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Possible Role of Fc Receptors on Cells Infected and Transformed by Herpesvirus: Escape from Immune Cytolysis

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Receptors for the Fc portion of nonimmune immunoglobulin G were demonstrated on B103 rat brain neuroma cells infected with herpes simplex virus type 1 (HSV-1) KOS by a radioimmunoassay using ^{125}I-labeled heat-aggregated Fc fragments. Immune F(ab')2 fragments specific for HSV antigens competed efficiently for Fc binding sites, suggesting that the binding of Fc fragments to infected cells is specific for viral cell-surface antigens. It has been suggested that the binding of immune complexes to Fc receptors on the surfaces of tumor cells in vivo plays a role in protecting these cells from immune destruction. In vitro evidence is presented for the ability of aggregated immunoglobulin G molecules bound to cell-surface Fc receptors to protect both HSV-infected and HSV-transformed cells against complement-dependent and cell-mediated immune lysis.

Receptors for the Fc portion of normal immunoglobulin G (IgG) have been detected on many lymphoid and reticuloendothelial cells and a wide variety of malignant cells (10, 24). Fc receptors (FcR) differ qualitatively in that they vary in their affinity for the classes and subclasses of immunoglobulins. The reaction between FcR and immunoglobulins may be stabilized by processes which alter the conformational structure of the Fc portion of the IgG molecule, as occurs in immune complexing or heat aggregation. Although the biological role of these receptors is not clearly understood, they appear to interact with a number of elements of the immune system. For example, Sinclair and Chan (17) suggest that FcR regulate lymphocyte function. It has also been suggested that the presence of FcR on such a diversity of tumor cells has important implications in the biology of tumor development. FcR on tumor cells may bind immune complexes and thereby mask tumor-specific antigen, protecting the tumor against immune cytolysis (10, 18).

Cells productively infected with herpes simplex virus (HSV) develop membrane changes which result in an affinity for the Fc portion of normal immunoglobulin (22). These receptors also have been detected on HSV type 1 (HSV-1)- and HSV-2-transformed hamster embryo fibroblasts (23). In this report, both lytically infected and transformed cells were used to test whether FcR may play a role in blocking immune destruction. The results presented here indicate that the binding of "blocking" antibody to the FcR of these cells in vitro is functional in protection from immune cytolysis mediated by either sensitized lymphocytes or complement and specific antibody.

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MATERIALS AND METHODS

Virus and target cells. The rat brain neuroma cell line B103 was obtained from D. Schubert (16), and the HSV-1-transformed hamster line 14-012-8-1 was from F. Rapp (5). All cells were grown in Eagle minimal essential medium supplemented with 10% fetal calf serum (Grand Island Biological Co.) and 100 U of penicillin and 100 μg of streptomycin per ml.

The KOS strain of HSV-1 was obtained from W. Munyon. Virus was propagated at low multiplicity in CV-1 cells.

Preparation of anti-HSV-1 antiserum. Serum against UV-inactivated HSV-1 KOS was obtained by injection of rabbits with 1 mg of partially purified virus in complete Freund adjuvant as reported earlier (19). The virus for injection was produced in primary rabbit kidney cells adapted for growth in rabbit serum.

Preparation of radiolabeled Fc fragments. Purified nonimmune rabbit Fc was prepared by the method of Porter (14) and radioiodinated by the chloramine-T procedure of Jensenius and Williams (8). The [^{125}I]sodium was obtained from Amersham/Searle. Aggregated fragments were prepared by heating for 20 min at 63°C, and gross precipitates were removed by centrifugation at 500 × g for 10 min. The iodinated Fc had a specific activity of approximately 3×10^6 cpm/μg.

Binding of ^{125}I-labeled Fc fragments. A 50-μl amount of aggregated Fc containing 10^6 cpm was added to unfixed confluent cultures grown on 5-cm^2

cover slips (Costar) and incubated at 37°C for 30 min. The coverslips were repeatedly washed in Veronal-buffered saline (VBS) and counted. All experiments were carried out in triplicate.

For the competition studies, the cover slips were first incubated at 37°C for 30 min with either nonimmune or immune rabbit F(ab')2 fragments prepared by the method of Nissonoff et al. (13). The cells were then washed in VBS and [^{125}I]Fc was added as described above.

Binding of fluorescein-labeled IgG. A 100-μl amount of fluorescein-conjugated complexes (Cappel Laboratories, Inc., Cochranville, Pa.) containing 500 μg of IgG was added to 100 μl of 2 × 10^5 HSV-infected cells in VBS and incubated for 30 min at room temperature. The cells were washed in cold VBS and centrifuged at 200 × *g*. Uninfected cells served as the negative control. The cells were examined with a Leitz Ortholux II microscope equipped with epifluorescence (selective fluorescein isothiocyanate excitation) and phase illumination. A minimum of 200 cells was examined.

^{51}Cr release and blocking assays. Cultures of B103 cells were incubated 12 to 16 h in the presence of 60 μCi of ^{51}Cr (sodium chromate; Amersham/Searle) per ml. The cells were dispersed with 0.01% ethylene/diaminetetraacetic acid and washed three times with VBS (19). Viability was greater than 90%, as determined by trypan blue exclusion. Portions (0.1-ml, 2 × 10^4 cells) were added to plastic tubes (12 by 76 mm). For the blocking experiments presented in Table 1, for the experiments in lines 3 to 5 and 7 and 9, either nonimmune aggregated IgG (agg-IgG) or agg-F(ab')2 was mixed with the cells for 1 h at 37°C. A 0.1-ml amount of complement-inactivated immune serum prepared from HSV-1-immunized rabbits was then added to all tubes for 1 h at 37°C. As a complement source, 0.1 ml of guinea pig serum was added, and the cells were incubated at 37°C for 90 min. In the experiments presented in line 9, the immune serum was added for 1 h before the nonimmune agg-IgG. After the complement incubation, 1 ml of cold VBS was added, and each tube was centrifuged at 1,000 × *g* for 5 min. A 1-ml quantity of supernatant fluid was removed for determination of ^{51}Cr activity. Results are expressed as percent ^{51}Cr released = (experimental release − spontaneous release)/[maximum release (2× freeze-thawed) − spontaneous release].

