INFECTION AND IMMUNITY, June 1973, p. 898-904

Vol. 7, No. 6
Printed in U.S.A.

Host Defense Mechanisms Against Herpes Simplex Virus

I. Control of Infection In Vitro by Sensitized Spleen Cells and Antibody

FRANCIS A. ENNIS

Division of Infectious Diseases, Department of Medicine, Boston University Medical Center, Boston, Massachusetts 02118

Received for publication 14 November 1972

Sensitized mouse spleen cells decrease the spread of herpes simplex virus infection in cell culture lines derived from human and murine tissues. These washed, sensitized cells act alone and additively in combination with antibody to diminish the ability of single virus-infected cells to spread infection to contiguous cells. This control of infection is not species specific, unlike interferon, and appears to be distinct from the effect of antibody. Lymphotoxin was not detected in this lymphocyte-mediated response. This control of herpes simplex virus infection in vitro by sensitized lymphoid cells is immunologically specific; spleen cells from donor animals immunized with a heterotypic virus do not cause herpesvirus plaque size reduction. The ratio of spleen cells from immunized animals to target monolayer cells needed to produce this effect is $> 4:1$. Plaque size reduction of herpes simplex virus by spleen cells requires intact, immune, non-glass-adhering lymphoid cells.

The importance of host factors in control and recovery from most viral infections, including herpes simplex virus infections, has not been completely delineated. In general, homotypic antibody may protect against subsequent viral challenge, but does not appear to be essential for recovery from infection (1) or to aid in recovery if passively administered after infection has commenced (6). Interferon treatment also is generally unsuccessful if initiated after viral challenge (2). Transfer of interferon-producing cells has been shown to be protective to mice challenged with Semliki Forest or encephalomyocarditis virus (7), and passive transfer of immune spleen cells to mice infected with ectromelia virus has been associated with a decrease in virus replication in the liver and spleens of challenged mice (4). Zisman et al. have reported an adverse effect of immunosuppression, by treatment with antilymphocyte and antimacrophage sera, in mice challenged with herpes simplex virus (14). The present experiments focus on the prevention of spread of herpes simplex virus in tissue culture by immune cells and serum after initiation of infection as a model to study host defense mechanisms against virus infection.

MATERIALS AND METHODS

Virus. *Herpesvirus hominis* (herpes simplex virus) strain Rhodanus was obtained from S. Kibrick. The virus was isolated from the lung of a child with eczema herpeticum and had been passed 18 times in human amnion cell cultures and maintained with Eagle minimum essential medium (MEM) containing 2% fetal calf serum (FCS). After cytopathic effect began in the nineteenth passage, supernatant fluid was removed, centrifuged to remove cell debris, and stored at −70 C as pooled virus.

Plaque assays. Clone 1 5-C 4 cells, a derivative of Chang's human conjunctival cell line, were obtained from E. D. Kilbourne and grown in medium 199 containing 10% FCS. L 929 cells were obtained from S. Cooperband and grown in Eagle MEM containing 10% FCS. Plaque studies were performed on monolayers of these cells in 60-mm plastic dishes. Each dish was infected with 50 plaque-forming units (PFU) of virus in 0.2 ml of Hanks balanced salt solution (HBSS) containing 1% FCS and was incubated at 36 C for 45 min. After this incubation period, monolayers were washed twice with 5 ml of phosphate-buffered saline (PBS), pH 7.2. Then 0.1 ml of serum diluted 1:10 in PBS, or 0.1 ml of spleen cells containing 3.0×10^7 erythrosin-excluding cells, was added to the monolayers. The dishes were reincubated at 36 C for 45 min, followed by addition of 10 ml of an agar overlay without removing serum or cells. The agar overlay

added to the clone 1 5-C 4 cells contained medium 199 and has been described (13). A similar agar overlay, substituting Eagle MEM for medium 199, was added to the dishes containing L 929 cells. After the agar solidified at room temperature for 15 min, dishes were incubated at 36 C in a humidified atmosphere containing 5% carbon dioxide. Three to four days later, agar was removed from the dishes, and monolayers were stained with 0.1% crystal violet in 95% ethanol. Plaques were measured on an inverted tissue culture microscope by using a linear grid in the eye piece. The number of cells in uninfected monolayers was determined after trypsinization by counting the number of erythrosin-excluding cells. The average plaque area was calculated after measuring 50 plaque diameters to determine the average plaque diameter. The number of cells in an average plaque was determined by the formula: average plaque area/total monolayer area = number of infected cells in average plaque/total number of cells in monolayer. Statistical analysis of plaque diameters was performed by using the Mann-Whitney U test.

