Role of Antibody-Dependent Cellular Cytotoxicity in Defense Against Herpes Simplex Virus Infections

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Antibody-dependent cellular cytotoxicity (ADCC) to cells infected with herpes simplex virus (HSV) is a mechanism of destruction of these cells by a combination of antiviral antibody and immunoglobulin Fc receptor–positive leukocytes. It has been well defined in vitro as a rapid lytic response utilizing minute amounts of IgG antibody. In vitro studies have shown ADCC restriction of the spread of virus. In vivo studies using adoptive transfer of human or murine ADCC effector cells plus antibody and ADCC-active, nonneutralizing $F(ab^1)_2$ antibody fragments or monoclonal antibodies have demonstrated the important role of this response in animal models of HSV infection. In humans, ADCC effector function and/or antibody levels have been associated with the outcome of infection, especially in immunocompromised patients and neonates. Reconstitution of this mechanism with appropriate antibodies or cytokines in high-risk hosts, with the resultant amelioration of severe HSV infection, will validate ADCC as a critical component of antiviral defense.

Cooperation between antibody and polymorphonuclear leukocytes to facilitate opsonophagocytosis is an important antibacterial defense mechanism. The coming together of the cellular effector system and humoral factors in viral infection is a relatively new biologic insight. The initial descriptions of sensitization of cells by antibody, rendering them susceptible to lysis by leukocytes, involved red blood cell or tumor cell target systems [1–3]. In these systems antibody (usually IgG) sensitizes a target cell by recognition of specific surface antigens. A nonimmune leukocyte effector cell with an Fc receptor then engages the fixed antibody and destroys the target cell in a process known as antibody-dependent cellular cytotoxicity (ADCC). The use of virus-infected target cells with a rapidly lytic virus such as herpes simplex virus (HSV) in an ADCC system was initially regarded as infeasible because of the expected virus-induced lysis of the target cells. It was with some surprise that two groups independently and simultaneously described ADCC to HSV-infected cells by human and murine effector cells [4, 5].

The basic technique of HSV ADCC as performed in most laboratories includes the use of three components. Target cells (generally HeLa, Chang liver, human amnion, or even human autologous cells [6]) are acutely infected with HSV 6–24 hours before use and labeled with radioactive chromium just before use. Effector cells (human or other animal leukocytes) at various concentrations (effector-to-target cell ratios) plus immune or nonimmune antiserum to HSV are then added, usually in a microtiter plate system. After incubation of this mixture for 4–24 hours, a fraction of the supernatant (typically one-half) is harvested, and both the supernatant and the remaining cells are counted in a γ counter to quantitate cell isotope release, which correlates extremely well with cell death. ADCC is then calculated by the following formula: {[(percentage isotope release of target cells + effector cells + immune serum) − (percentage isotope release of target cells + effector cells + nonimmune serum)]/[100 − (percentage isotope release of target cells + effector cells + nonimmune serum)]} × 100. Controls include well-characterized (usually by viral neutralization assays and ELISA) HSV-immune and HSV-nonimmune serum. A further control is that no ADCC is mediated against the uninfected target cells, although serum-independent natural-killer cytotoxicity is observed. Figure 1 is a schematic representation of ADCC. The assay for ADCC is remarkably reproducible in normal and immunodeficient individuals, although there is enough variation that the use of matched controls, appropriate statistical analyses, and multiple effector-to-target cell ratios is critical when populations are compared. Reports of results differing from group to group must be viewed with extreme caution when only one effector-to-target cell ratio has been used.

The initial description of the ADCC phenomenon showed that a relatively high ratio of effector cells to target cells (typically 100:1 to 30:1) was necessary. Subsequently, this requirement was found to be due to the mediation of ADCC by a small subset of leukocytes in human peripheral blood or murine spleen or peritoneal cells. The system was found to be exquisitely sensitive to small amounts of antibody. Indeed, previous reports of direct or T cell–mediated antiviral cellu-

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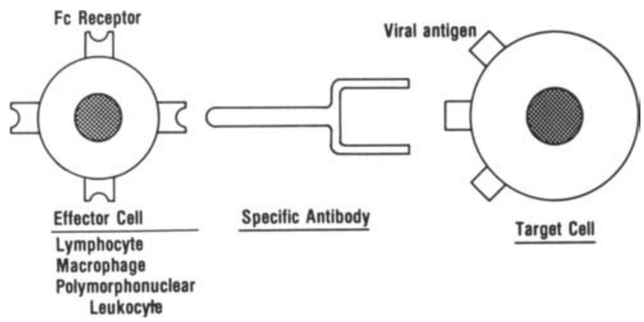

