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Lysis of Herpesvirus-Infected Target Cells by Immune Spleen Cells

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Spleen cells from herpes simplex-infected mice have been shown to lyse ^{51}Cr-labeled virus-infected target cells. The cell-mediated lysis was shown to be antibody dependent but not involving adherent cells. Lysis of infected cells by this mechanism may be one form of host defense in infection by some viruses.

Cell-mediated lysis of ^{51}Cr-labeled virus-infected target monolayers has now been shown for a number of virus systems (2, 4, 11). For ectromelia and lymphocytic choriomeningitis the effector cells have been shown to be specifically sensitized thymus-derived lymphocytes (T cells) (2, 4). The effector cells responsible for lysis of rubella-infected target cells (11) were not determined, but, in light of the recent finding that cytolysis due to T cells occurs only when target cells and lymphocytes share at least one set of H-2 antigenic specificities (13), the cells involved are unlikely to be T cells. Cytotoxic damage of murine sarcoma virus-transformed cells has been associated both with T cells and a thymus-independent lymphocyte population (6). Data presented here indicate that herpes-infected target cells can be lysed by spleen preparations from immunized mice by an antibody-dependent mechanism.

MATERIALS AND METHODS

Virus. Herpes simplex type 2 passaged in either Vero cells or mouse L cells was used.

Cytotoxic assay. Monolayers of Vero cells were infected with a high multiplicity of herpes simplex virus and incubated at 35 C. After a period of 48 h the cell monolayer remaining was removed by treatment with an ethylenediaminetetraacetic acid/trypsin mixture. The cells were then labeled with ^{51}Cr and incubated in Linbro plates with the spleen cell preparation to be tested. Viable infected Vero cells (1.0×10^5) were incubated with 10^7 spleen cells. The method is similar to that described previously (2). Cells were removed 24 h later, the supernatants were recovered by spinning at $800 \times g$ for 5 min, and the levels of radioactivity were determined. The percentage of specific ^{51}Cr release was calculated by the formula

$$\frac{\text{counts in supernatant} \times 100}{(\text{counts in supernatant} + \text{counts in cells})} \times \frac{100}{\%\ \text{water lysis}}$$

Values are quoted as mean ± standard error of mean, and groups were compared by Student's t test. The medium used throughout was Eagle minimal essential medium with added nonessential amino acids (Grand Island Biological Co., Grand Island, N.Y.) incorporating 10% heat-inactivated fetal calf serum.

Anti-θ ascitic fluid. Anti-θ ascitic fluid was prepared by injecting AKR/J mice with CBA/H thymocytes. Immune spleen cells (5×10^7/ml) were incubated with 1:4 dilutions of either anti-θ ascitic fluid or normal AKR ascitic fluid for 30 min at 37 C. Cells were then centrifuged, washed twice in medium, and suspended in guinea pig complement. The complement had previously been absorbed with agarose (80 mg/ml for 30 min at 4 C), clarified by centrifugation, and diluted 1:3 in medium. After a further 30 min at 37 C, cells were washed twice, suspended at 10^7 viable cells in 1.0 ml of medium, and overlayed on ^{51}Cr-labeled Vero cells (either normal or infected with herpes simplex).

Anti-immunoglobulin serum. Immune cells were treated with heat-inactivated (56 C for 30 min) rabbit anti-mouse immunoglobulin (Ig) serum and complement or normal rabbit serum and complement as described above. Full details of the antiserum are given elsewhere (2).

Immune spleen cells. Nine-week-old CBA/H mice were inoculated intravenously with herpes simplex virus grown in normal L cells at a 10^{-1} dilution (100 50% lethal doses). Mice were killed on day 8, and pools of spleens were assayed for cytotoxic activity.

RESULTS

Vero cells infected with herpes simplex virus were harvested at 24, 48, and 72 h after infection by treating the cell monolayer with ethylenediaminetetraacetic acid/trypsin and resuspending the cells in medium. The cells were labeled with ^{51}Cr and incubated with immune or normal spleen cells. Table 1 shows that with increasing time after infection the greater was the specific chromium release. Optimum release was found with cells infected for a period

of 72 h. However, fewer viable cells were recovered at this time interval; therefore, cells infected for a period of 48 h were routinely used.

To determine the cell responsible for cytolysis of the herpes-infected target cell, the immune spleen population was treated with various specific antisera. Specific release of ^{51}Cr was completely inhibited by prior treatment of spleen cells with rabbit anti-mouse Ig serum and guinea pig complement. Treatment with AKR anti-θ ascitic fluid and guinea pig complement had no effect on cytotoxicity (Table 2). These results indicate that the effector cell is not a T cell and that the cytotoxic mechanism is dependent on the Ig-bearing cells. One possible effector cell could be the macrophages present in the spleen population. Depletion of splenic macrophages by prior incubation (7.5×10^6 cells/ml) in plastic wells for 5 h at 37 C resulted in no significant alteration of cytotoxic activity (Table 2). Cells which adhere to the plastic under these conditions are apparently not involved in the cytolytic process.

