Human Leukocytes Kill Varicella-Zoster Virus-Infected Fibroblasts in the Presence of Murine Monoclonal Antibodies to Virus-Specific Glycoproteins

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Seven murine monoclonal antibodies reacting with major glycoproteins of varicella-zoster virus were tested for functional activity in assays for antibody-dependent cellular cytotoxicity (ADCC) and antibody-plus-complement-mediated lysis. Human peripheral blood mononuclear cells killed varicella-zoster virus-infected fibroblasts in the presence of three of four monoclonal antibodies directed against gp98/62 and a single monoclonal antibody directed against gp118. Neither of two monoclonal antibodies directed against gp66 was able to mediate ADCC. In 18-h assays, adherent effector cells were more active than nonadherent effector cells in mediating ADCC. Adherent cells treated with anti-Leu-11b and complement retained their cytotoxic activity, suggesting that monocytes are responsible for most of the adherent-cell-mediated cytotoxicity. Both immunoglobulin G1 and G2a murine monoclonal antibodies were able to participate in ADCC. Of the two immunoglobulin G2a monoclonal antibodies tested, both of which reacted with gp98/62, only one mediated lysis in the presence of complement. These results indicate that some murine monoclonal antibodies against major glycoproteins of varicella-zoster virus have functional activity in cytotoxicity assays.

The mechanisms by which specific antibodies contribute to the prevention of or recovery from varicella-zoster virus (VZV) infections remain largely undefined (3). The administration of high-titer antibodies soon after exposure can abort or modify a primary VZV infection; however, once the infection is established, the administration of antibodies seems to have little effect. Antibodies may neutralize extracellular virus, but since VZV is highly cell associated, antibodies may act primarily by mediating lysis by antibody and complement (C) or antibody-dependent cellular cytotoxicity (ADCC) against VZV-infected targets (4, 7; G. D. Cross, T. L. Cromeans, and S. L. Shore, *Fed. Proc.* **40**:988, 1981).

For antibody-dependent lysis to occur, antibodies must presumably bind to virus-specified antigens detected on the surfaces of VZV-infected cells (15). Recently, murine monoclonal antibodies reacting with major VZV glycoproteins on the membranes of infected cells were described (5, 6). In the present study, a panel of these monoclonal antibodies was tested for their ability to mediate cytotoxicity. In the process, the role of specific VZV glycoprotein antigens was further defined.

MATERIALS AND METHODS

Preparation of target cells. VZV-infected and noninfected target cells were prepared as previously described (7). Briefly, FS-4 strain human foreskin fibroblasts (National Institute of Allergy and Infectious Diseases, Bethesda, Md.) were infected with cell-free Ellen strain VZV (American Type Culture Collection, Rockville, Md.) or OKA strain VZV (kindly provided by Maurice Hilleman, Merck Sharpe & Dohme, West Point, Pa.) at a multiplicity of infection of 0.01. When 80 to 90% of the cell sheet showed a cytopathic

effect, cells were removed with 0.1% EDTA-0.25% trypsin and added to confluent, noninfected FS-4 cells at a ratio of one flask of infected cells to five flasks of noninfected cells. After 48 h of incubation at 37°C, the infected cells and the noninfected control cells were removed with EDTA-trypsin and suspended in 7.5% fetal bovine serum (FBS; HyClone, Logan, Utah)-10% dimethyl sulfoxide (Sigma Chemical Co., St. Louis, Mo.). Aliquots of 10⁶ cells in 1-ml volumes were kept at -70°C for 24 h and then transferred to a liquid nitrogen freezer. In assays for fluorescent antibody to membrane antigen (FAMA, [15]), 71% of the Ellen-infected targets and 69% of the OKA-infected targets demonstrated fluorescence. Greater than 95% of the target cells were viable after being thawed, as demonstrated by trypan blue exclusion.

