Protection against rabies in mice by a cytotoxic T cell clone recognizing the glycoprotein of rabies virus

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By the use of liposomes containing the purified surface glycoprotein (G) of rabies virus and the haemagglutinin neuraminidase (HN) and fusion (F) glycoproteins of Sendai virus, the target antigen of anti-rabies virus cytotoxic T lymphocyte (CTL) clones isolated in a previous study was identified as the G protein. Recognition of the H-2K determinant of the class I major histocompatibility complex (MHC) was necessary for target lysis by the CTL clones. One of the CTL clones was examined for the ability to protect mice against a lethal rabies virus infection. CTL were transferred into syngeneic mice which had been infected in the hind footpad with the ERA strain of rabies virus. The infection was converted into a lethal infection by cyclophosphamide treatment 1 day after virus infection. Transfer of CTL 2 to 3 days after virus infection protected approximately 50% of mice during the observation period of 4 weeks. Greater protection was obtained in mice receiving both anti-rabies virus antibodies and CTL cells.

Introduction

It is known that a high level of virus-neutralizing antibodies induced by inactivated vaccine before exposure to rabies virus confers complete protection against subsequent lethal infection (Sikes et al., 1971); however, the protective effect in post-exposure treatment is induced only by vaccine and not by anti-rabies virus antibodies (Sikes et al., 1971; Baer & Yager, 1977; Mifune et al., 1980). It has been suggested that the induction of specific cell-mediated immunity is a crucial factor in the defence of the host (Wiktor, 1978; Miller et al., 1978; Smith, 1981; Mifune et al., 1981). Attention has focused recently on the internal protein of rabies virus instead of the surface glycoprotein (G), in the light of observations with other pathogenic viruses where it has been shown that the internal viral protein is the major cytotoxic T lymphocyte (CTL) target, and that such proteins can contribute significantly to protection (Wraith et al., 1987; Taylor & Askonas, 1986). Indeed, priming of animals with the nucleoprotein of rabies virus gave rise to an efficient production of virus-neutralizing antibodies and resulted in the protection of animals against a subsequent lethal infection (Dietzschold et al., 1987).

In a previous study, we isolated 10 murine Thy-1+, Lyt-1-,2+ CTL clones which recognized three different strains of rabies virus and Duvenhage virus, a member of the rabies serogroup. The CTL clones were major histocompatibility complex (MHC) class I-restricted and produced low amounts of γ-interferon in response to antigen stimulation (Cho et al., 1987). These CTL clones provide useful tools in studying the role of cell-mediated immunity in recovery or protection from rabies virus infection in mice and in the characterization of the rabies virus antigen recognized by CTL.

In the present study, we identified the target antigen of some of these CTL clones and then examined their ability to protect mice against a lethal rabies virus infection.

Methods

Mice. Inbred A/J, C57BL/6 and BALB/c male mice and outbred ddy female mice, 7 to 12 weeks of age, were used.

Cell lines. Neuroblastoma (NA) cells, originating from an A/J mouse (N-18, H-2a), MC57G (H-2b), P815 (H-2d) and L929 cells (H-2k), and the NA cells persistently infected with the HEP-Flury (HEP) strain of rabies virus, and referred to as NA-HEP, were used. The NA, MC57G and L929 cells were maintained in Dulbecco's modified MEM supplemented with 5% foetal calf serum (FCS), 5% newborn calf serum and antibiotics. NA-HEP cells were grown in Dulbecco's MEM with 10% FCS and P815 cells were grown in RPMI 1640 medium with 10% FCS. Anti-rabies virus CTL clones from A/J mice, established in our laboratory, were grown in Iscove's modified Dulbecco's MEM supplemented with 5% foetal calf serum (FCS), 5% newborn calf serum and antibiotics (IMDM-S) with a weekly antigenic stimulation by mitomycin C-treated NA-HEP cells (Cho et al., 1987). An alloreactive
anti-H-2b CTL clone was established in vitro from spleen cells of A/J mice immunized with 137Cs-irradiated spleen cells of C57BL/6 mice. This clone lysed MC57G cells but not NA or NA-HEP cells as determined by the 51Cr release cytotoxicity test.

