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Inactivation of Varicella Zoster Virus In Vitro: Effect of Leukocytes and Specific Antibody

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Inactivation of varicella zoster virus in vitro by nonadherent, mononuclear peripheral blood leukocytes and antibody is described. When leukocytes and specific antibody were incubated with this virus, marked inactivation of the virus occurred. In contrast, leukocytes alone or with serum devoid of varicella zoster antibody caused only a small degree of inactivation of varicella zoster virus. The leukocytes involved appeared not to be monocyte-macrophages or T or B lymphocytes, and only minute amounts of specific antibody were required. We had found previously that leukocytes from unsensitized (varicella susceptible) as well as sensitized (varicella immune) donors could cause this reaction. We therefore propose that the reaction may be a form of antibody-dependent cellular cytotoxicity, as has been described for ^{51}Cr release by lymphoid (K) cells for other herpesviruses.

The means whereby the human host defends itself against varicella zoster (VZ) virus remains unclear. It appears that cellular rather than humoral immunity is critical (1). An in vitro cellular immune reaction involving sensitized T lymphocytes and monocyte-macrophages that may be important in host defense has previously been described (3, 4, 6). It is established, however, that passive immunization against VZ infection may successfully ameliorate varicella even in immunocompromised hosts (1). Thus, specific antibody may play some role in host defense against this strongly cell-associated virus. Years ago, Weller and Witton noted that specific antibody did not prevent cell-to-cell spread of VZ virus in vitro (12). The effect of specific antibody in conjunction with lymphoid cells on VZ virus in vitro, however, has not heretofore been studied. Such a reaction, utilizing lymphoid cells from immune as well as varicella-susceptible donors might be considered analogous to antibody-dependent cellular cytotoxicity (ADCC), that has been described against herpes simplex virus (HSV) (9-11). We therefore studied whether antibody and lymphoid cells could inactivate VZ virus in vitro.

MATERIALS AND METHODS

Cells, virus, and media. Human embryonic lung fibroblasts (HELF), obtained from the American Type Culture Collection, Rockville, Md., were used for propagation of VZ virus. Modified Eagle medium supplemented with 2% fetal calf serum was used for virus growth (12). Cell-free VZ virus was prepared by sonication of an infected cell pack. The Ellen strain (12) of VZ virus was used throughout.

Isolation of human leukocytes. Heparinized whole blood was obtained, diluted 1:3 in Hanks balanced salt solution, and subjected to density gradient centrifugation through Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.). Monocyte-macrophages were removed by adsorption of cells to plastic three times. T or B lymphocyte-depleted populations were prepared by rosetting, as described previously (3, 4, 6). In addition, in certain experiments, as stated, B lymphocytes and monocytes were removed by passage through a nylon wool column.

Serum samples and serological testing. Sera were obtained from human volunteers. Samples were stored at −20°C. Sera were assayed for VZ antibody by the fluorescent antibody to membrane antigen (FAMA) assay (13). Several sera were employed and gave similar results. One serum sample with a VZ FAMA titer of 1:128 but devoid of antibody to HSV I and II (kindly tested by radioimmunoassay by W. Rawls) was used for many of these experiments. Another serum used as noted in the text was tested for the presence of antibody to HSV by complement fixation; the titer was 1:64. The VZ FAMA antibody titer of this serum was <1:2.

Inactivation assay. The blood cells used in this assay were depleted of monocyte-macrophages by adsorbing them to plastic three times (or, where stated, on a nylon wool column). The adherent cells were discarded, and the remaining cells were counted in a hemocytometer. Usually, 25,000 cells were used, but if other numbers were employed, this is noted in the text. A smear and Wright stain of these cells showed them to be mononuclear; no polymorphonuclear leukocytes were found.

To test for VZ inactivation, monolayers of HELF grown in tissue culture tubes (16 by 125 mm) were

infected with approximately 50 plaque-forming units (PFU) of VZ virus. After 45 min, 1 ml of medium containing the following was added to each of duplicate tubes for each dilution of serum: (a) 0.1 ml of serum with a VZ FAMA titer of 1:128, diluted 1:32 and 1:64, plus 25,000 nonadherent leukocytes; (b) 0.1 ml of serum with a VZ FAMA titer of <1:2, diluted 1:32 and 1:64, plus 25,000 nonadherent leukocytes; (c) 0.1 ml of serum with a VZ FAMA titer of 1:128, diluted 1:32 and 1:64; (d) 25,000 nonadherent leukocytes; and (e) VZ virus alone.