Preparation of effector cells. Mononuclear cells from normal adults were obtained by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) centrifugation of peripheral blood. The cells were washed two times with Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) and suspended in RPMI-1640 (GIBCO) with 20% FBS. Adherent and nonadherent cells were obtained as previously described (9). Briefly, 60-mm petri dishes (Lux Scientific Corp., Newbury, Calif.) were incubated overnight with 2 ml of heat-inactivated FBS. On the next day, the dishes were rinsed three times with phosphate-buffered saline, and mononuclear cells obtained by Ficoll-Hypaque centrifugation were added at 10⁷ cells per dish in 5 ml of RPMI-1640 with 10% FBS. After 1 h of incubation at 37°C, the nonadherent cells were removed, and the dishes were rinsed five times with RPMI-1640. The adherent cells were then removed by incubation at 37°C with 0.37% EDTA in phosphate-buffered saline. Adherent cells were determined to be greater than 98% monocytes by nonspecific esterase staining, whereas fewer than 5% esterase-positive cells were found in the nonadherent populations.

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In selected experiments, adherent and nonadherent cells were treated with anti-Leu-11b (Becton Dickinson and Co., Mountain View, Calif.) and baby rabbit C (Pel-Freez, Rogers, Ariz.) to remove natural killer-killer cells (J. S. Thompson, N. E. Goeken, S. A. Brown, J. L. Rhoades, and J. L. Palsis, submitted for publication). Mononuclear cells (10^6) in 100 μ l of RPMI-1640 were added to microtiter plate wells and mixed with 0.1 μ g of anti-Leu-11b for 45 min at room temperature. The plates were then centrifuged at $100 \times g$ for 3 min. The supernatants were aspirated, and the cells were suspended in 100 μ l of baby rabbit C diluted 1:2 in RPMI-1640. The plates were rocked at room temperature for 60 min and washed once, and the cells were suspended in RPMI-1640. The number of viable cells recovered was determined by trypan blue exclusion.

Murine peritoneal exudate cells (PEC) were obtained from 8- to 10-week-old BALB/c mice (Jackson Laboratories, Bar Harbor, Maine). Mice were injected intraperitoneally with 2 ml of thioglycolate (29.5 g/liter; BBL Microbiology Systems, Cockeysville, Md.). Three days later, the mice were sacrificed, and 5 ml of RPMI-1640 with 10% FBS was injected into the peritoneal cavity. The fluid was then removed through a small peritoneal incision, and the peritoneal cavity was washed twice with media. The cells obtained were determined to be 80% monocytes on the basis of morphology and phagocytosis of latex particles.

Monoclonal antibodies. The preparation and characterization of six of the monoclonal antibodies used in this study have been reported elsewhere (5, 6). 6B7 is a monoclonal antibody against gp98/62 which was produced in the same fusion as that described in reference 5. Antibodies 3B3, 6B7, 10G4, and 14A7 precipitate VZV gp98/62, 9C6 precipitates gp118, and 151 and 158 precipitate gp66. 151 and 158 were raised against the OKA strain of VZV, whereas all of the other monoclonal antibodies were raised against the VZV-32 strain (5). All of the antibodies were positive in assays for FAMA; however, only 9C6 has neutralizing activity (5). A monoclonal antibody against human cytomegalovirus, kindly provided by Susan Michelson (Institut Pasteur, Paris, France) was used as a control. The antibodies used in this study were in the form of ascites fluid. In selected experiments, the ascites fluids were ultracentrifuged at $100,000 \times g$ for 20 min.

ADCC assays. ADCC assays were performed as previously described (7). Frozen target cells were rapidly thawed, washed two times in Hanks balanced salt solution with 5% FBS, and then labeled with $\text{Na}_2^{51}\text{CrO}_4$ (New England Nuclear Corp., Boston, Mass.). After 1 h of incubation, the labeled cells were washed two times in Hanks balanced salt solution with 5% FBS and suspended in RPMI-1640 with 20% FBS at a concentration of 5×10^4 /ml. Aliquots (0.1 ml) of target cells were added to round-bottomed microtiter plate wells (Flow Laboratories, Inc., McLean Va.), and 10 μ l-aliquots of monoclonal antibodies or pooled seropositive or seronegative human sera were added. The serum pools were prepared from the sera of individuals with known serological status and had titers of 128 (seropositive) and <2 (seronegative) in assays for FAMA. After 30 min of incubation at 4°C, 0.1-ml aliquots of effector cells or media were added. The plates were centrifuged at $100 \times g$ for 3 min and then incubated at 37°C for 4 h. In some experiments, 18-h incubation periods were used. The upper 100 μ l of media was gently removed from each well, and the ^{51}Cr activity was determined by gamma scintillation counting. The percent ^{51}Cr release was calculated with the following formula: $[(\text{cpm}_{\text{experimental}} - \text{cpm}_{\text{spontaneous}})/(\text{cpm}_{\text{total}} - \text{cpm}_{\text{spontaneous}})]$