Figure 1. Schematic representation of ADCC to HSV-infected cells. An Fc receptor–positive effector cell destroys a target cell displaying surface viral antigen via specific antibody. Reprinted with permission from [7].

lar cytotoxicity [8, 9] probably represented—at least in large part—instances of inadvertent ADCC mediated by contaminating antibody during the use of cells from seropositive donors.

In humans the effector cells for ADCC to HSV-infected cells are subpopulations of most Fc receptor–positive leukocytes, including polymorphonuclear leukocytes [10], monocyte-macrophages [11], and lymphocytes [12–14]. For each of these cell types, only a small subset of cells are the active effector cells. The lymphocyte effector cell has been characterized as the killer or natural killer subset and has been determined to be an Fc receptor–positive, non-T, non-B cell with CD16 markers [12–14]. The finer characterization of the human killer lymphocyte involved in ADCC to HSV remains to be achieved. Murine and human macrophages have been characterized as plastic-adherent, latex-sensitive, silica-sensitive cells [11, 15]. Unless otherwise specified, the usual populations of cells used in clinical and experimental conditions have included Ficoll-Hypaque-purified mononuclear cells (roughly 80% lymphocytes and 20% monocytes) or plastic-adherent purified monocyte-macrophages from a starting mononuclear cell population. The active subsets of the other two major classes of cells (polymorphonuclear leukocytes and monocyte-macrophages, usually thought of as homogeneous) must still be characterized in detail. Human lymphocyte effector cells require less antibody and mediate ADCC more rapidly than monocyte-macrophage and polymorphonuclear leukocyte effectors [10, 11]. In the mouse the effector cell found in spleen and peritoneal cell populations is the macrophage [15]. Murine monocyte-macrophages have kinetic and antibody characteristics remarkably similar to those of their human counterparts [15]. The antibody involved has been shown to be primarily of the IgG class, as determined by staphylococcal protein A adsorption and presence in human neonatal transplacental antibody [16, 17]. Complement is not necessary for ADCC, although in some systems complement enhances ADCC [18, 19]. Epitopes in the major HSV envelope glycoproteins B, C, and D are targets for polyclonal antibody- and monoclonal antibody-mediated ADCC [20–23].

In Vitro Significance of ADCC

With the observation and basic characterization of anti-HSV ADCC came the obvious question of its relevance. In particular, how could a defense mechanism utilizing antibody be separated and analyzed with regard to the effect of antibody alone? HSV can spread from cell to cell via intracellular bridges without being exposed to the extracellular fluid environment [24]. Thus the virus is protected against neutralizing antibody. Early observations of the limitation of plaque production by leukocytes in vitro often included small amounts of antibody in the overlay solution [25]. These experiments probably involved in vitro ADCC limitation of viral spread, which has more recently been confirmed [26]. This evidence was the earliest indication that ADCC was important against HSV.

The potential importance of ADCC was highlighted by the demonstration that HSV-infected cells were susceptible to lysis by mononuclear cell ADCC shortly (2–3 hours) after infection [27] as a result of virus-programmed surface antigen synthesized by the target cells [28]. Thus the antibody requirements (low) and kinetics (rapid) of mononuclear cell cytotoxicity suggested that ADCC could limit the spread of virus in vivo by destruction of infected cells before the release of new viral progeny and early during primary infection, when only low levels of antibody would be present.

Animal Models Supporting the Role of ADCC in Vivo

The importance of ADCC in vivo has been supported by the use of animal models. ADCC antibody appears very early after primary infection in mice (i.e., by 3 days) [29]. Neonatal mice known to be exquisitely susceptible to lethal HSV infection [30, 31] display marked defects in macrophage ADCC effector function [31]. However, like humans, neonatal mice have multiple immunologic defects with regard to HSV, including low natural-killer-cell activity [31], decreased or absent production of ADCC antibody to HSV [32], and macrophage defects [33]. The low levels of mononuclear cell ADCC effector activity may not be the only explanation for the susceptibility of neonatal mice to lethal HSV infection.

