# The Cytotoxic Effect of Human Immune Sera on *Herpesvirus hominis* Infected Cells[1] (37246)

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(Introduced by J. Thomas Grayston)

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Several studies have demonstrated that *Herpesvirus hominis* (HVH) infected cells are susceptible, in the presence of complement, to the cytotoxic effect of hyperimmune serum from HVH-infected animals (1–3). The presence of humoral factor(s) with cytolytic properties against HVH-infected cells in sera from humans who have had herpes infection, however, has not been demonstrated previously. Using a short, direct cytotoxicity test employed in an earlier study (3), we undertook the present investigation to examine a group of human sera for the presence of immune cytotoxicity also examined for virus-neutralizing activity.

*Materials and Methods. Virus.* Type 1 HVH strain UW-168, cytomegalovirus (CMV) strain UW-2 (4), and vaccinia virus (Lyster strain) propagated in human fetal tonsil fibroblasts were used.

*Sera.* Human serum samples were collected from 186 persons of lower socio-economic status, 2–65 years of age, from several areas in Taiwan. Children up to 10 years of age were bled during a vaccine trial in Taipei and were in good general health. Adolescents and adults were bled during an epidemiologic study of acute respiratory infections. Their general health was also good. All serum samples, obtained by venipuncture, were stored at —20°. Hyperimmune guinea pig serum was prepared as follows: UW-168, type 1 HVH strain, was inoculated onto scarified cornea of an adult animal. Two additional inocula were subsequently administered intramuscularly and intraperitoneally on two occasions one week apart three weeks after the corneal inoculation. One week following the second parenteral inoculation the animal was exsanguinated.

*Infected cells.* Preparation of HVH-infected target cells for the cytotoxicity test has been described (3). Briefly, human fetal tonsil fibroblasts (FT) were infected with 10 plaque-forming units type 1 HVH (UW-168 strain) per cell. After overnight incubation, infected cells were removed from the bottle by trypsin treatment and resuspended in Eagle's minimum essential medium supplemented with 10% calf serum and antibiotics.

*Cytotoxicity test.* The cytotoxicity test was performed as previously described (3). Serial twofold test serum dilutions were made starting at 1:10. For each serum dilution, four 25 μl volumes were delivered into each of four flat-bottomed wells in tissue culture microtest plates (Linbro). All test sera were first inactivated at 56° for 30 min and added to previously frozen pooled guinea

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pig serum. Approximately 150 dispersed, infected cells in 25 $\mu$l of tissue culture medium were then added to the appropriately diluted test serum in wells. The final reaction mixture contained 150 infected cells together with guinea pig serum and test serum each at a final dilution of 1:20. Following 40–50 min of incubation at room temperature during which time uninjured infected cells normally attached to the plate, the wells were washed, fixed with formalin, and stained with crystal violet. The number of cells attached to the bottom of each well was counted using a stero microscope. The plating efficiency for a test serum was derived by taking the average cell count of its four wells and dividing by the average count of four control wells containing the same concentration of guinea pig serum with infected FT cells but without test serum and multiplying by 100. In our previous report (3), we used the average count of the same cell inocula incubated with tissue culture medium without complement (guinea pig) serum as the control. Wells with greater than 50% reduction in the number of infected target cell plating as compared with controls were judged as showing cytotoxic activity. The cytotoxic titer of a test serum is expressed as $\log_{10}$ of the reciprocal of the positive end dilution determined by the method of Reed and Muench (5). To rule out nonspecific serum toxicity against the target cells, uninfected FT cells were reacted with the lowest dilution (1:10) of test serum tested.

*Micro neutralization test.* Micro neutralization tests (6) were performed to determine the virus neutralizing activity of all sera, using UW-168 stock virus as antigen. These sera were heated and supplemented with frozen guinea pig serum at the same concentrations used in the cytotoxicity test. The serum neutralization titer was determined using the method of Reed and Muench (5) and expressed as $\log_{10}$ of the reciprocal of the 50% neutralizing end point.