$\times 100$, where cpm is counts per minute, $\text{cpm}_{\text{experimental}}$ is the release occurring in the presence of effector cells, $\text{cpm}_{\text{total}}$ is the release occurring after the addition of 1% Triton X-100 to target cells, and $\text{cpm}_{\text{spontaneous}}$ is the release occurring with media alone. Spontaneous release averaged 18.8% for noninfected targets and 15.2% for infected targets in 4-h assays and 33.6% for noninfected targets and 38.9% for infected targets in 18-h assays. The percent ADCC was defined as the percent ^{51}Cr release occurring in the presence of seropositive sera minus the percent ^{51}Cr release occurring in the presence of seronegative sera. All determinations were done in triplicate.

Antibody-plus-C-mediated lysis. Aliquots (0.1 ml) of ^{51}Cr -labeled VZV-infected target cells were added to microtiter plate wells. Aliquots (20 μ l) of dilutions of either monoclonal antibodies or seropositive or seronegative human serum pools were added to triplicate wells. The plates were incubated for 30 min at 37°C. Aliquots (10 μ l) of nondiluted guinea pig C (GIBCO) and 0.07 ml of media were added to each well to yield a final volume of 0.2 ml (final C dilution, 1:20). The plates were incubated for 4 h at 37°C. The top 100 μ l was then removed for gamma scintillation counting. The percent ^{51}Cr release was calculated with the same formula as described above, except that $\text{cpm}_{\text{experimental}}$ was the release occurring in the presence of antibody and guinea pig C.

RESULTS

Monoclonal-antibody-mediated cellular cytotoxicity. As previously reported (7), peripheral blood mononuclear cells killed Ellen and OKA strain VZV-infected target cells in the presence of pooled seropositive human sera, whereas there was little or no killing in the presence of seronegative sera (Table 1). Infected target cells were also significantly lysed in the presence of four of the monoclonal antibodies tested, 3B3, 6B7, 9C6, and 10G4, whereas three of the monoclonal antibodies tested, 151, 158, and 14A7, did not mediate

TABLE 1. Mononuclear-cell-mediated cytotoxicity against VZV-infected targets in the presence of human and murine antibodies^a

Antibody	Mean % ^{51}Cr release $\pm$ SD from VZV-infected targets (no. of expt)	
	Ellen	OKA
Pooled human sera		
Seronegative	3.1 $\pm$ 1.8 (3)	1.2 $\pm$ 1.0 (14)
Seropositive	26.5 $\pm$ 3.9 (3)	32.7 $\pm$ 8.1 (14)
Murine monoclonal		
3B3	7.9 $\pm$ 0.8 (3) ^b	6.4 $\pm$ 1.5 (18) ^b
6B7	8.4 $\pm$ 0.6 (3) ^b	7.4 $\pm$ 2.6 (21) ^b
10G4	ND ^c	8.5 $\pm$ 2.7 (16) ^b
14A7	ND	1.3 $\pm$ 1.2 (15)
9C6	7.0 $\pm$ 1.8 (3) ^b	6.9 $\pm$ 3.0 (18) ^b
151	2.7 $\pm$ 0.6 (3)	2.0 $\pm$ 1.5 (16)
158	4.0 $\pm$ 1.1 (3)	ND
Cytomegalovirus monoclonal antibody 57/19	0.0 (3)	0.0 (4)

^a Pooled human sera and murine ascites fluids were used at final dilutions of 1:200. Effector cell/target cell ratios of 50:1 were used. The incubation time was 4 h.