Spleen cells. Sensitized spleen cells were prepared by immunizing 6-week-old white male inbred mice (strain CFW) purchased from Carworth Farms, New City, N.Y. Virus was diluted in HBSS containing 1% bovine albumin fraction V (Miles Laboratories, Inc., Kankakee, Ill.). Tail vein injection with 0.1 ml containing 100 PFU in HBSS-1% FCS was given, followed by similar doses 4 and 5 weeks after primary immunization. Adjuvants were not used. Spleens were removed from sacrificed animals 2 weeks after the last injection. The splenic capsule was removed, and tissue was extruded from the spleen into sterile plastic dishes containing HBSS - 1% FCS. After the tissues were minced with scissors, the mixture was filtered through sterile cotton gauze to remove large particulate material. The cells were then washed three times with this medium by pelleting in a centrifuge at 1,000 rpm for 10 min and discarding supernatant media. Cells were then counted on a hemocytometer and were diluted in fresh medium to contain 3×10^7 erythrosin-excluding cells in 0.1-ml vol. Spleen cells from immunized mice did not contain infectious virus, as determined by virus assay on clone 1 5-C 4 cells. Control mice of the same strain, age, and sex were injected at similar intervals with the same diluent not containing herpes virus or with 1,000 PFU of the AoNWS strain of influenza virus (obtained from E. D. Kilbourne; grown in the allantoic fluid of hens' eggs and diluted in HBSS containing 1% FCS), and their spleen cells were prepared in an identical fashion.

Spleen cells were exposed in certain experiments to either rabbit anti-mouse gamma globulin (Cappel Laboratories, Inc., Downington, Pa. lot 5975, 6.0 mg of antibody protein per ml) or a similar dilution of normal rabbit serum, after heat inactivation at 56 C for 30 min. A preliminary experiment revealed that this antiglobulin, diluted 1:20 in PBS, produced very significant lysis of CFW mouse spleen cells after 36 C incubation for 60 min, in the presence of complement diluted 1:4 in PBS ($\chi^2 = 48.45$, $P < 0.0001$ when compared to the effect of normal rabbit serum and complement as determined by erythrosin dye exclusion). Spleen cells, prepared as described above, from immunized mice were treated without complement in a similar manner with this rabbit anti-mouse globulin preparation or with normal rabbit serum. After 60 min of incubation at 4 C, a 0.2-ml sample containing 3×10^7 spleen cells was added, without washing, to each virus infected monolayer. The dishes received an agar overlay and were stained 3 days later as described above.

Antiserum. Herpes simplex virus antiserum was prepared in rabbits by administering 0.4 ml of virus (about 10^6 PFU of virus) in HBSS containing 1% FCS into the footpad without adjuvants at 6 and 2 weeks before bleeding. This serum titered 1:128 in a standard preinoculation plaque neutralization assay and was added at a dilution of 1:10 in the postinoculation plaque experiments described below. Normal rabbit serum with a herpesvirus plaque neutralization titer of <1:8 was used as the non-immune serum also at a dilution of 1:10 in PBS. All sera were inactivated by heating at 56 C for 30 min. Preliminary experiments revealed that addition of complement had no effect on plaque development; therefore, it was not added to the plaque system. Antisera to the AoNWS influenza virus was obtained from mice after immunization, as described above, when they were sacrificed for spleen cell samples.