*Absorption test.* A human serum with positive titer against HVH-infected cells was absorbed with HVH, CMV, and vaccinia virus infected FT cells. Each antigen used for absorption was harvested from two 32-oz bottles containing approximately $5 \times 10^7$ infected cells and showing 75% CPE. The absorbed serum was then subjected to the cytotoxicity test.

*Results. Cytotoxicity and neutralization.* Cytotoxicity and neutralization data of all sera tested are summarized by age of the subjects in Table I. *Herpesvirus hominis* (type 1 UW-168) neutralizing ability was found in 45% of sera from 2-year-olds. The prevalence of neutralizing antibody increased with age and reached 100% in all groups 8 years old and over. In each age group the percentage of sera which were cytotoxic to herpes-infected cells was either identical to or closely approximated that which showed neutralizing ability. All sera positive for cytotoxicity showed demonstrable neutralizing activity. No cytotoxicity could be shown in

TABLE I. Presence of Cytotoxic and Neutralizing Ability in Human Sera Collected in Taiwan According to the Age of the Subjects.

| | | Cytotoxicity[a] | | Neutralization[a] | |
|---|---|---|---|---|---|
| Age (years) | No. tested | No. | % positive | No. | % positive |
| 2 | 22 | 9 | 41 | 10 | 45 |
| 3 | 19 | 11 | 58 | 11 | 58 |
| 4 | 22 | 15 | 68 | 17 | 77 |
| 5 | 25 | 17 | 68 | 18 | 72 |
| 6 | 18 | 14 | 78 | 14 | 78 |
| 7 | 8 | 6 | 75 | 6 | 75 |
| 8–10 | 28 | 28 | 100 | 28 | 100 |
| 11–20 | 22 | 22 | 100 | 22 | 100 |
| 21–30 | 10 | 10 | 100 | 10 | 100 |
| 31– | | 12 | 100 | 12 | 100 |

[a] At dilution of 1:10.

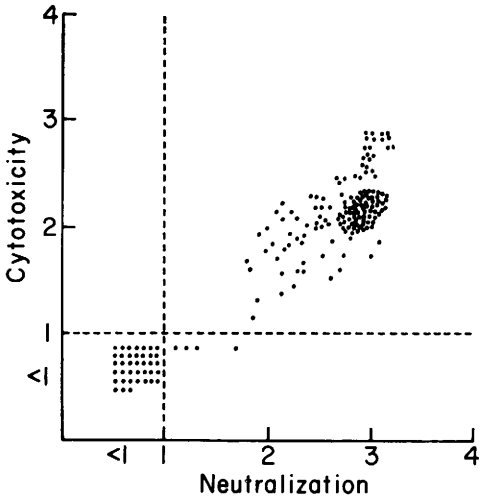


FIG. 1. Cytotoxicity vs neutralization titers of 186 serum samples. (Titer expressed as $\log_{10}$ of the reciprocal of end point dilution determined by method of Reed and Muench.)

38 sera without neutralizing activity. A total of 4 sera from children 2, 4, and 5 years of age with low neutralizing titers, ranging from 1.30 to 1.80, did not have demonstrable cytotoxic activity at dilutions of 1:10.

The scattergram (Fig. 1) includes all 186 serum specimens with their cytotoxic titers plotted against their neutralizing titers. In the present test system, the majority of the sera had higher neutralization than cytotoxicity titers, although the two were generally parallel.