**Viruses.** The HEP, CVS and ERA strains of rabies virus were grown in BHK-21 cells with Eagle’s MEM containing 2% FCS. Sendai virus was propagated in the chorioallantoic cavity of hen’s eggs. The HEP virus and Sendai virus were concentrated approximately 100-fold by ultracentrifugation and purified by sucrose density gradient centrifugation for further preparation of viral proteins.

**Preparation of TCGF.** TCGF was prepared by the method described by Gillis et al. (1978) using rat spleen cells. The activity of interleukin 2 (IL-2) was assayed as described elsewhere (Gillis et al., 1978) using an IL-2-dependent CTL clone which was established in our laboratory.

**Preparation of the viral proteins.** The G protein of the HEP virus was prepared by treatment of the purified virus with 20% non-ionic octylglucoside (Sigma) in 0.1 M-Tris-HCl pH 7.8, for 30 min at 37°C, and by ultracentrifugation of the mixture at 40000 r.p.m. for 60 min in the Beckman SW50.1 rotor. The haemagglutinin–neuraminidase (HN) and fusion (F) glycoproteins of Sendai virus were extracted similarly with 20% octylglucoside in 0.01 M-Dulbecco’s phosphate-buffered saline (PBS) solution (pH 7.4). Protein fractions were concentrated by precipitation with 20 volumes of cold butanol. The purity of these proteins was determined by staining the gels with Coomassie Brilliant Blue after SDS–PAGE (Laemmli, 1970).

**Preparation of liposomes.** As a lipid, asolectin (lecithin from soybean ‘DAIGO’, Wako), was used for the preparation of liposomes. Asolectin is composed of 35% phosphatidylcholine, 30% phosphatidylethanolamine, 22% phosphatidylinositol and 13% phosphatic acid. The G protein of the HEP virus (400 μg), the HN and F proteins of Sendai virus (400 μg) and asolectin (2 mg) were suspended in 2 ml of 200 mM-KCl solution with the vortex mixer and were then sonicated for 15 min on ice. After freezing with liquid nitrogen, the mixture was thawed on ice very slowly. The liposomes were packed by centrifugation (40000 g) and resuspended in the same volume of PBS.

**Preparation of target cells for the cytotoxicity assay.** NA, P815 and L929 cells were labelled with 100 μCi/ml of [51Cr]Na204 and incubated overnight at 37°C. After dispersal with trypsin and washing three times, labelled cells (5 x 105) were incubated with liposomes (containing 120 μg of viral proteins) in a volume of 0.3 ml of PBS for 20 min at 4°C, and for a further 60 min at 37°C. The cells were then washed twice by low speed centrifugation and resuspended at 2 x 105 cells/ml in IMDM-S.

**Cytotoxicity assays.** The 51Cr-labelled target cells were plated into wells of 96-well flat-bottomed microwell plates (Nunclon) at 2 x 104 cells per well and mixed with serial dilutions of CTL clones or spleen cells from immunized mice, to yield a variety of effector:target (E:T) ratios in a total volume of 200 μl of IMDM-S. The assay was performed in duplicate and terminated after an 18 h incubation at 37°C in an atmosphere of 5% CO2. The percentage specific lysis was calculated as (51Cr release with effector cells - 51Cr release in medium) / (51Cr release with 1% Triton X-100 - 51Cr release in medium) x 100.

**Adoptive transfer of the anti-rabies virus CTL clone.** Male A/J mice were infected with 0.03 ml of the ERA strain of rabies virus (2.0 x 105 p.f.u./mouse) in the left hind footpad on day 0 and injected intraperitoneally (i.p.) with cyclophosphamide (Cy) (150 to 160 mg/kg) on day 1 after infection. These mice were then injected intravenously (i.v.), at intervals after infection, with the anti-rabies virus CTL clone, the anti-rabies virus antibodies, or both. In parallel with the transfer of the CTL clone, 250 units of TCGF mixed with an equal volume of 20% gelatin was administered daily i.p. for 4 days to sustain the survival of the CTL clone in the mice (Donohue & Rosenberg, 1983). Control mice also received TCGF using the same schedule. Mice were observed daily for paralysis and death for 28 days after infection.

**Anti-rabies virus antibody.** ddy mice were injected i.p. at weekly intervals with a 10% CVS-infected suckling mouse brain suspension. At the last injection (Week 5), Ehrlich ascites tumour cells were also inoculated i.p. and the ascites fluid was harvested 10 to 14 days later.