^b $P < 0.001$ as compared with the percent ^{51}Cr release in the presence of pooled seronegative human sera or a monoclonal antibody to cytomegalovirus; determined by Student's *t*-test.

^c ND, Not determined.

FIG. 1. Mononuclear-cell-mediated cytotoxicity against VZV-infected targets in the presence of various dilutions of human and murine antibodies. Results of a representative experiment are shown as percent ^{51}Cr release in the presence of pooled seropositive human sera (●) and murine monoclonal antibodies 6B7 (▲), 3B3 (○), 9C6 (□), and 14A7 (■).

ADCC. Noninfected target cells incubated with monoclonal antibodies or with seropositive sera were not lysed (results not shown). OKA-infected targets were used in all subsequent experiments. The magnitude of killing of infected targets was significantly lower with monoclonal antibodies than with pooled seropositive human sera. This was also the case when various dilutions of selected monoclonal antibodies and pooled human sera were tested (Fig. 1). Failure to detect increased cytotoxicity with lower dilutions of monoclonal antibodies suggested that aggregates in the monoclonal antibody preparations might be inhibiting ADCC; however, similar results were obtained after ultracentrifugation ($100,000 \times g$ for 20 min) of ascites fluids. In subsequent experiments, final dilutions of 1:200 were used for both human sera and murine monoclonal antibodies.

Combinations of monoclonal antibodies were tested for their ability to mediate killing. A few combinations of two antibodies appeared to be slightly additive or antagonistic; however, none of the results was statistically significant (results not shown). Combinations of three or four monoclonal antibodies also failed to mediate significantly increased cytotoxicity.

Effector-cell-mediated ADCC. Peripheral blood mononuclear cells were separated into adherent and nonadherent populations, as described above. In 4-h assays, nonadherent cells appeared to be more effective than adherent cells in mediating ADCC in the presence of pooled human sera (Table 2). In contrast, adherent cells appeared to be more effective than nonadherent cells in mediating ADCC in the presence of murine monoclonal antibodies. These differences were not statistically significant, because of the small number of experiments performed, but suggested that longer incubation times should be tried, as monocytes, the predominant cells in adherent populations, were previously shown to require a longer incubation period than lymphocytes to mediate maximal ADCC against herpes simplex virus (HSV)-infected targets (8). In 18-h assays, nonadherent and adherent cells were equally active in mediating ADCC in the presence of pooled human sera (Table 2). In contrast, adherent cells were significantly more active than nonadherent cells in mediating ADCC in the presence of murine monoclonal antibodies. Adherent-cell-mediated killing in the presence of individual monoclonal antibodies was 56 to 76% of that detected in the presence of pooled human sera. In two experiments in which high effector cell/target cell ratios (150:1) were used, adherent-cell-mediated ADCC ranged from 21 to 38% with the four monoclonal antibodies previously shown to mediate ADCC, whereas ADCC remained nondetectable with nonadherent effector cells or in the presence of the other monoclonal antibodies tested (results not shown). As in earlier experiments with nonseparated cells, mixtures of monoclonal antibodies did not result in increased adherent-cell-mediated killing (results not shown). Adherent cells did not kill noninfected target cells in the presence of either pooled human sera or murine monoclonal antibodies (results not shown).

To define further the nature of the effector cells mediating ADCC against VZV-infected targets in the presence of pooled human sera and murine monoclonal antibodies, we treated effector cells with anti-Leu-11b, a monoclonal antibody against natural killer cells, and C or with C alone and then tested them for activity in 18-h assays. A representative experiment out of three which gave similar results is shown in Table 3. Treatment of nonadherent effector cells with anti-Leu-11b and C reduced viability by 8 to 12% and greatly reduced killing in the presence of seropositive sera (ADCC). Nonadherent effector cells treated with anti-Leu-11b and C were unable to kill VZV-infected targets in the presence of monoclonal antibodies. In contrast, adherent cells treated with anti-Leu-11b and C retained viability and ability to kill in the presence of either pooled human sera or murine monoclonal antibodies.

