV1 cells infected with the recombinant vaccinia viruses were tested for the expression of the gpII protein. In the first assay, we used an ELISA based on an anti-gpII MAb (a kind gift of Dr M. Grose, U.S.A.) and a rabbit polyclonal anti-VZV serum (a kind gift of Dr E. D'Hondt, SmithKline-Beecham Biologicals). The results showed that both types of recombinant vaccinia virus carrying engineered gpII coding sequences produced immunoreactive material, in contrast to the original VV2030 construct. In a second series of experiments, we performed immunoprecipitation with rabbit IgGs on crude RAT-2 cell extracts derived from infected cultures (m.o.i. of 5) labelled for 5 h with $^{[35]S}$methionine. Immunoprecipitates were analysed on 12% SDS-polyacrylamide gels and autoradiographed. As seen in Fig. 2, recombinant gpII was essentially produced as a single chain polypeptide of about 110K from infection with VV2050 (full-length coding sequence designated gpII s+a+) and of 90K from infection with VV2051 (truncated gpII species designated gpII s+a-). Processing of the gpII protein was not very efficient; bands corresponding to the dissociated heterodimers were rather faint.

Since the natural VZV gpII molecule is glycosylated (Davison et al., 1986), it was of interest to determine whether the unprocessed recombinant proteins also carried oligosaccharide side-chains. To this end, CV1 cells infected with VV2050 or VV2051 were labelled with $^{[35]S}$methionine for 5 h in the presence of tunicamycin at 5 μg/ml. Cell extracts were immunoprecipitated, separated by gel electrophoresis and autoradiographed. As seen in Fig. 2(b, lanes 1 and 5), the recombinant gpII species derived from tunicamycin-treated cells migrated with lower $M_r$ values, i.e. approx. 95K instead of approx. 110K for gpII s+a+ and approx. 75K instead of approx. 90K for gpII s+a-.

In another experiment, labelled recombinant gpII molecules immunoprecipitated from vaccinia virus-infected cells were treated in vitro with glycanase F (16 h at 37 °C, 2 units/50 μl reaction mixture, according to the manufacturer's specifications; Boehringer-Mannheim). As seen in Fig. 2(b, lanes 3 and 7), apparent $M_r$ values of both recombinant gpII species were also reduced although less prominently than in the in vivo tunicamycin experiment. Taken together, the results show that, although largely unprocessed, recombinant gpII molecules produced by vaccinia virus-infected cells undergo significant glycosylation.

To evaluate the immunogenicity of VZV gpII recombinants in laboratory animals, we inoculated a rabbit with VV2050, twice with a 2 month interval (2.5 x $10^7$ p.f.u. intravenously injected). The serum, Ranti-VV2050, was collected 14 days after the second inoculation and tested by ELISA. Immunoplates (Nunc) were coated with a 1:4000 dilution of ascitic fluid containing anti-gpII MAb, incubated for 2 h with 100 μl of a 10-fold dilution of total VZV antigens present in a lysate of VZV-infected, or control MRC5 cells (human embryonic lung cells, Behring Diagnostic), or with Webster isolate VZV (a kind gift of Dr M. Slaoui, SmithKline-Beecham Biologicals) followed by a 1 h incubation with various dilutions of the rabbit serum. The results demonstrated a specific reaction of the Ranti-VV2050 serum to VZV antigens with both preparations. Indeed, as seen in Fig. 3 for the cell lysate, a signal was obtained only when the rabbit was immunized with VV2050 and no signal was observed for the control non-infected cell lysate. Similar results were obtained with sera from eight VV2050-immunized mice. The rabbit serum was also tested for VZV Webster-neutralizing activity. Neutralization tests were performed as described previously (Vafai et al., 1987). The results, shown in Table 1, demonstrated a plaque reduction of about 50% with 1:32 dilution of Ranti-VV2050 independently of the presence of complement.

The data, in summary, indicate that the VV2050 recombinant produces gpII in vitro and in vivo. The recombinant gpII protein appears immunogenic for mice and rabbits and is thus appropriately presented to the immune system. The availability of recombinant vaccinia viruses expressing gpII will facilitate the identification of antigenic epitopes responsible for the induction of humoral and cell-mediated immunity.
Table 1. Neutralization of VZV infectivity by serum from a rabbit immunized with VV2050

<table>
<thead>
<tr>
<th>Serum Origin</th>
<th>Immunizing Agent</th>
<th>Dilution</th>
<th>Plaque Count*</th>
</tr>
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<tr>
<td>Rabbit 96</td>
<td>TK:VV†</td>
<td>1:8</td>
<td>27 (0)</td>
</tr>
<tr>
<td>Rabbit 97</td>
<td>None</td>
<td>1:8</td>
<td>26 (0)</td>
</tr>
<tr>
<td>Rabbit 97</td>
<td>VV2050</td>
<td>1:8</td>
<td>1 (96)</td>
</tr>
<tr>
<td>Rabbit 97</td>
<td>VV2050</td>
<td>1:10</td>
<td>ND ‡</td>
</tr>
<tr>
<td>Rabbit 97</td>
<td>VV2050</td>
<td>1:16</td>
<td>7 (73)</td>
</tr>
<tr>
<td>Rabbit 97</td>
<td>VV2050</td>
<td>1:32</td>
<td>14 (46)</td>
</tr>
</tbody>
</table>

* Plaque count in two independent experiments; mean of triplicate cultures. Figures in parenthesis indicate percentage of neutralization.
† Recombinant thymidine kinase-negative vaccinia virus expressing an unrelated viral protein.
‡ ND, Not determined.

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References


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