Vaccinia virus serpins B13R and B22R do not inhibit antigen presentation to class I-restricted cytotoxic T lymphocytes


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Vaccinia virus (VV) inhibits the presentation of certain epitopes from influenza virus nucleoprotein (NP), haemagglutinin (HA) and non-structural 1 (NS1) proteins to CD8+ cytotoxic T lymphocytes (CTL) by an unknown mechanism. We have investigated whether VV genes B13R and B22R, which encode proteins with amino acid similarity to serine protease inhibitors (serpins), are involved in this process. Recombinant VVs were constructed which express influenza virus proteins HA, NP or NS1 and which lack serpin gene B13R or both B13R and B22R. The lysis of cells infected with these viruses by influenza virus-specific CD8+ CTL was compared to the lysis of cells infected with viruses expressing both the influenza proteins and the serpin genes. Cytotoxicity assays showed that deletion of the VV serpin genes B13R and B22R and other genes between B13R and B24R did not increase the level of lysis, indicating that these genes are not involved in inhibition of antigen presentation of the epitopes tested.

CD8+ cytotoxic T lymphocytes (CTL) recognize short peptides that are presented on the cell surface in association with major histocompatibility (MHC) class I proteins. These peptides are derived from intracellular proteins by cytoplasmic proteolysis, possibly involving the proteasome, and are transported via ATP-dependent transporter proteins (TAPs) into the endoplasmic reticulum (ER) or the cis-Golgi compartment, where they bind to MHC class I molecules associated with β2-microglobulin (β2M). The peptide/MHC/β2M complex is then transported to the cell surface where it may be recognized by CD8+ CTL, reviewed in Germain & Margulies (1993).

Several animal viruses have developed mechanisms that enable infected cells to evade recognition by CTL (reviewed in McFadden & Kane, 1994). For example, some adenoviruses express a 19 kDa glycoprotein which binds and retains the MHC class I proteins in the ER (Andersson et al., 1985; Burgert & Kvist, 1985) and murine cytomegalovirus (MCMV) causes peptide-loaded MHC class I molecules to accumulate in the Golgi (del Val et al., 1992). Recently a herpes simplex virus (HSV) immediate early protein (ICP47) has been shown to block the transport of MHC class I molecules from the ER/cis-Golgi (York et al., 1994).

Vaccinia virus (VV), the prototypic orthopoxvirus, has a double-stranded DNA genome of 191 kb (Goebel et al., 1990) and replicates in the cell cytoplasm. As an expression vector it has been used extensively to study the recognition of virus peptides by CTL but it interferes with the presentation of certain T cell epitopes (reviewed in Bennink & Yewdell, 1990). This interference was initially reported with a recombinant VV expressing influenza virus haemagglutinin (HA) in which the presentation of HA epitopes to CTL was inhibited during the late phase of VV infection (Coupar et al., 1986). Another study revealed that VV also interfered with the presentation of one of two influenza nuclear protein (NP) epitopes examined (Townsend et al., 1988). The blockage was present during both the early and late phases of infection, although it was more extensive at late times and could be partially or completely overcome by expression of rapidly degraded antigen, such as a ubiquitin–NP fusion protein (Townsend et al., 1988), or by redirecting the HA into the cytoplasm by deletion of the NH2-terminal signal sequence (Gould et al., 1991; Townsend et al., 1988). The restoration of antigen presentation by rapid protein degradation led to the
suggestion that VV interferes with the proteolytic processing of antigens (Townsend et al., 1988). Candidate VV proteins responsible for this inhibition are the products of genes B13R and B22R which have amino acid similarity to serine protease inhibitors (serpins) (Kotwal & Moss, 1989; Smith et al., 1989). These proteins do not affect the virulence of the WR strain of VV (Kettle et al., 1995) but the cowpox virus equivalent of B13R (93% amino acid identity) affects virus pock morphology on the chorioallantoic membrane (Pickup et al., 1986), arachidonic acid metabolism (Palumbo et al., 1993), apoptosis (Gagliardini et al., 1994) and inhibits the interleukin-1β converting enzyme (Ray et al., 1992).

Here we have investigated whether the deletion of these genes affects the presentation of epitopes from the influenza virus HA, NP and non-structural protein 1 (NS1) to CD8+ CTL.

