A human monoclonal antibody against varicella-zoster virus glycoprotein III

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Hybridomas producing human monoclonal antibodies (HMAbs) against varicella-zoster virus (VZV) were generated by fusing murine myeloma cells with human lymphocytes immunized in vitro. An assay system was developed to select anti-glycoprotein (gp)III HMAbs from the pool of anti-VZV HMAbs. A murine anti-gpIII MAb, 4B7, did not react with a VZV-infected cell homogenate, but did react with a VZV-infected cell monolayer, whereas anti-gpI and anti-gpII MAbs reacted with both antigens. Hybridomas were screened to obtain HMAbs having a reaction profile similar to that of 4B7 and one such clone, V3, stably produces human IgG1 (x). HMAb V3 immunoprecipitated a VZV antigen of 115K to 120K, which was not immunoabsorbed by an anti-gpII HMAb, implying that V3 recognizes gpIII. V3 neutralized VZV independently of complement, unlike anti-gpI and anti-gpII HMAbs. All five strains of VZV tested were completely neutralized by V3, and the dose of V3 required to reduce the number of virus plaques by 50% ranged from 0-027 to 0-15 μg/ml. V3 was also able to inhibit the spread of virus infection from infected to uninfected cells, whereas anti-gpI and anti-gpII HMAbs could not. In addition, V3 mediated antibody-dependent cellular cytotoxicity but not complement-dependent cytotoxicity of VZV-infected cells. The results suggest that an anti-gpIII HMAb may provide a new means of passive immunoprophylaxis and also help to identify an antigenic epitope appropriate for a subunit vaccine.

Introduction

Varicella-zoster virus (VZV) can cause severe, life-threatening varicella and herpes zoster in immunocompromised patients (Feldman et al., 1975; Ljungman et al., 1986), even though these diseases are generally benign in immunocompetent subjects. The role of antibody in protection of the host against VZV infection remains controversial. Gershon & Steinberg (1979) have found a greater frequency and severity of infection in patients with depressed cellular immunity, but no correlation between the level of serum antibody and recovery from infection. Larkin et al. (1985), however, have shown that disseminated herpes zoster is associated with significantly low levels of antibodies to VZV glycoproteins.

Passive immunization with antibody can diminish the severity of varicella if sufficient antibody is administered shortly after exposure to VZV (Orenstein et al., 1981; Zaia et al., 1983; Miller et al., 1989). Monoclonal antibodies (MAbs), which consist of a pure antibody protein, have high antibody titres and some show very potent antiviral activity (Grose et al., 1983; Forghani et al., 1984; Keller et al., 1984; Kohl et al., 1990). All these MAbs are of rodent origin and are recognized as foreign substances by the human immune system (Khazaeli et al., 1988). Therefore, human MAbs (HMAbs) are more appropriate for passive immunoprophylaxis. Immunization and immortalization of human lymphocytes to obtain HMAbs is still more difficult than deriving mouse MAbs (for a review see Masuho, 1988). We have established a method for efficiently generating HMAb-producing hybridomas by a combination of in vitro immunization and cell fusion (Masuho et al., 1986; Matsumoto et al., 1986), and we have used that method to generate HMAbs to VZV (Sugano et al., 1987). These HMAbs recognize viral glycoprotein (gp) I or gpII, and are able to neutralize VZV particles; Foung et al. (1985) have also produced an anti-gpII HMAb. Studies with murine MAbs (Forghani et al., 1984; Keller et al., 1987) have shown that, unlike anti-gpI and anti-gpII MAbs, an anti-gpIII MAb not only neutralizes virus strongly without complement, but also inhibits the spread of...
infection. Therefore, it is likely that an anti-gpIII HMAb would be better than anti-gpI and anti-gpII HMAbs in protecting the host.

In this study, we prepared an anti-gpIII HMAb by developing an assay system for screening anti-gpIII HMAbs, and we studied its antiviral activity in comparison to those of anti-gpI and anti-gpII HMAbs.

**Methods**

**Virus and cell culture.** VZV strains were propagated and titrated in human embryonic lung (HEL) cells by standard procedures. The Kobayashi and Kubo strains were kindly provided by Dr. S. Shigeta, Fukushima Medical College, Japan. Cell-free virus was prepared by sonication of VZV-infected cells and centrifugation of the cell homogenate.