TABLE 2. Human adherent-cell- and nonadherent-cell-mediated ADCC against VZV-infected targets^a

Incubation period (h)	Effector cells	Mean ADCC $\pm$ SD mediated by (no. of expt):					
		Pooled seropositive human sera	Murine monoclonal antibody				
			3B3	6B7	10G4	14A7	9C6
4	Nonadherent	16.2 $\pm$ 5.5 (2)	ND ^b	1.5 $\pm$ 0.5 (2)	1.6 $\pm$ 2.2 (2)	ND	ND
	Adherent	5.9 $\pm$ 0.8 (2)	ND	5.6 $\pm$ 2.0 (2)	6.6 $\pm$ 1.9 (2)	ND	ND
18	Nonadherent	21.8 $\pm$ 3.1 (6)	3.9 $\pm$ 4.7 (5)	2.3 $\pm$ 3.4 (6)	1.8 $\pm$ 2.8 (6)	0.8 $\pm$ 0.9 (4)	3.9 $\pm$ 4.7 (4)
	Adherent	20.9 $\pm$ 5.7 (7)	15.6 $\pm$ 5.5 (6) ^c	11.7 $\pm$ 4.2 (6) ^c	15.9 $\pm$ 8.4 (6) ^c	1.9 $\pm$ 1.8 (4)	13.1 $\pm$ 5.9 (6) ^d

^a Effector cell/target cell ratios were 25:1. Final antibody dilutions were 1:200.

^b ND, Not determined.

^c $P < 0.01$ as compared with ADCC mediated by nonadherent cells; determined by a paired t -test.

^d $P < 0.05$ as compared with ADCC mediated by nonadherent cells; determined by a paired t -test.

TABLE 3. Effect of treatment of adherent and nonadherent cells with anti-Leu-11b plus C on the killing of VZV-infected targets^a

Effector cells	Treatment	% ADCC ^b mediated by:					
		Pooled seropositive human sera	Murine monoclonal antibody				
			3B3	6B7	10G4	14A7	9C6
Nonadherent	None	27.2	11.6	8.1	5.6	1.9	5.2
	C alone	35.5	7.2	11.9	12.7	0.1	16.0
	Anti-Leu-11b + C ^c	16.3	0.0	0.0	0.0	0.0	0.0
Adherent	None	19.2	21.6	9.4	20.1	1.7	7.1
	C alone	22.4	23.4	11.4	23.8	3.7	14.2
	Anti-Leu-11b + C ^d	25.4	25.0	12.5	26.0	1.2	6.2

^a Effector cell/target cell ratios were 25:1. The incubation time was 18 h.^b From one of three experiments with similar results.^c 90.5% of the recovered cells were viable, as compared with 98.5% of the cells treated with C alone.^d 97.0% of the recovered cells were viable, as compared with 98.0% of the cells treated with C alone.

PEC-mediated ADCC. Although the above-described experiments established that fresh human monocytes kill VZV-infected fibroblasts in the presence of certain murine monoclonal antibodies to VZV glycoproteins, the magnitude of ADCC was modest, except when extremely high effector cell/target cell ratios were used. These results might be explained by previous observations that murine monoclonal antibodies bind poorly to fresh human monocytes (14). For the purpose of comparison, PEC were used in cytotoxicity assays. PEC-mediated ADCC was detected in the presence of both seropositive human sera and those murine monoclonal antibodies previously shown to participate in ADCC with human monocytes (Table 4). The magnitude of killing was usually higher in the presence of monoclonal antibodies than in the presence of seropositive human sera. Modest amounts of ADCC were also detected with 14A7 and 151, which produced negative results with human monocytes. In the presence of murine monoclonal antibodies, PEC-mediated ADCC was of greater magnitude than ADCC obtained with comparable numbers of human monocytes (Table 2).

Antibody-plus-C-mediated lysis. The addition of pooled seropositive sera and C to VZV-infected targets resulted in lysis, as previously reported (Cross et al., Fed. Proc. 40:988, 1981). Of the monoclonal antibodies tested, only 6B7 mediated lysis of infected targets (Table 5).