Recombinant VVs were based on two parental viruses (vGS100 and vSSK2) which were derived from the Western Reserve (WR) strain. Both have a deletion at the right end of the virus genome. VV vGS100 lacks gene B22R and all genes rightward up to the gene encoding a 7.5 kDa polypeptide within the right inverted terminal repetition (ITR), whereas vSSK2 lacks genes B13R to B21R in addition to the region deleted in vGS100 (Fig. 1; N. W. Blake, S. Kettle, K. M. Law and G. L. Smith unpublished data). VVs vGS100 and vSSK2 thus lack one (B22R) or two (B22R and B13R) serpin genes (Fig. 1) and were used to investigate whether the deletion of these serpin genes would restore the presentation of those peptide epitopes which are poorly presented to CD8+ CTL from VV-infected cells. To do this, recombinant VVs were constructed from vGS100 and vSSK2 which express either the influenza virus HA, NP or NS1 proteins under the control of the early 7.5K or the late 4b promoters (Table 1). In each case the influenza virus protein was inserted into the VV thymidine kinase (TK) gene using established methodology (Mackett et al., 1984). These TK− VVs express the influenza A/NT/60/68 NP or A/PR/8/34 NS1 proteins from the VV 7.5K promoter, the influenza A/PR/8/34 HA from the VV 7.5K or 4b promoters, and the A/PR/8/34 HA lacking the signal peptide (L HA) from the 7.5K promoter (Table 1). Plasmids used to construct these recombinant VV have been described elsewhere and were pSC11/NT/60/68 (Townsend et al., 1988), pGS81 (Smith et al., 1987), pKG18/H1HA (Gould et al., 1987) pRK19/H1HA and pKG18/L1H1HA (Townsend et al., 1988). The recombinant VVs H1-VAC (Gould et al., 1987), NS1-VAC (Smith et al., 1987), 4bH1-VAC, Ub-R-NP VAC and WR-7.5NP have been described previously (Townsend et al., 1988).

Immunoblotting of infected cell extracts demonstrated that each recombinant VV expressed the influenza virus

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**Table 1. Recombinant vaccinia viruses used in CTL assays based on vGS100 and vSSK2 expressing influenza virus proteins**

<table>
<thead>
<tr>
<th>Parental viruses</th>
<th>Virus Promoter/protein</th>
<th>Virus Promoter/protein</th>
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<tr>
<td>vGS100 lacking B22R</td>
<td>vNWB20 7.5K/HA</td>
<td>vSSK4 7.5K/HA</td>
</tr>
<tr>
<td>vNWB9 4b/HA</td>
<td>vNWB12 7.5K/L-HA</td>
<td>vNWB11 7.5K/NP</td>
</tr>
<tr>
<td>vNWB12 7.5K/L-HA</td>
<td>vSSK3 7.5K/NP</td>
<td>vSSK9 7.5K/NS1</td>
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protein of the expected size (Fig. 2). Antiserum to HA recognizes a protein of approximately 70 kDa in lysates of cells infected with influenza A/PR/8/34 and VVs vNWB15 and vSSK9 but not in those infected with VV WR (Fig. 2b). Lastly, antiserum against influenza A/Cam/1/146 recognizes proteins corresponding to the HA (70 kDa), NS1 (25 kDa) and the NP (50 kDa) in cell lysates from influenza A/PR/8/34-infected cells, and a protein corresponding to the NP (50 kDa) in cells infected with vNWB11 and vSSK3 but not VV WR (Fig. 2c). These viruses were then used to examine the presentation of HA, NS1 and NP epitopes to CD8+ CTL.

Target cells for cytotoxicity assays were murine L929 (H-2Kb) cells and L929 cells transfected with the class I gene H-2Db(L929-Db) (Townsend et al., 1985). These cells were infected at 5–10 p.f.u./cell and labelled with 100 μCi 51Cr simultaneously for 90 min. For recombinants expressing the influenza proteins from early/late (7.5K—which hereafter referred to as early) or late (4b) promoters, infected cells were washed twice with assay medium (RPMI, 10 mm-HEPES pH 7.4, 10% FBS) and incubated in assay medium for 2 or 4 h, respectively. Cells were rewashed twice before being used in a standard 4-6 h 51Cr release assay as described previously (Gould et al., 1991). Effector cells for cytotoxicity assays were: (i) CTL clone HA8, specific for amino acids 10–18 of the HA2 subunit of influenza A/PR/8/34, which was maintained in vitro in medium containing 20 U/ml human recombinant interleukin-2 (R+D Systems) by weekly stimulation with feeder cells pulsed with 5 μM peptide (amino acids 10–18 of HA2) (Gould et al., 1991); (ii) polyclonal CTLs specific for amino acids 152–160 of the influenza A/PR/8/34 NS1 protein, maintained as described previously (Cossins et al., 1993); (iii) CTL clone F5, specific for amino acids 366–374 of influenza A/NT/60/68 NP maintained as described previously (Townsend et al., 1986) and (iv) CBA polyclonal CTL specific for peptide 50–63 of influenza A/NT/60/68 NP maintained as described previously (Townsend et al., 1988).