To determine if the cell responsible for cytolysis was present in the normal spleen population, herpes-infected Vero cells were incubated with normal spleen cells and 10-fold dilutions of antiserum obtained from herpes-infected mice. Low but specific cytotoxicity occurred with normal spleen cells and high dilutions of herpes antiserum but not with normal mouse serum (Table 3). This would indicate that the cytotoxic cell is not the immune B cell but some other nonspecific cell dependent on antibody for its cytotoxic activity.

TABLE 1. *Release of ^{51}Cr from herpes virus-infected and normal Vero cells by spleen preparations from normal and immune mice*

Spleen cells	Time after infection of Vero cells (h)	^{51}Cr release (%)
Immune	24	26.9 ± 0.4[a]
Normal	24	16.3 ± 0.3
Immune	48	38.1 ± 2.5[a]
Normal	48	17.9 ± 0.5
Immune	72	53.2 ± 1.9[a]
Normal	72	24.1 ± 0.9
Immune	Uninfected	31.5 ± 0.8
Normal	Uninfected	30.1 ± 0.7

[a] Difference by Student's t test of release of ^{51}Cr by immune and normal spleen cells on infected cells; $P < 0.001$.

TABLE 3. *Cytotoxicity of herpes immune mouse serum and normal spleen cells on infected Vero cells*

Serum	Dilution	^{51}Cr release from infected Vero cells (%)
Immune	10^{-1}	40.4 ± 2.5[a]
	10^{-2}	37.4 ± 1.7[a]
	10^{-3}	36.9 ± 1.4[a]
	10^{-4}	26.6 ± 0.7
	10^{-5}	27.5 ± 0.8
Normal	10^{-1}	25.4 ± 1.7
	10^{-2}	25.3 ± 1.0
	10^{-3}	26.6 ± 1.6

[a] Difference by Student's t test from release by cells treated with herpes-immune and normal mouse serum; $P < 0.001$.

DISCUSSION

The present report shows that Vero cells infected with herpes simplex can be lysed by

TABLE 2. *Effect of anti-θ or anti-mouse Ig serum on the cytotoxicity of immune spleen*

Spleen cells	Treatment[a]	Expt 1 (% ^{51}Cr release from Vero cells)		Expt 2 (% ^{51}Cr release from Vero cells)	
		Infected	Normal	Infected	Normal
Immune	Nil	51.8 ± 1.8	26.1 ± 0.2[b]	42.4 ± 1.2	28.4 ± 1.4[b]
	Anti-θ ascitic + C	47.4 ± 2.7[c]	ND[d]	40.1 ± 0.6[c]	ND
	Normal ascitic + C	51.0 ± 2.5[c]	ND	43.4 ± 1.6[c]	ND
	Anti-Ig + C	31.2 ± 1.6[b]	ND	32.8 ± 0.4[b]	ND
	NRS + C	46.5 ± 1.4[c]	ND	42.4 ± 1.6[c]	ND
	Macrophage depletion	ND	ND	48.2 ± 0.2[c]	ND
Normal	Nil	30.5 ± 0.3[b]	28.7 ± 0.5[b]	30.2 ± 0.8[b]	29.2 ± 0.8[b]

[a] C, Guinea pig complement; NRS, normal rabbit serum.
[b] Differences by Student's t test from release by untreated immune spleen cells on infected targets; $P > 0.05$.
[c] Differences by Student's t test from release by untreated immune spleen cells on infected targets; $P < 0.001$.
[d] ND, Not done.

immune spleen cells. Normal spleen cells were not cytotoxic in themselves but were able to lyse target cells coated with herpes antibody. Two recent reports (9, 10) have also demonstrated cytotoxicity of herpes-infected cells coated with antibody. Antibody-mediated lymphocyte cytotoxicity has been described in a variety of systems (7, 8, 12). It has been postulated that this form of cytotoxicity occurs in tumor immunity, auto-allergy (8), and as a mechanism of recovery from certain viruses (1). The cell or cells involved in antibody-mediated cytotoxicity have been confined to two subpopulations of cells. One subpopulation consists of macrophages, and the other appears to be a lymphocyte-like cell. The nature of the lymphocyte subpopulation is controversial. Forman and Möller (3) suggest that it is an Ig-bearing cell; however, other evidence (5; I. A. Ramshaw and C. R. Parish, unpublished data) indicates this is not the case and that the cell responsible for lysis does not normally bear Ig on its surface.

Cytotoxic T cells have been found to cause lysis of infected target cells. One recent report (13) indicates that lysis in this system only occurs when target cells and lymphocytes share at least one set of H-2 antigenic specificities. If this is a general rule, which seems to be the case, then the Vero cell infected with the herpes simplex virus in the system described in this paper would not be compatible with an effector T cell. However, even when infected mouse L cells, which are H-2 compatible to the effector spleen cells, are used as target cells no cytotoxic T cells can be demonstrated in mice infected with herpes simplex (I. A. Ramshaw, unpublished data). Whether the presence of cytotoxic T cells in an immune population is restricted to certain virus systems or whether some special requirement is needed to induce cytotoxic T cells, such as virus growth in lymphoid cells, has yet to be determined.

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