**Results**

**Properties of liposomes (G-HNF liposomes) containing the G protein of rabies virus and the HN and F proteins of Sendai virus**

Liposomes prepared by the procedures described in Methods were examined for some of their properties. Their size under light microscopy (x 400) was 1 to 1.5 mm on average and they were composed of the G protein of rabies virus and the HN and F proteins of Sendai virus, as determined by SDS–PAGE (Fig. 1). The liposomes had a haemagglutinating titre of 1/024/0.025 ml against goose erythrocytes (Halonen et al., 1968) and had an extensive haemolytic activity (OD540 of 2.042) at neutral pH against human O erythrocytes as measured by the method of Mannen et al. (1982). Control liposomes prepared with asolectin only did not exhibit any biological activity.

![Fig. 1. SDS–PAGE of G-HNF liposomes (lane 3). Lane 1, rabies virus; lane 2, G protein extracted from rabies virus; lane 4, HN and F proteins extracted from Sendai virus; lane 5, Sendai virus. The gel was stained with Coomassie blue. The G protein of rabies virus and the HN and F proteins of Sendai virus were extracted by treatment of purified virions with octylglucoside and the liposomes were prepared as described in Methods. Rabies virus membrane protein, M; nucleoprotein, N. F1 is the cleaved chain of the cell fusion protein F, of Sendai virus.](image-url)
Table 1. Susceptibility of various cells treated with G-HNF liposomes to lysis by CTL clones

<table>
<thead>
<tr>
<th>CTL clone</th>
<th>E:T</th>
<th>NA-HEP*</th>
<th>NA + liposome G-HNF†</th>
<th>L929 + liposome G-HNF (H-2 KdD+)</th>
<th>P815 + liposome G-HNF (H-2 KdD+)</th>
<th>NA + liposome HNF‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC-1</td>
<td>10:1</td>
<td>ND§</td>
<td>15 (4)</td>
<td>36 (6)</td>
<td>0 (4)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5:1</td>
<td>88</td>
<td>0 (0)</td>
<td>8 (0)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2:1</td>
<td>ND</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (2)</td>
<td>0</td>
</tr>
<tr>
<td>EI-8</td>
<td>10:1</td>
<td>91</td>
<td>23 (3)</td>
<td>16 (4)</td>
<td>0 (2)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5:1</td>
<td>ND</td>
<td>4 (1)</td>
<td>10 (0)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2:1</td>
<td>83</td>
<td>3 (0)</td>
<td>0 (0)</td>
<td>3 (3)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Murine neuroblastoma cells persistently infected with the HEP-Flury strain of rabies virus.
† NA cells treated with G-HNF liposomes.
‡ NA cells treated with HNF liposomes which did not contain the G protein of rabies virus.
§ ND. Not determined.
|| Figures in parentheses indicate percentage 51Cr release of control target cells which were not treated with liposomes.

Evidence that the G protein of rabies virus is a target antigen for CTL clones

G-HNF liposomes were then examined to determine whether they could modify and render uninfected target cells susceptible to lysis by anti-rabies virus CTL clones, which efficiently lysed syngeneic NA-HEP cells at E:T ratios of 2:1 to 10:1. Of 10 CTL clones established in the previous study (Cho et al., 1987), five were tested in this experiment. NA cells treated with G-HNF liposomes were lysed by all five CTL clones at an E:T ratio of 10:1. Table 1 shows the representative results of two CTL clones. Optimal lysis by CTL clones (at an E:T ratio of 10:1) was dependent upon the amount of G protein of rabies virus in the liposome preparations (Table 2). In contrast, NA cells treated with liposomes containing only the HN and F proteins of Sendai virus (HNF liposomes) were not lysed significantly by these CTL clones. Furthermore, NA cells treated with the G, HN and F proteins in the absence of lipid or non-treated NA cells, were not lysed by CTL clones (data not shown). The results suggest that the G protein is the target antigen recognized by these anti-rabies virus CTL clones.