Lymphotoxin assay. Virus was diluted in HBSS containing 1% FCS, and 0.2 ml containing 500 PFU of virus was added to monolayers of clone 1 5-C 4 cells in 35-mm plastic dishes. After 45 min of incubation at 37 C, 1.8 ml of medium 199 containing 2% FCS was added to the dishes which were then reincubated at 37 C in a humidified atmosphere containing 5% CO_2. Twenty-four hours after virus inoculation, the media was removed and monolayers were washed twice with PBS before receiving 5×10^7 viable spleen cells obtained from control or immune mice which had been immunized as described above and diluted to 0.5 ml in HBSS containing 1% FCS. After 30 min of incubation at 37 C, 3.5 ml of medium 199 containing 2% FCS was added to the dishes in each of the four experimental groups: (1) virus, no spleen cells; (2) virus, immune spleen cells; (3) virus, control spleen cells; (4) no virus, immune spleen cells. A 0.5-ml amount of supernatant fluid was removed from each dish at 12, 36, and 60 h after addition of cells to the infected monolayers. Samples from each group at each interval were pooled, centrifugated to remove cell debris, and stored at 4 C for 48 h. Live virus was inactivated in these samples by heating at 56 C for 120 min, which does not destroy lymphotoxin. The samples were then diluted 1:4 in medium 199 containing 2% FCS, and this mixture was added in 2.0-ml volumes to fully grown monolayers of primary rhesus monkey kidney, WI-38, primary rat embryo cells (Microbiological Associates, Inc., Bethesda, Md.), and L 929 cells. These cell cultures were examined daily for 10 days for cytotoxicity and cytopathic effect, as were cell cultures which contained media without added samples from the above experiment.

RESULTS

Effect of antibody. Specific antibody to herpes simplex virus, when added to monolayers of the two types of cell lines tested, decreases the subsequent plaque size. Infection does spread from the initially infected cell, but there is herpesvirus plaque size reduction (PSR) in the presence of immune rabbit serum. Table 1 demonstrates the significant PSR and the number of cells subsequently infected by the initial virus-infected cell. The number of cells infected by the originally infected cell was significantly less in cell monolayers treated with immune serum in comparison with those receiving non-immune rabbit serum. Table 1 demonstrates the decrease in plaque size in clone 1 5-C 4 cells (a variant of Chang's human conjunctival cell line), a similar decrease in plaque size in mouse L 929 cells, and the decrease in number of virus lysed cells between monolayers treated with immune serum and those receiving non-immune serum.

Antibody also caused an increase in the size of multinucleated giant cells in clone 1 5-C 4 cells, although total plaque size (lytic area and giant cell combined) was diminished (Table 2). Thus, it appears that antibody enhances the fusion of clone 1 5-C 4 cells infected with herpes simplex virus which form smaller giant cells when antibody is not present. Antibody did not induce giant cell formation in herpesvirus-infected L 929 cells, although it did cause PSR in these cells also. This may be associated with the complete absence of giant cell formation by herpesvirus in L 929 cells when antibody is not present.

Effect of immune spleen cells. The contribution of immune lymphoid cells to the control of herpesvirus infection was then evaluated. Sensitized lymphoid cells did not neutralize herpes simplex virus in a cell-free suspension as determined by pre-adsorption incubation of virus and sensitized cells before plaque virus titration (unpublished observation). However, sensitized cells do cause a reduction in spread of infection after herpes-virus infects cells. Figure 1 demonstrates the decrease in plaque size of herpes virus in L 929 cells. The reduction of the number of cells infected by one virus particle was 84% by sensitized cells alone, and this was increased to a 96% reduction when sensitized cells and immune sera were both present. The immune cells alone significantly decreased the number of cells infected by the initially infected cells ($Z = 4.7$;. $P < 0.001$); moreover, the combination of immune cells and sera was additive in limiting the number of infected cells compared with either alone ($Z = 6.0$; $P < 0.001$). Most plaques developing under agar containing immune sera and sensitized cells in L 929 cells are too small to be seen without a microscope (0.08 mm average with a range between 0.04–0.14 mm in diameter). Antibody or sensitized cells alone resulted in somewhat larger plaques (0.16 mm average) and control cells and sera allowed plaque development similar to untreated virus controls (0.40 mm average). The control of infection in 1 5-C 4 cells was similarly increased ($P < 0.001$) by immune cells and serum (0.20 mm average) from the non-immune cells and serum (0.60 mm average).