**Generation of hybridomas.** The in vitro immunization of human lymphocytes and fusion with myeloma cells were performed by the methods described previously (Masuho et al., 1986; Sugano et al., 1987), with minor modifications. Briefly, human lymphocytes were obtained from the spleen of a patient with idiopathic thrombocytopenia purpura who had not had an episode of VZV infection during the observation period. The splenic cells were cultured for 6 days in the presence of VZV antigens and B cell growth factor. VZV antigens were prepared by sonication of cells infected with the Oka strain and by centrifugation of the homogenate on a sucrose density gradient. The lymphocytes immunized in vitro were fused with murine myeloma P3 × 63Ag8U1 cells and fused cells were cultured in GIT medium (Wako Pure Chemical Industries) containing hypoxanthine, aminopterin and thymidine. When macroscopic cell colonies appeared, the culture supernatants were screened by the ELISA described below. The parental hybridoma cells producing an anti-gpIII HMAb were subcloned several times by limiting dilution to establish a stable clone.

**HMAbs and murine M Abs.** Hybridomas V1, V2 and V3 were cultured in serum-free medium, and HMAbs were purified from the culture supernatants on a Protein A-Sepharose column (Pharmacia). HMAb C23 is an IgG1 antibody specific for cytomegalovirus (CMV) (Masuho et al., 1987). The isotype of the HMAbs was determined by immunodiffusion with sheep antibodies specific for each subclass of human IgG (Miles Laboratories) and by ELISA with alkaline phosphatase-conjugated goat antibodies to each human λ or κ chain (Tago Inc.). The isoelectric point of each HMAb was determined by isoelectrofocusing in a pH 3 to 10 gradient.

A murine MAb to gpI, designated no. 9, and an MAb to gpII, designated no. 8, were provided by Professor K. Yamanishi, Osaka University, Japan. A murine MAb to gpIII, designated 4B7, has been described previously (Forghini et al., 1984).

**Cell homogenate ELISA and cell monolayer ELISA.** Culture supernatants of the hybridomas were screened for anti-gpIII HMAbs by two types of ELISA. In one ELISA, plates were coated with a homogenate of VZV-infected cells. HEL cells infected with the Oka strain were harvested when 80% of the cells showed c.p.e. and disrupted by sonication in PBS containing 1 mM-MgCl₂, 1 mM-CaCl₂ and 1 mM-PMSF. The homogenate was centrifuged at 2000 g for 20 min and the supernatant was used as a cell homogenate antigen. The antigen was coated onto plates at a concentration of 4 μg/ml. Further processing was as described previously (Sugano et al., 1987).

In the other ELISA, plates were coated with a monolayer of VZV-infected cells. HEL cells were cultured in 96-well, flat-bottomed culture plates and infected by dispensing 1000 cells infected with the Oka strain into each well. After 2 days, the plates were washed with PBS containing 1 mM-MgCl₂ and 1 mM-CaCl₂ [PBS(+)], and then fixed with 0.1% glutaraldehyde at 37 °C for 10 min. The plates were washed again and blocked with Hanks’ balanced salt solution containing 1% bovine serum albumin (BSA). In this ELISA, horseradish peroxidase-conjugated goat antibody to human IgG (Tago Inc.) and tetramethyl benzidine (Dojinkin Chemical) were used as the second antibody and the enzyme substrate, respectively. Hybridomas which generated culture supernatants which were more positive on the cell monolayer than on the cell homogenate were selected as possible anti-gpIII HMAb producers.

**Immunoprecipitation and SDS-PAGE.** HEL cells were infected with the Oka strain or mock-infected. When c.p.e. appeared in 80% of the cells, [35S]methionine (1.84 MBq/ml, 48 TBq/nmol) was added to the culture medium and the cells were cultured at 37 °C for 18 h. The cells lysed with detergents were mixed with each HMAb, immune complexes were precipitated with Protein A-Sepharose, and the precipitated proteins were analysed by SDS-PAGE and autoradiography as described previously (Fujinaga et al., 1987).