Specificity, isotype, and functional activity of monoclonal antibodies. Previously published data on the specificity, isotype, and neutralizing activity of monoclonal antibodies (5, 6) and data obtained in the present study on functional activity are summarized in Table 6. Three of four monoclonal antibodies against gp98/62 and the single monoclonal antibody against gp118 mediated ADCC with human adherent cells. Two of these monoclonal antibodies are of the immunoglobulin G2a (IgG2a) subclass, and two are of the

IgG1 subclass. Only 6B7, one of the IgG2a antibodies, mediated lysis by antibody plus C; 9C6, the only monoclonal antibody that has neutralizing activity, mediated ADCC but not lysis by antibody plus C.

DISCUSSION

Human effector cells have been shown to kill VZV-infected targets in the presence of murine monoclonal antibodies to two major glycoproteins of VZV. When nonseparated mononuclear cells were used as effector cells in 4-h assays, killing in the presence of murine monoclonal antibodies was considerably lower than that obtained in the presence of pooled seropositive human sera. The lower magnitude of killing with murine monoclonal antibodies did not appear to be due to lower quantities of antibodies to VZV, as the FAMA titers of the monoclonal antibodies were higher than those of the pooled human sera. Aggregates in murine ascites fluid also did not appear to inhibit ADCC, although it is possible that ultracentrifugation did not remove aggregates sufficiently.

Experiments in which the cytotoxic activity of adherent and nonadherent effector cells was compared provided a partial explanation for the lower magnitude of killing in the presence of murine monoclonal antibodies. Nonadherent

TABLE 5. Antibody-plus-C-mediated lysis of VZV-infected targets^a

Antibody	% ⁵¹ Cr release in expt:	
	1	2
Pooled human sera		
Seronegative	0.0	0.0
Seropositive	47.7	46.1
Murine monoclonal		
3B3	1.3	0.0
6B7	18.7	21.0
10G4	2.0	0.0
14A7	0.0	0.0
9C6	0.5	0.0
151	0.0	0.0
158	ND ^b	ND

^a Pooled human sera and murine ascites fluid were used at final dilutions of 1:50. Guinea pig C was added at a final dilution of 1:20. The incubation time was 4 h.^b ND, Not determined.TABLE 4. PEC-mediated ADCC against VZV-infected targets^a

Effector cell/target cell ratio	% ADCC ^b mediated by:						
	Pooled seropositive human sera	Murine monoclonal antibody					
		3B3	6B7	10G4	14A7	9C6	151
50:1	22.4	30.4	45.7	33.3	8.2	30.6	7.3
25:1	22.8	22.1	30.3	ND ^c	5.4	34.6	0.0
12.5:1	14.2	22.9	21.2	ND	1.0	31.0	0.0

^a The incubation time was 18 h. All antibodies were added at final dilutions of 1:200.^b From one of two experiments with similar results.^c ND, Not determined.

TABLE 6. Specificity, isotype, and functional activity of murine monoclonal antibodies to VZV^a

Clone	Reacting glycoprotein (from reference 5)	Isotype	FAMA titer	Functional activity ^b		
				Neutralization	ADCC	Antibody-plus-mediated C lysis
3B3	gp 98/62	IgG2a	1:512	—	+	—
6B7	gp 98/62	IgG2a	1:256	—	+	+
10G4	gp 98/62	IgG1	1:128	—	+	—
14A7	gp 98/62	IgG1	1:256	—	—	—
9C6	gp 118	IgG1	1:512	+	+	—
151	gp 66	IgG1	1:256	—	—	—
158	gp 66	IgG1	1:256	—	—	ND

^a Most of the results for reacting glycoprotein, isotype, FAMA titer, and neutralization were previously published (5, 6).

^b —, Not detected; +, detected; ND, not determined.

cells, which were responsible for most of the ADCC with human sera, were relatively inactive in the presence of murine monoclonal antibodies. Adherent cells appeared to be more effective than nonadherent cells in killing in the presence of murine monoclonal antibodies. Eighteen-hour assays were carried out, as monocytes, the predominant cells in adherent-cell populations, have been shown to require more time than lymphocytes to mediate ADCC against HSV-infected targets (8). In 18-h assays, adherent and nonadherent cells were equally effective in mediating ADCC in the presence of human sera, whereas adherent cells were more effective than nonadherent cells in mediating ADCC in the presence of monoclonal antibodies. Adherent-cell-mediated killing in the presence of some of the monoclonal antibodies, particularly 10G4, approached the magnitude of killing detected in the presence of pooled human sera.