After 3 days of incubation at 37°C, the monolayers were trypsinized and passed onto fresh HELF monolayers. At 2 to 3 days later, the number of VZ plaques was counted microscopically (5). The percent plaque reduction was calculated as follows: 100 − (no. of VZ PFU with leukocytes and serum with VZ antibody/no. of VZ PFU with leukocytes and serum without VZ antibody) × 100.

In one experiment, serum was tested before and after heating to 56°C for 30 min. This treatment did not seem to influence the assay in any way.

RESULTS

Inactivation of VZ virus. The ability of nonadherent lymphoid cells to inactivate VZ virus is shown in Fig. 1. Few VZ plaques were present 2 days after passage, but after 3 days, distinct differences after various treatments were apparent. The number of VZ PFU in controls b, c, and d was somewhat lower than the number of PFU in control e, VZ virus alone. Controls b to d, therefore, show that nonadherent lymphoid cells either alone or with VZ antibody-negative serum, as well as specific antibody alone without lymphoid cells, somewhat depressed the growth of VZ virus in vitro. The decline in the number of VZ PFU was most striking, however, in cultures treated with leukocytes and specific antibody, as shown in control a. For purposes of calculation, the values in experimental a were compared with those of control b to obtain the percent VZ plaque reduction. For example, in Fig. 1, the percent plaque reduction for experimental a compared with control b was 69%.

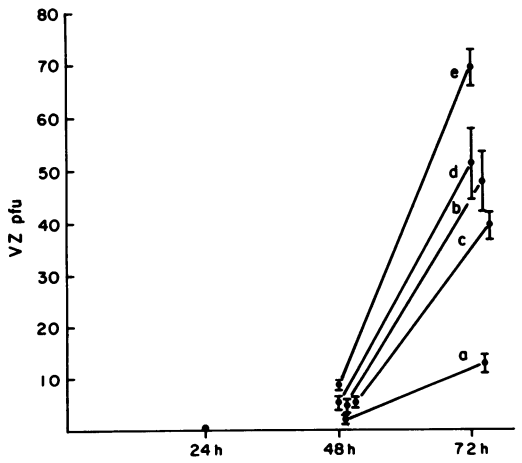

FIG. 1. *The number of VZ plaques observed after various treatments of tissue cultures is shown. In each instance, the lymphoid cells had been previously adsorbed to plastic three times to remove monocyte-macrophages. a, VZ virus, 25,000 lymphoid cells, VZ antibody; b, VZ virus, 25,000 lymphoid cells, antibody-negative serum; c, VZ virus, antibody-positive serum; d, VZ virus, 25,000 lymphoid cells; e, VZ virus alone. The controls are b through e. Data from at least six determinations are shown, along with the standard error of the mean.*

Specificity of the assay. The specificity of the assay was examined by comparing the ability of leukocytes and VZ antiserum with leukocytes and HSV antiserum to inactivate VZ virus. Dilutions of serum from 1:32 to 1:1,024 were used, and the percent plaque reduction at each dilution was calculated (Fig. 2). When serum containing antibody to HSV but not to VZ virus was used, there was no decrease in VZ PFU. In contrast, when serum containing VZ antibody but no HSV antibody was used, significant plaque reduction occurred at dilutions of serum ranging from 1:32 to 1:256. The ability of lymphoid cells to reduce VZ plaques was dependent on the concentration of VZ antibody. Over 75% plaque reduction occurred at dilutions of serum between 1:32 and 1:128. At a dilution of 1:256, however, there was only 50% plaque reduction, and at higher dilutions there was no plaque reduction.