Although it was observed in the previous study that these CTL clones lysed rabies virus-infected cells in an H-2-restricted fashion, the fine specificity of the H-2 antigens recognized by the clones was not determined. This was examined here using P815 and L929 cells, which were treated with G-HNF liposomes (as described for NA cells), followed by determination of lysis by the CTL clones. As shown in Table 1, L929 cells sharing the H-2K gene with NA cells were susceptible to lysis by CTL clones but P815 cells were not, suggesting that the CTL clones were restricted to the H-2K end of the MHC class I.

Properties of a rabies virus–mouse model used in subsequent experiments to determine the protective action of CTL clones

The ERA strain of rabies virus was used as the challenge virus. This virus, although lethal if inoculated intracerebrally into young adult mice, is known to cause an abortive infection if inoculated peripherally, as described by Smith (1981). However, immunosuppression of these mice on day 1 after virus infection with 150 to 160 mg/kg Cy converted a non-lethal ERA virus infection into a lethal infection, as shown in Table 3. The virus replication in the brains of these immunosuppressed mice was significantly greater than that of immunocompetent mice. Virus was cleared from the brains of immunocompetent mice by day 14. In contrast, there was
Table 3. Effect of adoptive transfer of the anti-rabies virus G protein CTL clone into immunosuppressed mice infected peripherally with the ERA rabies virus*

<table>
<thead>
<tr>
<th>CTL used for transfer</th>
<th>Day of transfer after virus infection</th>
<th>No. of cells transferred</th>
<th>No. of survivors/ no. of mice</th>
<th>Survival ratio (%)</th>
<th>Average day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>None†</td>
<td>0</td>
<td>0/8</td>
<td>0</td>
<td>14 (10-19)‡</td>
<td></td>
</tr>
<tr>
<td>DC-1</td>
<td>2</td>
<td>7 x 10⁷</td>
<td>6 (1)/13</td>
<td>46</td>
<td>19 (15-24)</td>
</tr>
<tr>
<td>DC-1</td>
<td>3</td>
<td>7 x 10⁷</td>
<td>7 (1)/14</td>
<td>50</td>
<td>18 (13-21)</td>
</tr>
<tr>
<td>DC-1</td>
<td>3</td>
<td>1.4 x 10⁷</td>
<td>3 (1)/10</td>
<td>30</td>
<td>16 (14-19)</td>
</tr>
<tr>
<td>DC-1</td>
<td>3</td>
<td>7 x 10⁶</td>
<td>0/10</td>
<td>0</td>
<td>16 (14-18)</td>
</tr>
<tr>
<td>DC-1</td>
<td>4</td>
<td>7 x 10⁷</td>
<td>3 (1)/8</td>
<td>38</td>
<td>16 (14-19)</td>
</tr>
<tr>
<td>DC-1</td>
<td>5</td>
<td>7 x 10⁷</td>
<td>2 (2)/6</td>
<td>33</td>
<td>17 (16-20)</td>
</tr>
<tr>
<td>Allogeneic CTL‖</td>
<td>3</td>
<td>7 x 10⁷</td>
<td>0/10</td>
<td>0</td>
<td>14 (11-17)</td>
</tr>
</tbody>
</table>

* The results of two or three experiments were summarized in this table. No significant difference was noticed between the results of each experiment.
† The same doses of TCGF were administered as for groups of mice receiving CTLs.
‡ Numbers in parentheses represent the time range.
§ Numbers in parentheses represent the number of mice with paralysis of the legs in surviving mice.
‖ Allogeneic CTL directed to C57/BL6 mice.

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no clearance of virus from the brains of immunosuppressed mice during this time (Fig. 2a), suggesting that some aspect of the immune response of mice was involved in viral clearance. Therefore, the humoral and cell-mediated immune responses were compared at various times after infection. As shown in Fig. 2(b and c), antibody was first detected in immunocompetent mice on day 4 and the levels rose rapidly thereafter, whereas no serum antibody was detected in immunosuppressed mice until day 13 after the addition of Cy. Cytotoxic activity of spleen cells was first detected after 2 or 3 days in immunocompetent mice and reached a peak on day 7, gradually declining thereafter. No significant cytotoxic activity of spleen cells was detected in immunosuppressed mice during the 13 days of study after Cy treatment.

