Cross-reactive Target Antigen in Cell-mediated Cytolysis of Cells Infected with a Temperature-sensitive Mutant of Sindbis Virus

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(Accepted 31 December 1984)

SUMMARY

Cytotoxic T lymphocytes (CTL) against alphavirus-infected L929 cells were generated in mice by two in vivo immunizations of one virus or by in vitro immunization followed by in vitro stimulation of splenocytes with infected peritoneal exudate cells or splenocytes. These CTL caused specific H-2-restricted cytolysis equally well with homologous, heterologous or Sindbis virus ts20 mutant-infected cells. Thus, specific CTL appear to be cross-reactive components in normal immunity to alphaviruses and the E1 glycoprotein is implicated as the target antigen.

Sindbis virus (SIN) and Semliki Forest virus (SF) are members of different subgroups of the genus alphavirus, family Togaviridae, as defined by the inability of antiserum prepared against either virus to neutralize infectivity of the second virus (for review, see Porterfield, 1980). However, immunization or infection with one alphavirus has been shown to cross-protect against infection by a member of a different alphavirus subgroup (Casals, 1963; Cole & McKinney, 1971; Latif et al., 1979; Peck et al., 1975). The mechanisms that provide cross-protection in the absence of cross-neutralization are not completely understood. We and others have provided evidence for cross-reactive cytotoxic T lymphocytes (CTL) in alphavirus cross-protection (Latif et al., 1979; Peck et al., 1975, 1979; Wolcott et al., 1982a; Mullbacher et al., 1979). There is also evidence for cross-reactive non-neutralizing antibody that recognizes viral determinants unique to the infected cell surface (Gates et al., 1982; Wolcott et al., 1984; Schmaljohn et al., 1982). This communication describes the use of a temperature-sensitive (ts20) mutant of SIN virus (Strauss & Strauss, 1980) to help determine which viral antigens participate in homologous and cross-recognition of infected cells by alphavirus-immune CTL.

C3H mice were immunized intraperitoneally with 10⁷ p.f.u. SIN or 10³ p.f.u. of SF virus propagated as described (Wolcott et al., 1982a, b). Spleen cell suspensions were prepared 21 to 28 days post-immunization and enriched for T lymphocytes by passage over nylon wool (Wolcott et al., 1982a). T-enriched cells (responders) were co-cultured 5 days in vitro with syngeneic, thioglycollate-induced peritoneal cells (PEC stimulators) which had been incubated 4 h with SIN or SF at a multiplicity of about 5 p.f.u./cell and exposed to 2000 R of gamma radiation. Alternatively, syngeneic infected non-immune spleen cells could be substituted for PEC in the 5 day stimulation cultures. Responder to stimulator ratios were typically 1:5 or 1:10 in RPMI 1640 medium (Gibco) with 10% foetal bovine serum (Sterile Systems, Logan, Utah, U.S.A.), 10 mm-glutamine, 5 × 10⁻⁵ m-2-mercaptoethanol, 10 mm-HEPES and 20 μg/ml gentamicin. After culture, T cells were harvested, washed in RPMI 1640 and viability was determined by trypan blue dye exclusion. Viable effector cells were added at indicated ratios to microtitre wells containing 10⁴ target cells which had been previously infected with the specified alphavirus and labelled with Na⁵¹CrO₄ (New England Nuclear) as previously described in detail (Wolcott et al., 1982a, b). Syngeneic (H-2k from C3H mice) target cells were L929; allogeneic (H-2b from BALB/c mice) target cells were A31 3T3. After a 6 h incubation, supernatant fluids were harvested for measurement of ⁵¹Cr release in a gamma spectrometer.

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### Table 1. Percent specific cytolysis of infected target cells by alphavirus immune effector cells after primary stimulation in vivo and secondary stimulation in vitro*

<table>
<thead>
<tr>
<th>Expt. (a)</th>
<th>Target cells infected with</th>
<th>Target:effectors (SIN in vivo, SIN in vitro)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target E:T†</td>
<td>Uninfected SIN</td>
</tr>
<tr>
<td>L929</td>
<td>5:1</td>
<td>13:1</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>20:2</td>
</tr>
<tr>
<td>A31</td>
<td>5:1</td>
<td>5:2</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>9:8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Expt. (b)</th>
<th>Target cells infected with</th>
<th>Stimulating virus in vivo/in vitro</th>
<th>L929 target cells infected with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target E:T†</td>
<td>Uninfected SIN</td>
<td>SF</td>
</tr>
<tr>
<td>L929</td>
<td>5:1</td>
<td>10:9</td>
<td>31.3</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>15:1</td>
<td>43.5</td>
</tr>
<tr>
<td>A31</td>
<td>5:1</td>
<td>5:0</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>9:9</td>
<td>8.7</td>
</tr>
</tbody>
</table>

* In these experiments, infected, irradiated PEC were used as stimulators; assays were performed at 40°C.
† Effector:target cell ratio.
†† ND, Not done.
§ Cytolysis of L929 target cells; E:T, 6:25:1.

and percent specific $^{51}$Cr release was calculated as percent specific cytolysis, \[\frac{(\text{c.p.m. released with effector cells}) - (\text{c.p.m. released in medium})}{(\text{total c.p.m. released by Triton X-100}) - (\text{c.p.m. released in medium})} \times 100\]. In other experiments, C3H mice were immunized twice with SIN or SF, as described above and elsewhere (Wolcott et al., 1982a, b), with secondary immunization carried out 14 days after primary immunization. Four days after secondary immunization, T-enriched splenocytes were prepared and immediately assayed for cytolysis activity on target cells. All target cell assays involving SIN ts20 were performed at restrictive temperature, 40°C.