Ratio of concentration of leukocytes to amount of VZ virus inactivated. The ability of various concentrations of lymphoid cells to inactivate VZ virus was examined. Data from a representative experiment are depicted in Fig. 3. Plaque reduction was dependent on the number of lymphoid or effector cells with a standard 50-PFU input of VZ (targets). For example, when 100,000 lymphoid cells were used, there was 97% reduction of VZ plaques. The percent reduction with 1,000 to 2,500 lymphoid cells was roughly similar, 83 to 87%. Evidence of plaque reduction was seen, however, if 500 or even 100 lymphoid cells were used.

In similar untreated culture tubes in which VZ virus was propagated, the average number of VZ plaques was 50, 3 days after passage. Therefore, a rough estimate of the ratio of the number of effector (lymphoid) to target (VZ infected) cells may be made (Fig. 3). Even at effector-target ratios of 2 to 1, plaque reduction of VZ virus occurred.

Depletion experiments. Peripheral blood leukocytes were depleted of monocyte-macrophages and B cells by passage over a nylon wool column. This was followed by depletion of T

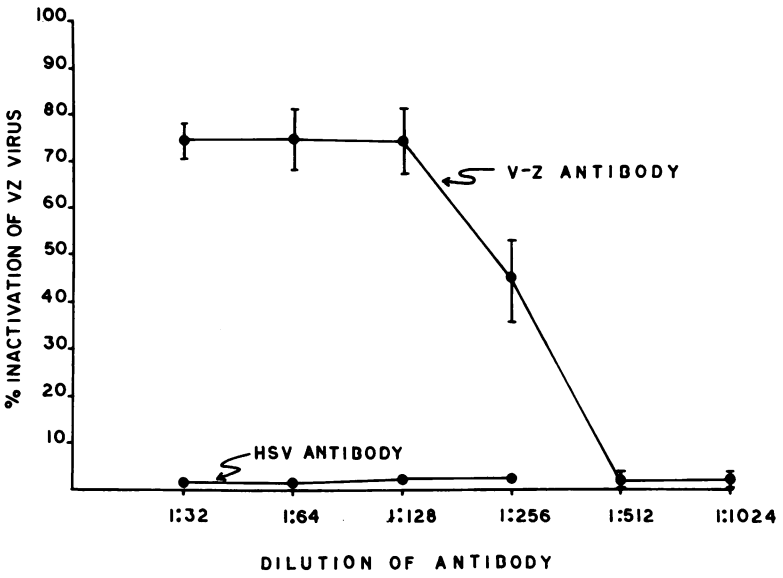

FIG. 2. *The influence of specific antibody in the presence of nonadherent lymphoid cells on VZ virus is shown. With VZ antibody, plaque reduction by ADCC falls at a dilution of 1:256. The VZ antibody titer (FAMA) of the serum used was 1:128; there was no antibody to HSV I or II. When serum containing antibody to HSV I (complement-fixing antibody titer 1:64; VZ FAMA titer <1:2) was used, plaque reduction was less than 1% at all dilutions. The results of one representative experiment run in triplicate are shown.*

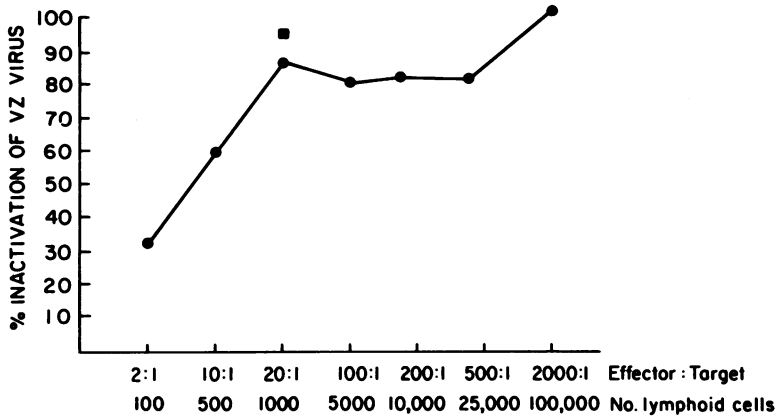

FIG. 3. *Percent plaque reduction of VZ virus, using a 1:32 dilution of serum containing VZ antibody and varying numbers of nonadherent lymphoid effector cells (●). The target number of VZ PFU, 50, was kept constant. Even at an approximate effector-target ratio of 2:1, inactivation of VZ virus occurred. When rosette-forming-depleted, nonadherent mononuclear cells (■) were used, virus inactivation was demonstrated. Results shown are from one representative experiment run in duplicate.*

cells by rosetting and subjection to a second Ficoll gradient to isolate nonrosetted lymphoid cells. The remaining cells were still capable of inactivating VZ virus in the presence of VZ antibody. For example, when 1,000 such depleted cells were used, 95% plaque reduction occurred in the presence of specific antibody (Fig. 3).