To determine whether monocytes or contaminating natural killer-killer cells (2) were responsible for killing, we treated effector cells with anti-Leu-11b, a monoclonal antibody against natural killer cells, and C and then tested them for cytotoxic activity. After nonadherent cells were treated with anti-Leu-11b and C, ADCC in the presence of human sera was greatly reduced. Killing in the presence of monoclonal antibodies was completely eliminated, indicating that natural killer-killer cells are the predominant effector cells in nonadherent-cell populations. In contrast, killing by adherent cells in the presence of human sera or murine monoclonal antibodies was not altered by treatment with anti-Leu-11b and C, indicating that monocytes are the predominant effector cells in adherent-cell populations. These experiments suggest that the low magnitude of cytotoxicity with nonseparated effector cells and murine monoclonal antibodies is due in part to the relative paucity of monocytes in such populations and the necessity for longer incubation periods for maximal lysis.

Monocytes killed VZV-infected targets sensitized with monoclonal antibodies of both the IgG1 and IgG2a subclasses. Steplewski et al. previously reported that human monocytes kill human colon carcinoma cells in the presence of IgG2a, IgG2b, and IgG1 murine monoclonal antibodies, although the magnitude of killing was highest with antibody of the IgG2a subclass (14). These workers also reported that all murine IgG subclasses bind poorly to fresh human monocytes; however, Perussia et al. recently reported that IgG2a and IgG3 murine monoclonal antibodies compete with human IgG1 for binding to human monocytes (11). These reports suggest that weak binding of murine monoclonal

antibodies to fresh human monocytes may explain the relatively modest amounts of ADCC detected with human adherent cells. In this regard, it is of interest that PEC-mediated ADCC was of greater magnitude than that obtained with human adherent cells, although the effector cell(s) involved was not further characterized. Experiments are in progress to determine whether the magnitude of killing will be increased with cultured human macrophages, which were reported to develop receptors for IgG2a and to mediate high levels of cytotoxicity in the presence of IgG2a antibodies to human colon carcinoma cells (14).

The major glycoproteins of VZV have been identified as gp62, gp98, and gp118 (5). The preparation of monoclonal antibodies to these glycoproteins and to gp66, a newly identified structural glycoprotein, has been described previously (5, 6). Four of the monoclonal antibodies precipitate gp98/62, indicating that these two glycoproteins share antigenic properties. Of the seven monoclonal antibodies tested in the present study, three of the four against gp98/62, the single monoclonal antibody against gp118, and neither of the two monoclonal antibodies against gp66 mediated ADCC in the presence of human effector cells. Only one of the antibodies, 6B7, which reacts with gp98/62, lysed VZV-infected targets in the presence of guinea pig C. 6B7 was one of two IgG2a monoclonal antibodies; the other, 3B3, did not mediate lysis by antibody and C. The other monoclonal antibodies were of the IgG1 subclass, and as this subclass fixes C poorly (13), it is not surprising that they failed to mediate lysis. Both Ellen- and OKA-infected targets were lysed in the ADCC assay, indicating that the antibodies are directed against antigens shared by the two strains. The results of cytotoxicity assays did not correlate with assays for neutralizing activity, in which only 9C6, the monoclonal antibody against gp118, had activity. The failure of one of the monoclonal antibodies against gp98/62 to mediate ADCC and of one of the IgG2a monoclonal antibodies to mediate lysis by antibody and C suggests that only certain epitopes of gp98/62 are involved in the lytic process. The failure to detect ADCC with the two monoclonal antibodies against gp66 does not rule out the possibility that other antibodies to different epitopes of gp66 might have activity. It will be of interest to test other monoclonal antibodies to other VZV glycoproteins as they become available.