**Protection against a lethal infection by a G-specific CTL clone**

Mice, infected with rabies virus and treated with Cy as described above, were injected i.v. with 7 x 10⁷ DC-1 CTLs/mouse on days 2, 3, 4 or 5 after virus infection. The timing of the transfer of the CTL clone into mice mimicked the development of the cell-mediated immune response observed in immunocompetent mice. As shown in Table 3, adoptive transfer of the cloned CTL on days 2 or 3 after infection resulted in the most effective protection (46% to 50% survival) with the average day of death occurring later. Survival rates were reduced when the transfer was done later than day 3 after virus infection. However, in this group, there was an increase in ataxia or paralysis of the legs in the surviving animals. The survival ratio was dependent on the number of cells transferred into mice when tested on day 3 after
infection. No virus was recovered and no fluorescent viral antigen was detected in the brains of some of these surviving mice sacrificed 2 weeks after virus infection (data not shown). Low levels of circulating interferon (1:20 to 1:32) were detected after 24 h in the mice given $7 \times 10^7$ CTLs; however, almost the same levels of interferon (1:20 to 1:28) were detected also in mice given decreased numbers of CTL cells and which were not protected against lethal infection. This suggests that the interferon observed in the serum of the recipients of CTL is not playing an important role in the protection, under our experimental conditions. In parallel control experiments, mice treated similarly with virus and Cy and transferred with an alloreactive CTL clone, instead of the anti-rabies virus CTL clone, were not protected.

The effect of anti-rabies virus antibodies and CTL cells in protecting mice against rabies

Passive protection experiments were carried out with anti-rabies virus antibodies in the groups of mice treated with virus and Cy. One ml per mouse of anti-rabies virus ascitic fluids (neutralizing activity 1:1600/0.1 ml) was injected i.p. on days −1, 2 or 5 after virus infection. When antibody was given up to 2 days after the virus infection, 100% of mice survived the infection. The number of survivors declined when the antibody was given at 5 days post-infection, with only 25% of mice surviving.

To virus-infected mice treated with Cy on day 1 and transferred with $7 \times 10^7$ CTL DC-1 cells on day 3, anti-rabies virus antibodies were given on day 5 after virus infection. The survival rate of these mice was greater (80%) than that of mice given CTLs alone (46%) or than that of mice given antibody alone (25%), suggesting that the concurrent presence of antibodies with CTLs facilitates the protection (Table 4).

Discussion

In the present study, liposomes were used to determine the target antigen recognized by CTL clones specific for rabies virus. Similar studies have been described in vesicular stomatitis virus and influenza virus systems (Hale et al., 1981; Stitz et al., 1985). It was demonstrated that anti-rabies virus CTL clones recognize the G protein of rabies virus and that the recognition is restricted to the H-2K$^k$ determinant of the MHC class I antigens. There was no direct evidence from the present study that G-HNF liposomes fused with the membrane of the various target cells. However, the fact that such liposomes had haemolytic activity against human erythrocytes and that uninfected NA cells treated with liposomes containing G-HNF were susceptible to CTL lysis, suggests that the rabies virus G protein was probably incorporated into the surface of target cells as a result of membrane fusion through the F protein of Sendai virus in the liposomes.

Recent reports have defined the internal viral nucleoprotein (NP) as a major target antigen of the CTLs induced by influenza virus (Townsend & Skehel, 1984; Yewdell et al., 1985; Bennink et al., 1987) and vesicular stomatitis virus (Puddington et al., 1986). Furthermore the NP of influenza virus (Wraith et al., 1987) and CTLs specific for this protein (Taylor & Askonas, 1986) are involved in the induction of protective immunity. Although it has not yet been determined whether CTL recognition of the NP of rabies virus is induced by immunization, the NP has been shown to induce protective immunity against a lethal challenge of rabies.