The stock SIN ts20 was plaque-purified four times and titrated at $4 \times 10^9$ p.f.u./ml at the permissive temperature of 30°C. Reversion frequency was determined as $10^{-5}$/ml.

SIN or SF immune effectors from secondary stimulation in vitro by infected PEC were assayed for their ability to lyse virus-infected target cells. Two representative experiments are shown in Table 1. Effector cells efficiently lysed virus-infected syngeneic (L929) cells but not allogeneic (A31) cells, suggesting that effector cells were CTL. Failure of effector cells to lyse A31 target cells was not attributable to lack of viral surface antigen or to inherent resistance to cytolysis, since infected A31 cells were equally or more susceptible than L929 cells to lysis by antiviral antibody plus complement (data not shown). The effector cells from stimulation in vitro expressed both Thy 1 and Lyt 2.1 antigens, since treatment of effector cells with rabbit anti-mouse T cell serum or mouse monoclonal anti-Lyt 2.1 antibody and Low-Tox-M rabbit complement (Accurate Chemical & Scientific, Westbury, N.Y., U.S.A.) before addition to target cells reduced cytotoxicity to insignificant levels.

Alphavirus CTL generated by immunization and stimulation in vitro with a single alphavirus were shown to lyse syngeneic target cells infected with the same virus or with an alphavirus...
Table 2. Ability of cytolytic T-enriched splenocytes to lyse alphavirus-infected L929 cells after primary (1) and secondary (2) immunization in vivo*

<table>
<thead>
<tr>
<th>Virus for immunization</th>
<th>E:T†</th>
<th>Net specific $^{51}$Cr release from cells infected with</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIN (1), SIN (2)</td>
<td>50:1</td>
<td>SIN</td>
</tr>
<tr>
<td>SF (1), SF (2)</td>
<td>25:1</td>
<td>24.8</td>
</tr>
</tbody>
</table>

* Immunization was given intraperitoneally and T-enriched splenocytes prepared 4 days after secondary immunization with SIN (10¹ p.f.u.) or SF (10³ p.f.u.).
† Effector:target ratio yielding maximal cytolysis. E:T ratios greater than 50:1 were not tested.
‡ The amount of $^{51}$Cr release from uninfected cells (< 4%) was subtracted from the observed release of infected cells.

belonging to a different subgroup (Table 1). These data support those of Mullbacher et al. (1979) and our previous reports (Gates et al., 1982; Wolcott et al., 1982a; Peck et al., 1979) on cross-reactivity of alphavirus-induced CTL.

These data demonstrate the ability of alphavirus-induced CTL to lyse syngeneic target cells infected with SIN $ts20$. Both homologous (SIN, exp. a, part 1) and heterologous (SF, exp. a, part 2) virus-induced CTL recognized SIN $ts20$-infected targets when assayed at restrictive temperature.

Unlabelled target competition studies were performed with wild-type virus- or SIN $ts20$-infected competitor cells and wild-type-infected, $^{51}$Cr-labelled target cells mixed with SIN or SF effector cells. Inhibition of cytolysis increased with increasing numbers of competitor cells, using 1 : 1, 5 : 1 and 10 : 1 ratios of competitor to target (data not shown). At a ratio of 10 : 1, after subtracting the percent inhibition of cytolysis by uninfected competitors, SIN $ts20$-infected competitors produced a 22% to 38% inhibition of cytolysis of wild-type-infected target cells. Over a series of triplicate experiments, inhibition by SIN $ts20$-infected competitors was comparable to that observed with competitors infected with wild-type virus (data not shown).

At restrictive temperature, SIN $ts20$-infected cells are known to express only the E1 glycoprotein at the plasma membrane surface (Strauss & Strauss, 1980; Bell & Waite, 1977; Bracha & Schlesinger, 1976; Smith & Brown, 1977). We found that rabbit anti-E1 serum, but not anti-E2 serum, will cause cytolysis of SIN $ts20$-infected L929 cells in antibody-dependent, complement-mediated cytotoxicity assays (data not shown). Thus, the evidence supports the interpretation that E1 glycoprotein serves as a target antigen for homologous and cross-reactive alphavirus-immune CTL. Cross-reactivity of E1 has been previously demonstrated in antibody-mediated reactions involving cytolysis (Wolcott et al., 1982b), immunoblotting (Wolcott et al., 1984), immunoprecipitation (Wolcott et al., 1984), and haemagglutination-inhibition (Gates et al., 1982).

In addition to homologous and cross-cytolysis of alphavirus-infected targets by CTL stimulated in vitro, effector cells stimulated by immunization in vivo similarly lysed syngeneic target cells (Table 2; Wolcott et al., 1982a).

These data and previous reports (Peck et al., 1975; Gates et al., 1982; Wolcott et al., 1982a, b, 1984; Mullbacher et al., 1979; Peck et al., 1979; King et al., 1977) suggest a role for cross-reactive CTL in alphavirus-induced cross-protection in vivo and further implicates E1 glycoprotein as one of the target antigens in protection.

The authors wish to thank John W. Bulrice for his excellent technical assistance in these studies. J.A.W. was a postdoctoral fellow supported by Public Health Service Research Service Award training grant T32 AI07123 from the National Institute of Allergy and Infectious Diseases.

REFERENCES


Short communication


(Received 30 August 1984)