Previous studies have documented the role of HSV glycoproteins in ADCC and antibody-plus-C-mediated lysis. Norrild et al. reported that sera raised in rabbits against gA/B, gC, and gD of HSV mediated both ADCC against HSV-infected targets in the presence of human effector cells and lysis by antibody plus C (10). Two reports have appeared on the ability of murine monoclonal antibodies to mediate ADCC and lysis by antibody plus C against HSV-infected targets (1, 12). All but one of the monoclonal antibodies tested against gA/B, gC, gD, gE, or gF mediated ADCC, and more than half mediated lysis by antibody plus C. These results cannot be directly compared with ours, as rodent (mouse or hamster) target and effector cells were used, but it is of interest that Balachandran et al. noted differences in the functional activity of two monoclonal antibodies to gC (1).

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LITERATURE CITED

1. Balachandran, N., S. Bacchetti, and W. E. Rawls. 1982. Protection against lethal challenge of BALB/c mice by passive transfer of monoclonal antibodies to five glycoproteins of herpes simplex virus type 2. *Infect. Immun.* 37:1132-1137.
2. Freundlich, B., G. Trinchieri, B. Perussia, and R. B. Zurier. 1984. The cytotoxic effector cells in preparations of adherent mononuclear cells from human peripheral blood. *J. Immunol.* 132:1255-1260.
3. Gershon, A. A., and S. P. Steinberg. 1981. Antibody responses to varicella-zoster virus and the role of antibody in host defense. *Am. J. Med. Sci.* 282:12-17.
4. Gershon, A. A., and S. P. Steinberg. 1981. Inactivation of varicella zoster virus in vitro: effect of leukocytes and specific antibody. *Infect. Immun.* 33:507-511.
5. Grose, C., D. P. Edwards, W. E. Friedrichs, K. A. Weigle, and W. L. McGuire. 1983. Monoclonal antibodies against three major glycoproteins of varicella-zoster virus. *Infect. Immun.* 40:381-388.
6. Grose, C., D. P. Edwards, K. A. Weigle, W. E. Friedrichs, and W. L. McGuire. 1984. Varicella-zoster virus-specific gp 140: a highly immunogenic and disulfide-linked structural glycoprotein. *Virology* 132:138-146.
7. Kamiya, H., S. E. Starr, A. M. Arbeter, and S. A. Plotkin. 1982. Antibody-dependent cell-mediated cytotoxicity against varicella-zoster virus-infected targets. *Infect. Immun.* 38:554-557.
8. Kohl, S., S. E. Starr, J. M. Oleske, S. L. Shore, R. B. Ashman, and A. J. Nahmias. 1977. Human monocyte-macrophage-mediated antibody-dependent cytotoxicity to herpes simplex virus-infected cells. *J. Immunol.* 118:729-735.
9. Kumagai, K., K. Itoh, S. Hinuma, and M. Tada. 1979. Pretreatment of plastic petri dishes with fetal calf serum: a simple method for macrophage isolation. *J. Immunol. Methods* 29:17-25.
10. Norrild, B., S. L. Shore, and A. J. Nahmias. 1979. Herpes simplex virus glycoproteins: participation of individual herpes simplex virus type 1 glycoprotein antigens in immunocytolysis and their correlation with previously identified glycopolypeptides. *J. Virol.* 32:741-748.
11. Perussia, B., E. T. Dayton, R. Lazarus, V. Fanning, and C. Trinchieri. 1983. Immune interferon induces the receptor for monomeric IgG1 on human monocytic and myeloid cells. *J. Exp. Med.* 158:1092-1113.
12. Rector, J. T., R. N. Lausch, and J. E. Oakes. 1982. Use of monoclonal antibodies for analysis of antibody-dependent immunity to ocular herpes simplex virus type 1 infection. *Infect. Immun.* 38:168-174.
13. Spiegelberg, H. L. 1974. Biological activities of immunoglobulins of different classes and subclasses. *Adv. Immunol.* 19:259-294.
14. Steplewski, Z., M. D. Lubeck, and H. Koprowski. 1983. Human macrophages armed with murine immunoglobulin G2a antibodies to tumors destroy human cancer cells. *Science* 221:865-867.
15. Williams, V., A. Gershon, and P. Brunell. 1974. Serological response to varicella-zoster membrane antigens measured by indirect immunofluorescence. *J. Infect. Dis.* 130:669-672.