In an additional experiment, gpII was removed before immunoprecipitation with HMAb V3. VZV antigens labelled by the above method were mixed with HMAb V1 and then with Protein A-Sepharose, and the immune complexes were removed along with the Sepharose by centrifugation. Residual antigen in the supernatant was immunoprecipitated with HMAb V3.

**Neutralization of virus infectivity.** This study was based on the method described previously (Sugano et al., 1987). Briefly, 400 p.f.u. of each virus was treated with serially diluted antibodies in the presence or absence of 20-fold diluted fresh guinea-pig serum as a complement source. Residual virus was titrated by plaque assay. The neutralization activity of an antibody was expressed as the concentration necessary to reduce the number of virus plaques by 50% (ED50).

**Inhibition of spread of virus infection.** The Kawaguchi strain was inoculated onto HEL cells on 96-well plates at a dose of 500 p.f.u./well and the plates were kept at 37 °C for 4 h. Infected cells were washed three times with PBS(+) and then cultured in Eagle’s MEM supplemented with 5% foetal calf serum (FCS) containing HMAb at 37 °C for 2 to 3 days. When c.p.e. appeared in 80 to 100% of the cells in the wells without HMAb, the cell monolayers were washed three times with PBS(+) and then fixed with 0.1% glutaraldehyde at 37 °C for 10 min. After washing with PBS(+)-BSA, VZV antigens on the cells were allowed to react with 1 μg/ml HMAb V2 at room temperature for 1 h. After washing, they were allowed to react with peroxidase-conjugated goat antibody to human IgG (diluted 200-fold; Tago Inc.) at room temperature for 1 h. VZV antigens were finally visualized with 0.2 mg/ml 4-chloro-l-naphthol and 5 mM-H₂O₂ in 0.05 M-Tris-HCl pH 7.0. Infected cells were observed under a microscope. For quantification of virus spread, tetramethylbenzidine was used as the enzyme substrate and the absorbance at 450 nm was measured using an ELISA reader.

**Antibody-dependent cellular cytotoxicity (ADCC).** Uninfected HEL cells were mixed with HEL cells infected with the Oka strain at an infected:uninfected cell ratio of 5:1. The cells were cultured for 24 h, detached from the flask and suspended in 20% FCS-RPMI 1640 at a density of 1 x 10⁶ cells/ml. Na⁺¹CrO₄ (7.4 MBq, 9.3 to 19 GBq/mg Cr; Amersham) was added to 1 ml of the cell suspension and cells were labelled by incubation at 37 °C for 60 min. After washing, they were resuspended in 20% FCS-RPMI 1640 at a density of 5 x 10⁶ cells/ml. A 100 μl aliquot of the target cell suspension was dispensed into each well on a round-bottomed, 96-well plate and a 50 μl aliquot of various antibody solutions was added.

Human spleen cells were used as effector cells. Mononuclear cells were separated by centrifugation on a Ficoll-Paque layer and
A human anti-VZV gpIII MAb

Figure 1. Reactivity of murine MAbs to gpI (no. 9; △), gpII (no. 8; □) and gpIII (4B7; ○) in a cell homogenate ELISA (a) and cell monolayer ELISA (b).

suspended in 20% FCS-RPMI 1640 at a density of 1 × 10⁷ cells/ml. A 100 µl aliquot of the suspension was dispensed into each well containing target cells. The cell mixtures were incubated in a CO₂ incubator for 18 h, and the culture supernatants were harvested using a Skatron supernatant collection system. The radioactivity was determined using a gamma scintillation counter (Aloka). Total ⁵¹Cr release and spontaneous release were measured by the addition of 2% Triton X-100 to the cell mixture and using a control which contained no antibody, respectively. The ⁵¹Cr release of test samples (%) was calculated from the formula [(sample d.p.m. - spontaneous d.p.m.)/(total release d.p.m. - spontaneous d.p.m.) × 100]. The values are expressed as the mean of three experiments.

Complement-dependent cytotoxicity (CDC). ⁵¹Cr-Labelled target cells were prepared as described above. Each 100 µl aliquot of the target cell suspension (5 × 10⁴ cells/ml) was mixed with 50 µl of serially diluted antibody solution and 100 µl of baby rabbit complement diluted four-fold (Pel-freeze), and incubated at 37 °C for 2 h. The negative control was a mixture of target cells and complement without antibody. The values for ⁵¹Cr release were determined in the same way as for ADCC.