Target cells infected with VV expressing the influenza virus HA present the HA8 peptide epitope (through class I H-2Kb) to CTL inefficiently when the HA is expressed from the 7.5K early promoter (H1-VAC) and very poorly when expressed from the 4b late promoter (4bH1-VAC) (Fig. 3). In contrast, cells infected with a VV expressing a L-HA (vNWB12) were efficiently lysed by CTL, as previously reported for other L-HA viruses (Gould et al., 1991; Townsend et al., 1988). When the HA was expressed from an early promoter in the absence of serpin B22R (vNWB20) or serpins B13R and B22R (vSSK4), the level of presentation of peptide epitope HA8, although slightly higher than previously reported (Gould et al., 1991), was unaltered compared to the control (H1-VAC) expressing both serpins (Fig. 3a). Similarly, for recombinant VV expressing the HA from
the 4b late promoter, deletion of the serpins did not restore lysis by the HA8 CTL clone (Fig. 3b). Lysis of target cells infected with VV expressing the HA from the 4b promoter (4bH1-VAC) was barely above the background for cells infected with vNWB11, a virus expressing the influenza NP. Recombinant VV expressing the HA from the 4b promoter and lacking serpin B22R (vNWB9) or serpins B13R and B22R (vSSK5) gave levels of lysis slightly greater than 4bH1-VAC in the experiment shown in Fig. 3(b). However, over a number of experiments there was no consistently greater lysis of target cells infected with the serpin deletion viruses than with 4bH1-VAC.

Whether the deletion of the serpins would affect the presentation of other influenza epitopes which are normally poorly presented from VV-infected cells was investigated next. First, the recognition of the NP epitope 366–374 (through class I H-2D^b) by CTL clone F5 was analysed (Fig. 4a). Cells infected with WR-7.5NP, a VV expressing the NP from 7.5K promoter, are only poorly lysed by the NP-specific F5 CTL clone, whereas cells infected with a VV expressing NP fused to ubiquitin via an arginine residue (Ub-R-NP; Townsend et al., 1988) are efficiently lysed by the NP-specific F5 CTL clone, as previously reported (Cossins et al., 1993). Cells infected with viruses lacking serpin B22R (vNWB15) or B13R and B22R (vSSK9) and expressing the NS1 protein from the 7.5K promoter were also lysed poorly and at levels equivalent to the NS1-VAC-infected cells (Fig. 4c). Thus presentation of epitopes from NP and NS1 was not restored by deletion of serpins B13R and B22R. The defect in the presentation of the influenza epitopes studied appears to be at the level of epitope generation and not related to the restriction element concerned. Although there is an inhibition in presentation of influenza HA (Fig. 3) and NS1 (Fig. 4c) epitopes (both restricted through class I H-2K^b) there is no such defect in presentation of the H-2K^b-restricted NP epitope 50–63 (Fig. 4b).

Evasion of immune surveillance by CTL is a mechanism employed by many viruses to enable them to survive better in the host. Adenovirus, herpes simplex virus and murine cytomegalovirus reduce the expression of MHC class I and peptide on the cell surface by blocking the synthesis or transport of MHC class I antigens. Previous reports have suggested that VV may
employ a different strategy to avoid CTL recognition of infected cells. Coupar et al. (1986) reported that presentation of influenza HA epitopes from a recombinant VV was inhibited at late times of infection. This phenomenon was further investigated for influenza proteins HA and NP by Townsend et al. (1988) who showed that the blockage in antigen presentation was antigen and epitope specific, was more profound late during infection and could be overcome by expression of rapidly degraded forms of the proteins. This suggested that a defect in the processing of specific antigens to peptides might explain the inhibition of presentation of certain epitopes (Townsend et al., 1988). The identification of VV proteins with amino acid similarity to serpins (Kotwal & Moss, 1989; Smith et al., 1989) led to the suggestion that these may somehow inhibit the cellular proteolytic machinery involved in generating peptides for MHC class I molecules (Smith et al., 1989).

We have tested this hypothesis by constructing recombinant VVs in which serpin B22R or serpins B22R and B13R have been deleted and which express the influenza virus HA, NP or NS1 proteins. Cytotoxicity assays with epitope-specific CTL showed that there was no increase in the level of lysis as a result of deletion of the serpins, or other VV genes located between B13R and B24R. Thus, under the conditions tested, these genes play no role in the VV-induced reduction of antigen presentation. Consequently the mechanism by which VV inhibits the presentation of certain peptide epitopes to CTL remains unknown. It seems unlikely that this phenomenon is due to a general reduction in MHC class I expression caused by the virus-mediated inhibition of cellular protein synthesis, because other peptide epitopes are expressed from within the same cell and are recognized by CTL (Townsend et al., 1988). Nor is the defect in presentation related to the type of restriction element since there is an inhibition of NS1 and HA2 epitopes restricted through H-2Kk but presentation of NP epitope 50–63 through the same restriction element. Which of the approximately 200 virus genes is/are involved in the inhibition of antigen presentation remains to be determined, but given the numerous and diverse mechanisms that VV displays to counter the host response to infection (Smith, 1994), it seems likely that a specific gene will be involved.

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References


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