Representative data on the cytotoxic effect on HVH-infected FT cells of sera from 3-year-olds or preimmune and hyperimmune guinea pig sera tested at a dilution of 1:10 are shown in Table II. Six of ten human sera inhibited plating of 70–90% of the HVH-infected cells. The remaining 4 sera inhibited less than 20% of infected cells. More than 87% of uninfected cells attached to the wells in the presence of all these sera. The post HVH-immunized guinea pig serum also inhibited the plating of 80% of HVH-infected cells. Similar cells were not appreciably affected by preimmune serum from the same animal. More than 99% of uninfected cells attached to the plates when incubated with either guinea pig serum. In all instances, including the sera not shown in Table II, positive human and guinea pig sera substantially reduced HVH-infected cells.

*Absorption test.* The cytotoxic property of sera for HVH-infected cells could be removed by absorption with HVH-infected cells but not by CMV or vaccinia virus infected cells (Table III).

*Discussion.* Oncogenic viruses are well

TABLE II. Cytotoxic Effect Against HVH-Infected Fetal Tonsil Fibroblasts and HVH Neutralization Ability of 12 Selected Human[a] and Guinea Pig Sera.

| Serum no. | Cytotoxicity[b]: av. cell count ± SE & % plating | | | | Neutralization[b] |
|---|---|---|---|---|---|
| | Uninfected cells | | HVH-infected cells | | |
| C′ only | 86.5 ± 2.8 | 100.00 | 57.0 ± 2.0 | 100.0 | — |
| 012 | 92.3 ± 5.9 | 106.7 | 54.0 ± 3.2 | 94.7 | — |
| 014 | 86.8 ± 2.7 | 100.3 | 6.8 ± 0.7 | 11.9 | + |
| 018 | 75.3 ± 4.3 | 87.1 | 6.3 ± 1.4 | 11.1 | + |
| 023 | 93.3 ± 3.4 | 107.9 | 15.8 ± 1.3 | 27.7 | + |
| 029 | 83.0 ± 3.2 | 96.0 | 45.8 ± 4.0 | 80.4 | — |
| 046 | 84.5 ± 4.1 | 97.7 | 10.8 ± 1.8 | 18.9 | + |
| 062 | 87.0 ± 4.0 | 100.6 | 47.8 ± 2.4 | 83.9 | — |
| 084 | 90.0 ± 4.0 | 104.0 | 5.3 ± 0.4 | 9.3 | + |
| 104 | 89.8 ± 4.0 | 103.8 | 49.0 ± 4.4 | 86.0 | — |
| 134 | 98.0 ± 3.1 | 113.3 | 5.0 ± 1.1 | 8.8 | + |
| G.P. AS[c]–#11 | 85.4 ± 4.2 | 98.8 | 11.3 ± 1.9 | 19.8 | + |
| G.P. PS[c]–#11 | 103.3 ± 3.1 | 119.4 | 53.5 ± 1.5 | 93.9 | — |

[a] Sera represent portion of 3-year-old group tested.

[b] Both the cytotoxicity and neutralization tested at 1:10.

[c] Pre- (PS) and Post (AS) HVH-immunization of guinea pig (G.P.) serum, #11.

TABLE III. Specificity of the Abolition of Cytotoxicity by Absorption with HVH-Infected Cells.

| | | Absorbed with cells | | | |
|---|---|---|---|---|---|
| | | | Infected by | | |
| | Nonabsorbed | Noninfected | HVH | CMV | Vaccinia |
| Cytotoxic titer against HVH-infected cells | 2.20[a] | 2.20 | <1.30[b] | 2.05 | 2.20 |

[a] $Log_{10}$ of the reciprocal of 50% end point dilution.
[b] Negative at final dilution of 1:20.

recognized for their role in inducing new surface antigenicity in transformed cells and rendering them susceptible to immune killing by specific antibody and sensitized lymphocytes (7, 8). A number of nononcogenic viruses including herpes (1–3), rabies (9), $SV_5$ (10), Newcastle disease, Sendai (11), and lymphocytic choriomeningitis (12) virus can similarly confer new antigenicity upon infected cells and make them susceptible to specific immune cytotoxicity. Cytotoxic activity in human serum so far has been shown only against rabies-infected cells (9). One individual was infected with rabies and another had had the Pasteur treatment with rabies vaccine.