TABLE 2. *Effect of antibody on total plaque diameters and plaque giant cell diameters produced in clone 1 5-C 4 cells by herpes simplex virus*

Expt	Non-immune serum/avg diameter (mm)	Immune serum/ avg. diameter (mm)
1	0.66/0.05[a]	0.41/0.21
2	0.54/0.08	0.35/0.22
3	0.69/0.14	0.29/0.27

[a] Numerator: total diameter of average plaque; denominator: giant cell diameter in average plaque.

TABLE 1. *Herpes simplex virus plaque size reduction and decrease in number of secondarily infected cells by antibody in clone 1 5-C 4 cells and L 929 cells*

Treatment	1 5-C 4 cells			L 929 cells		
	Plaque count	Avg diameter (mm)	No. of infected cells in avg plaque	Plaque count	Avg diameter (mm)	No. of infected cells in avg plaque
Immune serum	64	0.35	253	49	0.16	66
Non-immune serum	69	0.54 ($P < 0.001$)[a]	557	55	0.39 ($P < 0.001$)	394

[a] Statistical analysis by Mann-Whitney U test.

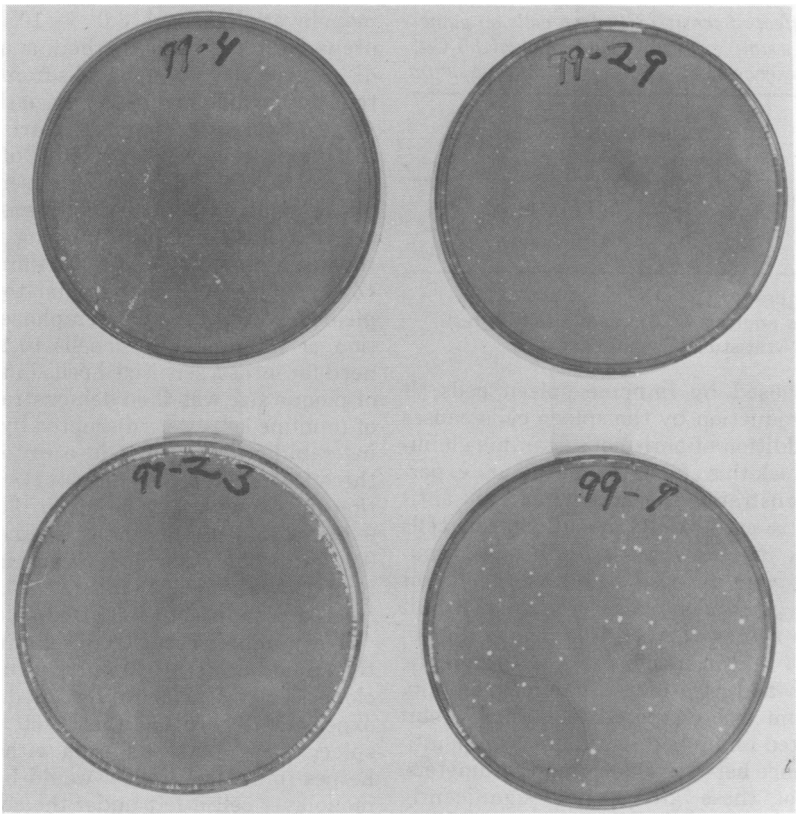

FIG. 1. *Effect of immune cells and serum on herpes simplex virus plaques in L 929 cells. Upper-left dish received immune serum, and non-immune cells. Upper-right dish received immune cells and non-immune serum. Lower-left dish received immune serum and immune cells. Lower-right dish received non-immune cells and non-immune serum.*

This control of herpes simplex virus infection by sensitized spleen cells and serum was specific. Table 3 demonstrates that spleen cells obtained from mice immunized in a similar fashion with the AoNWS strain of influenza did not cause herpesvirus PSR, unlike the action of specifically sensitized spleen cells or serum.

The role of antibody in the immune lymphoid cell-mediated PSR was then considered. There are two markers for antibody activity on herpes simplex virus plaques in clone 1 5-C 4 cells, namely, PSR and enhancement of giant cell development in the plaque. If immune cells cause PSR by release of antibody, similar plaque changes might occur in immune cell treated plaques. Table 4 demonstrates the decrease in plaque size secondary to immune cells, both with normal and immune serum added, but there is no increase in the plaque giant cell size associated with immune cell treatment as opposed to treatment with antibody alone. The difference in the giant cell diameters between antibody treated or immune cell-treated dishes is significant ($Z = 5.6$; $P < 0.001$), although both reduce total plaque diameter over control dishes treated with non-immune serum or cells.