More elegant and convincing demonstrations of the importance of intact mouse macrophage ADCC effector cell activity have come from adoptive transfer experiments. Antibody alone offers some protection against murine HSV infection. Rager-Zisman and Allison demonstrated in cyclophosphamide-treated mice that optimal protection against lethal HSV infection was afforded by transfer of antiviral antibody and adherent spleen cells (macrophages) from nonimmune mice [34]. These are the components of ADCC. In a series of works by Oakes's group with antithymocyte serum [35] or irradiation [36, 37], it was shown that antibody-mediated protection against ocular or subcutaneous HSV infection was dependent on an intact cellular element in normal mice. While this group attributed the effect to T cells, the purity of the antileukocyte antibodies then available and the recent recognition of some T cell antigens on macrophages necessitate caution regarding the identity of the effector cell.

Similar evidence for the role of ADCC in animal models of HSV infection has emerged from studies using antibody components and, more recently, monoclonal antibodies (Mabs). In mice, intact IgG is protective in certain models of HSV infection. The use of derived $F(ab^1)_2$ fragments retaining antiviral neutralizing activity but lacking antibody-complement or ADCC functional activity failed to provide protection [38–40], even when comparable in vivo levels of $F(ab^1)_2$ fragments (as of IgG) were maintained. The role of complement did not appear to be important, since intact IgG was as effective in complement-deficient mice [38, 39]. Removal of neutralizing activity from IgG by adsorption with virus-infected cells did not ablate protection against HSV infection [41].

Studies of Mab-mediated protection against HSV infection demonstrated that protection was not associated with antiviral neutralization activity [22, 42–45]. When tested, protection by Mab was associated with in vitro ADCC activity [22, 23, 42, 43], with one exception. The mechanism of protection afforded by Mab F3AB (Dr. John Oakes, University of Alabama, Mobile)—a nonneutralizing, non-ADCC-active Mab (as confirmed in our laboratory with use of murine or human effector cells)—remains to be understood [42]. These results also demonstrate that there may be a discordance between ADCC antibody and neutralizing antibody. The discrepancy is to be expected since, for instance, IgM antibody can neutralize virus but cannot mediate ADCC because the IgG Fc receptor is critical on the effector cell. In the typical polyclonal response to a viral infection, both ADCC and neutralizing antibody are produced, although with different kinetics (see below and [46–49]).

Thus, based on the examination of effector cell function, the definition of cell populations that participate in antibody-mediated protection, and the delineation of functional activities of digested polyclonal immunoglobulin or Mab, ADCC appears to be an important in vivo protective mechanism in murine models of HSV infection.

Studies Associating ADCC Activity with Human Resistance to HSV Infection

Several avenues of investigation have begun to link ADCC activity with human resistance to severe HSV infection. Studies of children with primary HSV gingivostomatitis [46] and of adults with primary genital HSV infection [47–49] have defined the kinetics of production of ADCC antibody to HSV. This has been demonstrated to be a very early antibody response; at least in one study, its appearance was associated with defervescence [46]. While ADCC to HSV may be studied with either HSV-1 or HSV-2 [16], nearly all work has used HSV-1. Our laboratory has used a strain (HE) obtained from a human neonate with disseminated disease. There is a high degree of cross-reactivity of sera positive for HSV-1 and HSV-2 in the ADCC assay. Indeed, HSV-1-infected cells have been shown to define the kinetics of ADCC antibody in primary HSV-2 genital infections very nicely [48, 49]. In the future formal comparisons of HSV-2- and HSV-1-infected target cells with regard to the type of infection in a patient, or even use of the patient's own virus, may yield fine specificity data on the antibody response or (in the case of neonates) protective epitopes. It is surprising that such comparisons have not yet been made. Neonates may acquire antibody by passive transfer from their mothers. In this setting the presence of high titers of ADCC antibody ($\geq 10^{-4}$) at presentation of an infected neonate was associated with local infection (either of the skin or of the CNS) and not with disseminated illness [50]. Levels of antibody were highest in babies exposed to HSV but not infected by the virus [50]. There was no association between ADCC levels and virus neutralization levels in mothers or neonates.