Results

Screening for HMAbs to gpIII

The reactivity of a murine anti-gpIII MAb, 4B7, was examined by our cell homogenate ELISA, but no reactivity could be detected (Fig. 1a). However, the immunofluorescence of VZV-infected cells using 4B7 was as intense as that using murine anti-gpI or -gpII MAbs (data not shown). We therefore developed a cell monolayer ELISA and 4B7 showed high reactivity with gpIII in this test (Fig. 1b). Therefore, we concluded that HMAbs which were negative in a cell homogenate ELISA but positive in a cell monolayer ELISA are directed against gpIII.

The culture supernatants of hybridomas in 96-well culture plates were screened by the two ELISA techniques (Fig. 2). By screening five culture plates (480 wells), 125 wells (26%) were found to be positive in both ELISAs, whereas five wells (1.1%) were negative or very weak in the cell homogenate ELISA but positive in the cell monolayer ELISA. One of these clones, V3, was established successfully.

Characteristics of hybridoma V3 and its HMAbs

VZV antigens were immunoprecipitated and analysed by SDS-PAGE to identify the antigens recognized by HMAb V3. Unlike gpI and gpII, HMAb V3 immunoprecipitated a single molecule of 115K to 120K (Fig. 3, lane 7) which was indistinguishable from the largest molecule of gpII (lane 5). However, if gpII was removed before immunoprecipitation with V1, the antigen recognized by V3 remained (lanes 11 and 12). These results imply that HMAb V3 recognizes gpIII of VZV. The reactivities of V3 with a homogenate and monolayer of VZV-infected cells were compared with the reactivities of HMAbs V2 and V1, which are directed against gpI and gpII, respectively (Fig. 4). Similarly to its murine counterpart, V3 was only slightly reactive in the cell homogenate ELISA but as strongly reactive as V1 and V2 in the cell monolayer ELISA.

The V3 hybridoma cells generated by cell fusion between human lymphocytes and murine myeloma cells had between 90 and 120 murine, and between six and 11 human chromosomes. They grew in serum-free medium
Fig. 3. Immunoprecipitation analysis of the viral antigen recognized by HMAb V3. VZV-infected HEL cells (lanes 1, 3, 5, 7, 9, 11 and 12) and mock-infected HEL cells (lanes 2, 4, 6, 8 and 10) were radiolabelled with [35S]methionine and lysed with detergents. The cell lysates were immunoprecipitated with human VZV-positive serum (lanes 1 and 2), HMAb V2 (lanes 3 and 4), HMAb V1 (lanes 5, 6 and 11), HMAb V3 (lanes 7, 8 and 12), and murine MAb 4B7 and rabbit anti-mouse IgG serum (lanes 9 and 10). In lanes 11 and 12, gplI was removed before immunoprecipitation using HMAb V1.

with a doubling time of 26.5 h and continued producing the V3 HMAb for at least 6 months at a production rate of 8.5 µg/10⁶ cells/24 h. The isotype of HMAb V3 was IgG1, and its isoelectric point ranged from pI 8.5 to pI 9.5.

Fig. 4. Reactivity of HMAbs to gpl (V2; △), gpII (V1; □) and gpIII (V3; ○) in the cell homogenate (a) and cell monolayer (b) ELISA. HMAb C23, to CMV, was used as a control (▲).

Virus neutralization by HMAb V3

The ability of HMAb V3 to neutralize virus infectivity was determined by measuring the reduction in the number of plaques. V3 showed very high neutralization activity against the Kawaguchi strain of VZV (Fig. 5); the ED₅₀ value was about 0.03 µg/ml and virus infectivity was completely neutralized at V3 concentrations of 1 µg/ml or more. Addition of complement did not affect neutralization. The neutralization activity of V3 was 15000-fold that of normal human serum gammaglobulin (NHSG) in the absence of complement and 2600-fold greater in the presence of complement.