TABLE 3. *Herpes simplex virus plaque size reduction by spleen cells and serum obtained from mice immunized with herpes simplex or influenza (AoNWS) virus*

Group	Avg plaque diameter (mm) vs. virus control[a]
Cells after immunization with:	
Herpes simplex	0.35 ($P < 0.001$)
Influenza (AoNWS)	0.55 (NSS)[b]
Serum after immunization with:	
Herpes simplex	0.53 ($P < 0.05$)
Influenza (AoNWS)	0.65 (NSS)
Virus controls	0.60

[a] Statistical analysis by Mann-Whitney U test of plaques in clone 1 5-C 4 cells.
[b] NSS, Not statistically significant.

An experiment was performed to study the effect of rabbit anti-mouse gamma globulin on

TABLE 4. *Effect of sensitized spleen cells on giant cells of herpes simplex virus plaques in clone 1 5-C 4 cells in the presence of immune or non-immune serum*

Serum	Avg diameter (mm) of giant cells[a]		
	None	Sensitized	Control
Immune	0.54	0.05	0.20
Non-immune	0.28	0.05	0.06
	($P < 0.001$)[b]	NSS[c]	($P < 0.01$)

[a] In 50 plaques.
[b] Statistical analysis by Mann-Whitney U test.
[c] NSS, Not statistically significant.

the PSR caused by immune spleen cells. If antibody production by the spleen cells causes PSR, the addition of anti-mouse gamma globulin might block this effect. A preliminary experiment demonstrated the activity of this anti-globulin in a cytotoxicity assay. Spleen cells from herpes simplex virus- or influenza-immunized mice were exposed to either the rabbit anti-mouse gamma globulin or normal rabbit serum. The average herpesvirus plaque diameter was 0.44 mm in dishes exposed to immune spleen cells and anti-mouse gamma globulin, and 0.47 mm when exposed to normal rabbit serum-treated immune cells. There is no significant difference between these plaque diameters, but both of these groups had significantly reduced plaque size diameters when compared to dishes treated with spleen cells from influenza immunized mice, exposed to anti-mouse gamma globulin (0.86 mm average diameter) or virus controls (0.90 mm average diameter). This experiment does not support a definitive role for antibody in the immune spleen cell control of herpesvirus infection in vitro.

Elaboration of lymphotoxin, a factor released from sensitized lymphocytes which can destroy non-specific target cells (5), was sought in the supernatant fluids of herpes simplex virus-infected clone 1 5-C 4 cells after addition of spleen cells from immune and control animals. This factor has been reported as being released by the interaction of sensitized lymphocytes with the specific antigen to which they have been sensitized, viral (12) or non-viral (5). Table 5 depicts the negative assays for lymphotoxin which resulted when supernatant fluids from herpesvirus-infected tissue culture cells were exposed to sensitized and nonsensitized mouse spleen cells, when assayed on a variety of target cells.

The PSR caused by immune spleen cells was studied in an experiment designed to decrease the number of macrophages present in the spleen cell population. One group of infected monolayers received 3.0×10^7 spleen cells prepared in the usual method; another group of dishes received 3.0×10^7 cells from the supernatant fluid which had been passed three times, at 15-min intervals on glass petri dishes. This treatment removed over 90% of the macrophages from this sample; however, this macrophage-depleted population of sensitized spleen cells remained capable of causing a reduction in the mean plaque size from 0.44 mm to 0.15 mm ($Z = 5.39$; $P < 0.001$) similar to that accomplished by the original macrophage-rich population of immune spleen cells (0.13 mm). The need for intact, sensitized cells in this reduction of plaque size was then demonstrated. Samples of immune cells were disrupted by freeze-thawing rapidly three times in a dry-ice bath, and these fragmented cells did not cause any reduction in plaque size. The mean plaque size was 0.38 mm in dishes receiving lysed immune cells, 0.42 mm for dishes receiving intact control lymphocytes, and 0.13 mm ($P < 0.01$) for dishes treated with intact, sensitized spleen cells.