Defective ADCC effector function has been associated with severe HSV infection. The human neonate who has low mononuclear cell ADCC activity [51, 52] is susceptible to unusually severe HSV infection. The defect is due in part to the inability of effector cells from human neonates to adhere normally in the presence of target cell–sensitizing antibody [52]. In addition, a lytic defect is present; interferon can increase adhesion but not lysis in the presence of antibody [52]. The apparent paradox of low mononuclear cell ADCC effector function in the neonate despite protection associated with high levels of mononuclear cell ADCC-active antibody [50] is probably reconciled by the low but detectable effector function in the neonate, which may explain the necessity for such high levels of antibody for protection. Like neonates, patients with Hodgkin's lymphoma had low macrophage-mediated ADCC at the time of chemotherapy, their period of highest risk for HSV infection [53]. Comparable results have been obtained for patients with serious burns during the third week of convalescence [54], a time of increased risk for serious HSV infection [55].

In the most recent study of this type, patients with leukemia and those with renal transplants were analyzed for HSV excretion and illness [56]. While the two groups had high and similar rates of virus excretion (47%–50%), the incidence of lesions associated with virus shedding was three times higher among patients with leukemia than among those with transplants (100% vs. 32%). These differences were associated with low levels of mononuclear cell ADCC effector activity in the leukemic patients. The association of low mononuclear cell ADCC activity with increased susceptibility to HSV infection is confounded by concomitant immune defects in these hosts. Thus these studies offer suggestive but not conclusive evidence for the importance of ADCC in humans.

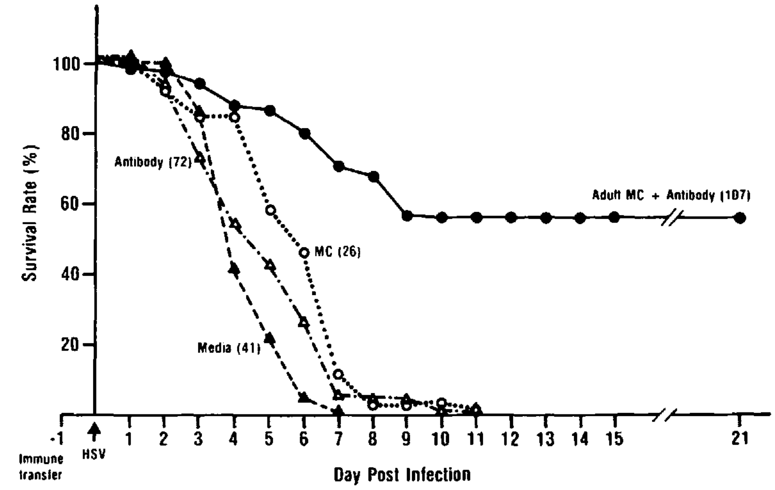

Figure 2. Protection of neonatal mice from lethal HSV infection by antibody and mononuclear cells (MC; 5 × 10^6 per mouse, primarily lymphocytes) from adult humans. One-week-old mice received an immune transfer 1 day before intraperitoneal inoculation with a uniformly lethal dose of HSV-1. In parentheses is the number of mice in each group. Reprinted with permission from [57].

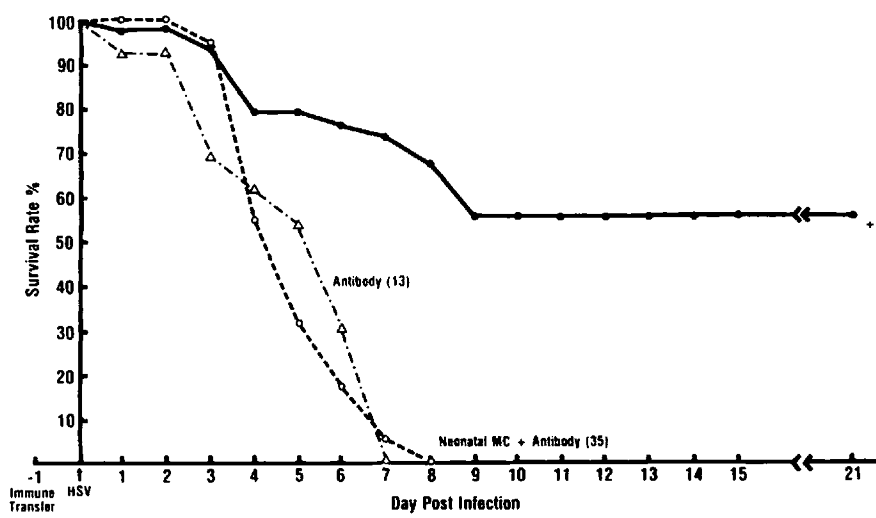

Figure 3. Lack of protection against murine neonatal HSV infection by antibody and mononuclear cells (MC) from human neonates. The experiments were conducted as described for figure 2. Reprinted with permission from [58].