The neutralization of different strains of VZV by V3 was compared with that of an HMAb to gpII, V1, and an HMAb to gpl, V2 (Table 1). The dependence on complement of these three HMAbs differed markedly. Complement augmented neutralization by V1 was essential for neutralization by V2 and hardly affected neutralization by V3; V3 was as active as V2 and more active than V1 in the presence of complement. The ED₅₀ value of V3 in the presence of complement was within a narrow range between 0.03 and 0.05 µg/ml, suggesting that the epitope recognized by V3 is conserved in different VZV strains.

Inhibition of virus spread by HMAb V3

VZV is a cell-associated virus and infection spreads not only through production of cell-free virus but also by cell-to-cell transmission. Experiments were conducted to determine the effect of HMAbs on virus spread from infected to uninfected cells. After establishment of virus infection at low multiplicity, cell monolayers were cultured in the presence of different HMAbs and infectious foci were visualized by immunostaining with V2 (Fig. 6). Neither V1 nor V2 had any effect, virus
A human anti-VZV gpIII MAb

Fig. 6. Inhibition of virus spread by HMAbs as observed by microscopy. HEL cell monolayers were infected with the Kawaguchi strain (a to e) or mock-infected (f), and, 4 h post-infection, 50 µg/ml V1 (a), 50 µg/ml V2 (b), and 12.5 (c) or 6.3 (d) µg/ml V3 was added to the culture medium. After a 3 day culture, infected cells were immunostained with V2.

Table 1. Neutralization of VZV strains by HMAbs to gpI, gpII and gpIII*

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>V1 (anti-gpII)</th>
<th>V2 (anti-gpI)</th>
<th>V3 (anti-gpIII)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C−</td>
<td>C+</td>
<td>C−</td>
</tr>
<tr>
<td>VZV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kawaguchi</td>
<td>4.0</td>
<td>0.84</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Oka</td>
<td>1.8</td>
<td>1.2</td>
<td>&gt;10</td>
</tr>
<tr>
<td>CaQu</td>
<td>3.5</td>
<td>0.28</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Kobayashi</td>
<td>1.4</td>
<td>0.50</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Kubo</td>
<td>4.5</td>
<td>0.46</td>
<td>&gt;10</td>
</tr>
<tr>
<td>HSV-1 KOS</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>HSV-2 UW-268</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
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* Neutralization titres of HMAbs in the presence (+) or absence (−) of guinea-pig complement (C) were determined by virus plaque assay and expressed as the ED_{50} (µg/ml).

c.p.e. appearing in all the cells in these culture wells, as in the negative control. By contrast, V3 restricted the viral antigen-positive cells to small foci. Virus spread was quantified by measuring the absorbance at 450 nm using an ELISA reader (Fig. 7). V3 completely inhibited virus spread at about 10 µg/ml, whereas neither V1 nor V2, or the control HMAb, C23, caused inhibition, even at high concentration. NHSG did not inhibit virus spread at a concentration of 1 mg/ml (data not shown).

ADCC and CDC activities of HMAb V3

An antibody can inhibit virus replication in infected cells by exerting cytotoxicity in collaboration with effector cells and effector molecules. The cytotoxic activity of V3 against infected cells was determined in comparison with that of V1 or V2. Human splenic mononuclear cells were used as effector cells for ADCC. In this experimental system VZV-seropositive serum showed cytotoxicity in a
Fig. 7. Dose–response curves for inhibition of virus spread by HMAbs. VZV-infected cells were cultured in the presence of V1 (□), V2 (△), V3 (○) or C23 (▲). After immunostaining of infected cells, virus spread was assessed by measuring the absorbance at 450 nm. An increase in absorbance at high HMAb concentrations was caused by non-specific binding of HMAbs to cells.

Fig. 8. ADCC activity of HMAbs against VZV-infected cells. VZV-infected cells were labelled with $^{51}$Cr and incubated with human splenic mononuclear cells at a ratio of 1:100 in the presence of V1 (□), V2 (△), V3 (○), and VZV-seropositive (●) or seronegative (▲) serum. $^{51}$Cr release was calculated as a percentage using the formula in Methods.

Fig. 9. CDC activity of HMAbs against VZV-infected cells. VZV-infected cells were labelled with $^{51}$Cr and incubated with a combination of rabbit complement and V1 (□), V2 (△), V3 (○), and VZV-seropositive (●) or seronegative (▲) serum.