The number of sensitized cells needed to limit the spread of herpes simplex virus infection in clone 1 5-C 4 cells was studied. Preliminary experiments revealed that $>60 \times 10^6$ mouse spleen cells, obtained from either control or herpes immunized mice, would be toxic to the monolayer cell if left under the agar for 4 days. Therefore, serial dilution of immune spleen cells was performed in HBSS and 1% FCS (Table 6). There needs to be more than 15×10^6 spleen cells, obtained from specifically sensitized mice to significantly reduce herpesvirus plaque size when spleen cells are added to a 60-mm petri dish containing 5.0×10^6 clone 1 5-C 4 cells. When there are enough viable sensitized spleen cells present to limit the spread of virus, addition of more cells does not appear to further decrease plaque size.

TABLE 5. *Lymphotoxin assays on supernatant fluids obtained from herpes simplex virus-infected clone 1 5-C 4 cells exposed to mouse spleen cells*

Group	Release of lymphotoxin			
	WI 38	L929	PMK[a]	PRE[b]
1. Virus, no spleen cells	0	0	0	0
2. Virus, immune spleen cells	0	0	0	0
3. Virus, control spleen cells	0	0	0	0
4. No virus, immune spleen cells	0	0	0	0

[a] Primary rhesus monkey kidney.
[b] Primary rat embryo.

TABLE 6. *Effect of number of spleen cells on herpes simplex virus plaque size in clone 1 5-C 4 cells*

Group	Avg plaque diameter (mm) vs. virus controls
Sensitized cells ($\times 10^6$)	
60	0.26 ($P < 0.001$)[a]
45	0.29 ($P < 0.001$)
30	0.24 ($P < 0.001$)
15	0.50 (NSS)[b]
Control cells ($\times 10^6$)	
60	0.55 (NSS)
Virus control, no cells	0.57

[a] Statistical analysis by Mann-Whitney U test.
[b] NSS, Not statistically significant.

DISCUSSION

The PSR model demonstrates control of the spread of herpes simplex virus infection in vitro by immune serum and lymphoid cells. Immune serum presumably causes PSR by inactivating cell-free virus. It fails to completely halt plaque development because some herpesvirus succeeds in infecting contiguous cells. This might explain the greater decrease in plaque size by antibody in herpes simplex virus lytic infection of L 929 cells, than in the fused cell-containing plaques in clone 1 5-C 4 cells. Virus released from lysed cells is more accessible to inactivation by antibody, than virus in the polykaryons seen in herpesvirus plaques in clone 1 5-C 4 cells.

PSR has also been observed with influenza virus plaques when specific anti-neuraminidase antibody is added to agar after virus adsorption (11). Specific anti-hemagglutinin antibody, however, completely prevents the development of influenza plaques. Anti-neuraminidase antibody acts to prevent spread of virus from cell to cell without neutralizing the influenza virus; however, herpesvirus antibody neutralizes herpesvirus in cell-free suspension. It appears that herpesvirus antibody acts to limit the spread of virus by neutralizing extracellular virus. This ability to control spread of virus in infected cultures was also noted by Black and Melnick with herpes type B virus when a liquid medium containing specific antibody was used (3).