DISCUSSION

We have previously demonstrated that sensitized T lymphocytes plus monocytes (from peripheral blood of patients immune to varicella), in the presence of VZ antigen, inactivate VZ virus in vitro (3, 4, 6). We have termed this reaction T/M cytotoxicity. In this study, we

describe another apparent means whereby VZ virus may be inactivated in vitro. Peripheral blood lymphoid cells depleted of monocytes inactivated VZ virus in the presence of specific antibody. Earlier, we demonstrated that this reaction, in contrast to T/M cytotoxicity, occurs whether the lymphoid cells are obtained from previously sensitized donors or not (5).

Since this reaction requires VZ antibody and since lymphoid cells from unsensitized donors are effective, it is possible that this reaction is a form of ADCC. ADCC, which is usually measured by release of ^{51}Cr from target cells rather than by virus inactivation, has been proposed as important in host defense against HSV (9–11), although others (2) have disputed this.

Additional features of this reaction also appear to be similar to those of ADCC. For example, this reaction appears to be highly efficient, requiring minimal amounts of antibody, as does ADCC (8). Since in the VZ cultures the serum was diluted roughtly 1:10, the assay is about 10 times more sensitive than FAMA. This is of interest because it has been found that FAMA antibody titers correspond roughly to neutralizing antibody titers (7).

In addition, this reaction against VZ virus requires a remarkably small number of lymphoid cells, as does ADCC (8). Inactivation of VZ virus was demonstrated with as few as 100 nonadherent lymphoid cells to 50 PFU of VZ virus. Roughly, this could be considered to be an effector/target ratio of only 2:1. Further experiments, however, using different concentrations of cells depleted of monocytes and T and B cells, will be required to obtain a more accurate ratio of effectors to targets.

T/M cytotoxicity against VZ virus (3, 4, 6) appears clearly to be a different form of cell-mediated immunity than that described here. For example, in T/M cytotoxicity, only sensitized T cells (from varicella-immune donors) and macrophages are effective, and antibody blocks rather than aids the reaction (3). We also had found earlier that large concentrations of monocytes (over 1,000) alone are nonspecifically toxic to VZ virus in vitro. We did not observe this phenomenon in the experiments described here because we adsorbed the monocytes before performing our assay.

We may also be describing yet another form of cell-mediated immunity against VZ virus in these experiments. We noted that nonadherent lymphoid cells alone caused some decrease in the number of VZ plaques, as seen in one of the controls for the assay. This may have been due to activity of natural killer cells. Natural killer cell activity has also been proposed as a form of cell-mediated immunity operative against HSV (2). We therefore plan to further explore this phenomenon.

What functional role might nonsensitized lymphoid cells and specific antibody play against VZ virus in vivo? Possibly it is important, along with other immune reactions, during recovery from varicella and zoster infection after specific antibody has been formed. It may also play a significant role against VZ virus during successful passive immunization (1). It has been assumed that neutralization of virus by antibody is the major event in this process. VZ virus, however, is strongly cell associated, and it is difficult to envision how virus neutralization could occur after the virus has invaded cells. If lymphoid cells and specific antibody could inactivate viruses in vivo, it might explain why passive immunization is effective against intracellular viruses, such as VZ. It is of note in this regard that immunocompromised persons require greater amounts of specific antibody for passive immunization than do normal persons (1). Perhaps the lymphoid cells involved in this reaction in immunocompromised persons are fewer in number or less efficient, therefore requiring more antibody. Additional experiments to explore this possibility are planned. In addition, further depletion and blocking experiments are planned to determine whether the reaction described is indeed ADCC, as we have proposed.

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