Dose-dependent manner whereas seronegative serum did not (Fig. 8). The cytotoxicity of V3 caused a maximum $^{51}$Cr release of about 10% at a V3 concentration as low as 20 ng/ml; this value was lower than that caused by polyclonal antibodies. Of three HMAbs, V3 (anti-gpIII) showed the most potent ADCC activity, and V1 (anti-gpII) was more potent than V2 (anti-gpl). The CDC activities of HMAbs were determined with rabbit complement, which works better than human complement in this system (Fig. 9). Seropositive serum showed $^{51}$Cr release of about 70%, whereas seronegative serum did not cause release. In this system, V3 did not exert cytotoxicity even at high concentrations, whereas V1 and V2 did, V1 again being more potent than V2. Therefore, the order of the CDC activity of these three HMAbs was different from that of their ADCC activity.

Discussion

Unlike polyclonal antibodies, an MAb is directed against a single epitope of a single antigenic molecule in a virus particle. Although only the antibody titre to a virus has been considered in passive immunization with human serum gammaglobulin, it is important to study the antiviral activity of MAbs that recognize different viral antigens. Some MAbs protect a host against virus infection by means of virus neutralization and/or cytotoxicity to infected cells, but others show no protection. For example, we have obtained many HMAbs to CMV, most of which recognize an immunodominant tegument antigen and are incapable of either neutralization or binding to the surface of infected cells (Masuho et al., 1987). Others not only fail to protect the host but even enhance infectivity. HMAbs to gp41 of human immunodeficiency virus, for example, enhance virus infection in the presence of complement (Robinson et al., 1990). Some murine MAbs to viruses including herpesviruses cross-react with uninfected human tissues, suggesting that such antibodies may be capable of...
Table 2. Antiviral activity of HMAbs against gpI, gpII and gpIII

<table>
<thead>
<tr>
<th>HMAb</th>
<th>Isotype</th>
<th>Antigen</th>
<th>Neutralization</th>
<th>Inhibition of virus spread</th>
<th>ADCC</th>
<th>CDC</th>
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<tbody>
<tr>
<td>V1</td>
<td>IgG1, λ</td>
<td>gpII</td>
<td>C−</td>
<td>−</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>V2</td>
<td>IgG1, λ</td>
<td>gpI</td>
<td>C−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V3</td>
<td>IgG1, κ</td>
<td>gpIII</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>−</td>
</tr>
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</table>

The ED50 values of V3 in the presence of complement range between 0.03 and 0.05 μg/ml, and the activity of V3 is 2600-fold that of NHSG. Passive immunization against VZV infection has been attempted with varicella-zoster and zoster immunoglobulins (VZIG and ZIG), which have high titres to VZV (Orenstein et al., 1981; Zaia et al., 1983); however these titres are only up to 10-fold that of NHSG (Paryani et al., 1984). Therefore, V3 should be much more potent than VZIG and ZIG for passive immunization.

V3 is capable of inhibiting virus spread from infected to uninfected cells, whereas V1 and V2 are not. The mechanism of the inhibition remains to be elucidated, but it may be explained in terms of neutralization. The neutralization activity of V3 is very high even in the absence of complement, and therefore V3 may neutralize virus within the short period of time in which virus is shed from infected cells and penetrates adjacent cells. Oakes & Lausch (1984) have shown that non-neutralizing MAbs to HSV suppress virus replication which occurs inside cells. Similar suppression may also be involved in the inhibition of virus spread by V3.

Cytotoxicity to infected cells is one of the mechanisms by which an antibody protects a host against virus infection. Since V1, V2 and V3 induce membrane fluorescence on infected cells, and belong to the IgG1 isotype which binds to the Fc receptors of effector cells and activates complement, they were expected to be capable of ADCC and CDC. In fact, they all show ADCC activity, with V3 being the most active. Kohl et al. (1990) compared the ADCC, neutralization and in vivo protective activity of antibodies to different antigenic epitopes of HSV, and found that ADCC activity is associated with in vivo protection at high challenge dose. Their finding suggests that V3 will protect the host by ADCC.