The present investigation of 186 human sera documents the existence of a complement-dependent cytotoxic reactivity in serum which also had herpesvirus neutralizing antibody, presumably implying previous infection with this agent. The high degree of correlation between cytotoxicity and neutralization and the selective cytotoxicity of positive sera on HVH-infected and not on uninfected cells demonstrates the specificity of the present cytotoxic reaction. This assumption is further strengthened by the finding HVH-infected cells specifically absorbed out the serum cytotoxicity against HVH-infected cells whereas cytomegalovirus or vaccinia-infected cells did not.

The age-specific prevalence of neutralizing antibodies to HVH in the present study population increased with age, although the age at which 50% and 100% of persons tested had antibody was much younger than reported elsewhere (13). The higher prevalence of antibody at all ages of this population may be related to the considerably lower socioeconomic and hygienic factors that exist in Taiwan as compared with England. It is also possible that addition of complement in the present test has had a potentiating effect on the neutralization by sera which would not have shown activity without complement (14).

The antigenic nature of the newly acquired HVH-infected cell membrane specificity is not entirely clear. Studies by Roizman and co-workers indicate that it is either identical with, or shares common antigenic constituents with the viron envelope (15). Data derived from the investigation of cell membranes altered by other herpes viruses varies: the pseudorabies virus-induced new membrane antigens appear to be largely nonstructural viral glycoprotein (16). The membrane antigens of Epstein–Barr virus transformed lymphoid cells and Burkitt's lymphoma cells are thought either to contain EB viral envelope components (17) or to be distinctive of the structural viral antigen (18).

The role of cytotoxicity against HVH-infected cells in the natural course of herpes infection is not known. *In vitro,* HVH-infected cells are found to acquire new antigenicity and become susceptible to cytotoxicity 4–6 hr after infection (3) considerably before the time infected cells reach maximum virus replication (19). Whether this *in vitro* time relation between cytotoxic susceptibility and virus multiplication can be extrapolated to the *in vivo* situation is not clear. If so the measurement of cytotoxicity can be of some importance, in addition to

neutralization, in understanding the parameters of host immunologic defense against virus infection. Theoretically, cytotoxicity may be envisioned as assisting in control of infection by (a) prompt elimination of infected cells accompanying early acquisition of new antigenicity thereby reducing potential sources of virus multiplication and, (b) by making the infected cells containing virus permeable to the neutralizing antibodies thus diminishing the availability of virus for dissemination. The later mode of defense might have an added significance in view of the possibility that transmission of herpes infection can be accomplished through cell-to-cell contact without the virus having the intracellular environment (20). It is our view that cytotoxicity against infected cells may be an important adjuvant in bringing about the termination of a herpes infection.

*Summary.* *Herpesvirus hominis* infected cells are susceptible in the presence of complement to an immune cytotoxic effect of human anti-herpes sera (as judged by neutralization). The immune cytotoxicity was demonstrated by the loss of ability of herpesvirus-infected cells to attach to plastic cell culture plates after reaction in suspension with immune sera and complement. The ability of uninfected cells similarly treated to attach to the plates was not affected. Human serum without neutralizing antibody to herpesvirus showed no cytotoxic effect against herpesvirus-infected cells. The herpesvirus neutralizing titers were generally higher than the cytotoxic titers. Cytotoxic effect of human herpes immune sera can be abrogated readily by absorbing sera with *Herpesvirus hominis* infected cells but not with cytomegalovirus or vaccinia virus infected cells.

*Addendum*: Since submission of this manuscript, an article by Smith *et al.* (21) has been published describing a $^{51}Cr$ cytotoxic assay. The cytolytic activity of Herpes type I and type II human sera against type I and type II infected cells is shown. Sera from patients with varying clinical manifestations of Herpes type I and type II infection were tested and absorption studies were performed.

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