The mechanism of control of virus spread by sensitized lymphoid cells is not yet determined. It is unlikely that sensitized cells inactivate cell-free virus under agar because washed, sensitized cells do not neutralize herpes simplex virus in liquid medium. Also, it is not probable that immune cells act by releasing antibody at the plaque site because they do not cause enhanced fused cell formation in the plaques in clone 1 5-C 4 cells, unlike antibody. Treatment of immune spleen cells by anti-mouse gamma globulin did not remove the PSR effect of the immune cells. However, the possibility of antibody being the mediator of this effect still exists. Sensitized cells probably do not cause PSR by release of interferon because herpesvirus plaques were reduced in cell lines derived from human and murine tissues, and sensitized mouse spleen cells were the constant source of immune cells. Interferon is species specific; therefore, production of interferon by immune spleen cells is probably not responsible for this cell-mediated effect. Lymphotoxin release by immune cells exposed to specific viral antigen might inhibit viral spread by injuring target cells in the plaque area, but no lymphotoxin, was detected in this system. Lysis of infected cells with herpes virus antigen on their surface before infectious virus is produced might be the mechanism. Ito and Barron have demonstrated that a surface antigen appears and peaks before infectious virus is produced in herpesvirus-infected cells (10). Destruction of these target cells by sensitized cells early in infection would result in limited infection with a decrease in total virus yield. The release of a factor distinct from antibody, interferon, or lymphotoxin from the sensitized lymphoid cells, which might alter cellular synthesis (e.g., proliferation inhibition factor) (9), might also result in a decrease in virus spread and PSR. These questions must be resolved in non-agar systems measuring the effect of immune lymphoid cells and serum on the spread of herpesvirus infection.

These experiments demonstrate an immune-specific control of herpes simplex virus infection in cell cultures by sensitized spleen cells and antibody. Although the mechanism of this control is not clearly defined, it appears not to be secondary to elaboration of antibody, interferon, or lymphotoxin by sensitized lymphocytes. The relationship of these in vitro observations to in vivo recovery from herpes simplex virus challenge is discussed in another paper which focuses on the adoptive immunity conferred by sensitized syngeneic spleen cells to mice challenged with herpes simplex virus (J. Infect. Dis., July, 1973).

ACKNOWLEDGMENTS

The author thanks Martha Wells for technical assistance, and Bernard Kreger for statistical analysis.

LITERATURE CITED

1. Babbott, F. L., Jr., and J. E. Jordon. 1954. Modern measles. Amer. J. Med. Sci. **228**:334-361.
2. Baron, S. 1969. The defense and biological roles of the interferon system, p. 268-280. *In* N. B. Fintner (ed.), Interferons. North-Holland, Amsterdam.
3. Black, F. L., and J. L. Melnick. 1955. Microepidemiology of polio and herpes B infection—spread of virus within tissue cultures. J. Immunol. **74**:236-242.
4. Blanden, R. V. 1971. Mechanisms of recovery from a generalized viral infection. J. Exp. Med. **133**:1074-1104.
5. David, J. R. 1971. Lymphocytic factors in cellular hypersensitivity, p. 146-165. *In* R. A. Good (ed.), Immunobiology. Sinauer Assoc., Stamford, Conn.
6. Evans, L. A., H. B. Slavin, and G. P. Berry. 1946. Studies on herpetic infection in mice. IV. The effect of specific antibodies on the progression of virus within the central nervous system. J. Exp. Med. **84**:429-447.
7. Glasgow, L. 1970. Transfer of interferon producing macrophages: new approach to viral chemotherapy. Science **170**:854-855.
8. Green, J., S. R. Cooperband, and S. Kibrick. 1969. Immune specific induction of interferon production in cultures of human blood lymphocytes. Science **164**:1415-1417.
9. Green, J., S. R. Cooperband, J. A. Ruttstein, and S. Kibrick. 1970. Inhibition of target cell proliferation by supernatants from cultures of human peripheral lymphocytes. J. Immunol. **105**:48-54.
10. Ito, M., and A. L. Barron. 1972. Surface antigen produced by herpes simplex virus. J. Immunol. **108**:711-718.
11. Kilbourne, E. D., F. S. Lief, J. L. Schulman, R. J. Jahiel, and W. G. Laver. 1967. Antigenic hybrids of influenza viruses. Perspect. Virol. **5**:87-106.
12. Oldstone, M. B. A., and F. J. Dixon. 1970. Tissue injury in lymphocytic choriomeningitis virus infection: virus induced immunologically specific release of a cytotoxic factor from immune lymphoid cells. Virology **42**:805-813.
13. Sugiura, A., and E. D. Kilbourne. 1965. Genetic studies of influenza virus in a clone of variant human heterophoid cell line. Virology **26**:478-488.
14. Zisman, B., M. S. Hirsch, and A. C. Allison. 1970. Selective effects of antimacrophage serum, silica and anti-lymphocyte serum in herpes virus infection of young adult mice. J. Immunol. **104**:1155-1172.