Table 4. Enhancement of protection by combined transfer of anti-rabies antibodies (Ab) and anti-rabies virus G protein CTLs against a lethal rabies virus infection

<table>
<thead>
<tr>
<th>CTL used for transfer</th>
<th>Day of transfer after virus infection</th>
<th>No of survivors/ no. of mice</th>
<th>Survival ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab*</td>
<td>−1</td>
<td>5/5</td>
<td>100</td>
</tr>
<tr>
<td>Ab</td>
<td>2</td>
<td>7/8</td>
<td>88</td>
</tr>
<tr>
<td>Ab</td>
<td>5</td>
<td>2/8</td>
<td>25</td>
</tr>
<tr>
<td>CTL(DC-1 clone)$^\dagger$</td>
<td>3</td>
<td>6 (21/13)</td>
<td>46$^\dagger$</td>
</tr>
<tr>
<td>CTL(DC-1 clone) + Ab$^\dagger$</td>
<td>3 and 5</td>
<td>16 (5/20)</td>
<td>80$^\dagger$</td>
</tr>
</tbody>
</table>

* One ml/mouse of anti-rabies virus mouse ascites fluid (neutralizing titre: 1:1600/0.1 ml) was administered i.p.
$^\dagger$ The results of three experiments were summarized. No significant difference was noticed between the results of each experiment.
$^\dagger$ Figure in parentheses indicates the number of mice with paralysis of legs in surviving mice.
§ CTL ($7 \times 10^7$ cells) and Ab were transferred on day 3 and 5, respectively.
$^\dagger$ Fisher's test indicated that the survival ratio of mice given both CTLs and Ab is significantly higher than that of mice given CTL alone ($P = 0.043$).
virus (Dietzschold et al., 1987). All CTL clones examined in the present study recognized the G protein of rabies virus; there was no evidence that any of the clones recognized the internal proteins. The reason for this is not known at present but it may be due to an intrinsic property of rabies virus or differences in experimental procedures for the isolation of CTLs. Also differences in the H-2 background of the mice used in the induction of CTL might be responsible, as suggested in the lymphocytic choriomeningitis virus system described by Whitton et al. (1988).

Although it has been suggested that cell-mediated immunity plays an important role in recovery from attenuated rabies virus infection and in the post-exposure prophylaxis of rabies (Miller et al., 1978; Smith, 1981; Mifune et al., 1981), there have been no experiments using CTL clones with known viral antigen specificity. We therefore examined their role in protection against rabies by transferring CTL clones to infected animals. The animal model used in this study was mice infected peripherally with an attenuated strain of rabies virus and converted into a lethal infection by treatment with Cy. The overall features observed in the mice, that is, limited virus replication in the brain without immunosuppression, 100% conversion into a lethal infection by Cy treatment on day 1 after virus infection and the complete suppression of humoral and cell-mediated immune responses during 14 days after Cy treatment, are in good agreement with the findings originally described by Smith (1981). Introduction of CTLs into such mice 2 to 3 days after virus infection conferred the most effective protection (approximately 50%). The level of protection decreased when CTLs were administered later. However the number of mice with paralysis of the legs increased when CTLs were given 4 days after virus infection. In the present study we could not identify the sites of virus clearance by CTLs. However other studies in animals have shown that rabies virus replicates in peripheral myocytes and neuromuscular junctions, near the site of virus infection, before the virus enters the peripheral and central nervous systems (Murphy et al., 1973). These experimental results suggest that CTLs probably act near the site of infection where the virus can be eliminated more efficiently. However, even in the central nervous system, virus clearance can occur under certain conditions.

Enhancement of protection against a lethal rabies virus infection was observed by combined administration of neutralizing antibody and CTLs. Antibody alone can inhibit cell-to-cell spread of rabies virus but cannot eliminate it from the infected cells (Lodmell & Ewalt, 1987). Furthermore, passive administration of antibody is not significantly effective in reducing the mortality of infected animals if given late after infection (Sikes et al., 1971; Baer & Yager, 1977; Mifune et al., 1980), as demonstrated also in the present study. The underlying mechanism(s) by which the protection was enhanced by combined administration of neutralizing antibody with CTLs is unclear but an explanation might be that inactivation of extracellular virus released from infected cells by G-specific CTL lysis is necessary for more efficient protection. The possibility of antibody-mediated enhancement of the proliferation of rabies virus-reactive T cells via antigen-presenting cells (Celis et al., 1985), cannot be excluded. Further studies are necessary to elucidate the mechanism. It will be interesting to examine whether CTLs recognizing NP can be induced in immunized animals and to examine to what extent they contribute to protection, especially as NP is synthesized first in rabies virus-infected cells which therefore would be expected to be lysed by CTLs before virus maturation is completed.

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References


Protection against rabies by CTL


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