V3 does not exert CDC even at a concentration 1000-fold greater than that used for ADCC, whereas V1 and V2 have CDC activity. A murine MAb to gpIII also induces ADCC but not CDC activity (Ito et al., 1985). Their and our experiments on CDC were performed with animal complement instead of human complement. Since human cells bear membrane proteins which inhibit causing autoimmune disease (Srinivasappa et al., 1986). These findings led us to investigate different HMAbs for their potential application to passive immunization.

HMAbs to gpI and gpII have been described previously (Sugano et al., 1987), and an HMAb to gpIII was prepared in this study. Initially, we tried to obtain anti-gpIII HMAbs from many HMAbs found to be positive by a cell homogenate ELISA, but none of these were directed to this antigen. Subsequently, a murine anti-gpIII MAb, 4B7, was found to be completely negative in the cell homogenate ELISA but positive in a cell monolayer ELISA, whereas murine anti-gpI and -gpII MAbs were positive in both ELISAs. Thus, 4B7-like HMAbs to VZV were selected on the basis of results like HMAbs to VZV were selected on the basis of results

The antiviral activity of V3 (anti-gpIII) was compared with that of V1 (anti-gpII) and V2 (anti-gpI) (Table 2). Neutralization by V3 is more potent and less dependent on complement than that by V1 and V2; murine anti-gpIII MAbs have similar neutralization profiles (Grose et al., 1983; Forghani et al., 1984; Keller et al., 1984). These results suggest that gpIII plays an important role in virus infection.

VZV gpIII is homologous with gH of herpes simplex virus (HSV) (McGeoch & Davison, 1986; Gompels & Minson, 1986; Davison & Scott, 1986). Both V3 (anti-gpIII) and an anti-gpIII murine MAb inhibit virus spread (Keller et al., 1987), which is similar to the activity of anti-gH antibodies (Buckmaster et al., 1984; Gompels & Minson, 1986). These results suggest similarities between the biological properties of gH and gpIII. Fuller et al. (1989) have shown that antibodies to gH permit virus attachment to cells, but prevent penetration. Therefore, V3 may inhibit the penetration of VZV into cells.
attack by human complement (Okada et al., 1989), the CDC observed in vitro may not occur in the human body.

Which HMAb is expected to be most effective in passive immunization against VZV infection? V3 (anti-gpIII) is more active than V1 (anti-gpII) and V2 (anti-gpI) in terms of neutralization, inhibition of virus spread and ADCC, but less effective in CDC. Therefore, V3 appears to be the best candidate for passive immunization. GpIII may be essential for infection because HSV mutants lacking gH, a homologue of gpIII, are incapable of causing infection (Desai et al., 1988). Dubey et al. (1988) reported that leukaemic vaccinees who have serum antibodies to gpIII are protected against varicella upon household exposure, although the relevance of this is still under debate (Brunell et al., 1987).

There are two animal models of VZV infection: guinea-pigs infected with VZV (Myers et al., 1980) and monkeys infected with simian varicella virus (Soike et al., 1987), although the pathogenicity of the former is limited. V3 does not cross-react with simian varicella virus, whereas V1 and V2 cross-react slightly (K. F. Soike, personal communication). Our previous study has shown the protective efficacy of an anti-HSV HMAb in vivo (Fujinaga et al., 1987). Anti-gB HMAbs are much more effective in an animal infection model than in neutralization experiments compared to polyclonal NHSG, suggesting that the protective effect of an MAb is amplified by the combination of various effectors in vivo. The neutralization activity of V3 is much greater than that of NHSG and so V3 should be even more effective for passive immunization.

Passive immunization with NHSG, VZIG or ZIG has been shown to modify the clinical course of infectious disease only when administered at high dose and at an early stage of infection (Orenstein et al., 1981; Zaia et al., 1983; Miller et al., 1989). A high titre HMAb to VZV could be useful for prophylaxis in high-risk patients, especially immunocompromised patients, who respond poorly to vaccines. We have finished a phase I clinical trial on an HMAb to CMV (Azuma et al., 1991). This HMAb, which is of the same IgG1 isotype as V3, has a long half-life of 20 to 25 days. No antibody responses, including an anti-idiotypic response, have been detected in immunocompetent volunteers, and no side-effects have been observed. In conclusion, V3 is anticipated to provide a very safe means of passive immunoprophylaxis against VZV infectious disease.

References


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