Human-to-Murine Adoptive Transfer Model

We have taken a novel approach to further understanding the role of ADCC to HSV. In our model the ADCC-deficient 1-week-old mouse was used to determine the importance of human ADCC activity [57]. Neither human mononuclear leukocytes (lymphocytes plus macrophages) nor subneutralizing doses (10^{-6} dilution) of antibody to HSV alone could protect neonatal mice from lethal HSV infection (10^6 pfu of virus; >100 LD_{50}). In contrast, intraperitoneal injection of the combination of leukocytes plus antibody 1 day before infection provided solid protection [57] (figure 2). The effector cell was either a lymphocyte or a monocyte-macrophage. The antibody was effective at remarkably high dilutions (10^{-6}– 10^{-8} in normal human immune serum). In this model protection was not afforded by ADCC-inactive $F(ab^1)_2$ fragments, which neutralize virus. Transfer of cells and antibody after the infection was not protective. It is possible that with less lethal infections (that is, with use of either lower doses of virus or older, more resistant animals), a treatment model may be developed.

Mononuclear cells from patients unable to mediate ADCC in vitro, such as neonates [58] (figure 3) or patients with the leukocyte adhesion defect (absence of CD11,18 adhesive glycoprotein molecules [59]), could not protect in vivo in combination with antibody. When mononuclear cell ADCC activity of leukocytes from neonates was increased by incubation in γ interferon, the cells were able to mediate protection in vivo [60]. Antibody with high mononuclear cell ADCC activity in vitro, as in certain preparations of intravenous immune globulin [61] or polyclonal antisera raised in guinea pigs to cloned HSV glycoproteins B and D [62], was particularly able to mediate protection in vivo in combination with effector cells. Finally, with use of a battery of Mabs to HSV glycoprotein B and polyclonal antisera to synthetic peptides of glycoprotein D, an extremely strong association between in vitro ADCC activity and protection against HSV infection was discerned ($P < .001$) [63].

Table 1. Importance of ADCC in HSV infection.

Type of study	Findings
In vitro	Restriction of viral plaques in cell monolayers by antibody and leukocytes Early lysis of infected cells by ADCC mechanism
Mouse model	Appearance of ADCC antibody within 2–4 days after primary infection Low-level ADCC activity in HSV-susceptible mice (e.g., neonates) Cell requirement for optimal antibody protection in vivo Requirement for intact IgG, not $F(ab^1)_2$ fragments (which are unable to mediate ADCC), for protection Monoclonal antibody protection associated with ADCC, not neutralization activity
Human immunoepidemiologic	Early appearance of ADCC antibody in primary infection Appearance of ADCC antibody at time of defervescence in primary gingivostomatitis ADCC antibody titers inversely correlated with severity of neonatal infection Low effector activity in susceptible groups (e.g., neonates; patients with Hodgkin's lymphoma, burns, or leukemia)
Human-to-mouse transfer	Protection of infant mice by leukocytes from adult humans plus antibody No protection by cells from ADCC-deficient humans (e.g., neonates, leukocyte adherence–deficient patients) Association of polyclonal sera and Mab ADCC activity in vitro and protection in vivo

Conclusion

Multiple studies – in vitro, animal model, human immunoepidemiologic, and animal adoptive transfer – have supported the important role of ADCC in controlling HSV infection (table 1). Further proof of the significance of this mechanism must come from in vivo work in humans with defective ADCC and increased susceptibility to HSV infection. If provision of active cells, cell stimulators, or (when cellular function is adequate) high levels of ADCC antibody ameliorates or prevents serious HSV infection, a role for ADCC in humans will have been documented. Thus the challenge of the next decade is to translate our knowledge of the potential importance of ADCC into the clinical arena as we move into the era of epitopic vaccine selection, hyperimmune serum replacement, and recombinant cytokine-mediated immunomodulation.

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