Effector cell assays. Blood was taken from a rabbit immunized with UV-inactivated extracts from rabbit cells infected with HSV-1 KOS. The mononuclear cells were separated by Ficoll-Hypaque flotation (6). After three washings with VBS, these cells were incubated with target cells for 4 h at 37°C with frequent shaking. The optimum ratio of effector: target cell was determined and carried out at a ratio of 100:1. The reaction was stopped by the addition of 1 ml of cold VBS, and ^{51}Cr release was determined as described above.

Complement fixation assay. Complement fixation was determined by the method of Kabat and Mayer (9).

RESULTS

Detection of FcR. The presence of FcR on infected cells can be demonstrated by the adsorption of radiolabeled agg-Fc fragments. The experiment presented in Fig. 1 shows the appearance with time of receptors on B103 cells after infection by HSV-1 KOS at a multiplicity of five. The binding of [^{125}I]Fc increased exponentially from 3 h postinfection, and by 10 h after infection the amount of Fc binding was approximately 50 times greater than that observed with uninfected cells. Similar curves were found using nonimmune agg-IgG in place of purified Fc. These results with IgG confirm the findings of Westmoreland and Watkins (22).

Effect of immune F(ab')2 on Fc binding. Competition experiments were carried out by using either nonimmune F(ab')2 fragments or F(ab')2 directed against HSV antigens to establish that the binding of Fc fragments to infected cells was specific for viral surface antigens. When infected cells were preincubated with nonimmune fragments, no effect was seen on the binding of [^{125}I]Fc. However, when infected cells were preincubated with immune F(ab')2 fragments, the level of Fc binding was reduced greater than 90% (Fig. 2). These results suggest that Fc specifically binds to infected cell membranes.

Blocking of complement-mediated cytolysis. Protection of infected cells from immune destruction was first tested by using a complement-mediated cytolysis assay. To avoid nonphysiological levels of IgG, blocking experiments employed less than 10 mg of IgG per ml. The normal concentration found in human serum is approximately 10 to 12 mg/ml. Both uninfected and KOS-infected B103 cells were labeled with ^{51}Cr. Incubation of the infected cells in the presence of anti-HSV serum and complement resulted in the immune destruction of infected cells, with release of the chromium (Table 1, line

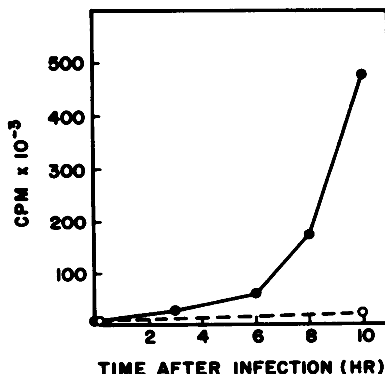

FIG. 1. *Binding of ^{125}I-labeled aggregated rabbit Fc fragments to B103 cells infected with HSV. Binding to either KOS-infected (●——●) or mock-infected (○----○) cells was measured.*

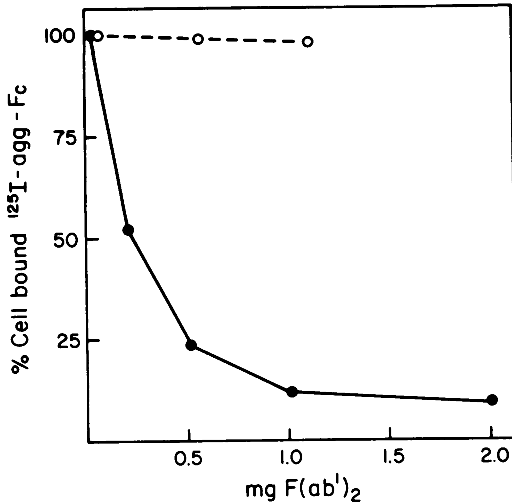

FIG. 2. *Binding of [^{125}I]Fc to cells preincubated with immune F(ab')2 (●——●) or nonimmune F(ab')2 (O-----O). One hundred percent represents 7 × 10^4 cpm bound.*

2). Uninfected cells were unharmed (Table 1, line 1). However, when infected cells were preincubated with aggregated nonimmune IgG as an Fc reagent (3), immune destruction was reduced from 40 to 60% (Table 1, lines 3 and 4). In some experiments as much as 90% protection against immune destruction was seen. Preincubation with the F(ab')2 fraction of normal IgG had no effect on the level of immune cytolysis (Table 1, line 5).

Primary cells transformed by HSV express virus-specific surface antigens, some of which have been reported to possess FcR activity (23). Experiments similar to those above were carried out by using the HSV-1 hamster embryo fibroblast-transformed cell line 14-012-8-1. We found that these transformed cells express FcR by a standard rosette assay (23) using sheep erythrocytes coated with anti-sheep erythrocyte IgG (unpublished data). Incubation in the presence of anti-HSV serum and complement resulted in the immune destruction of the cells (Table 1, line 6). In contrast, preimmune serum plus complement gave only about 1% release of ^{51}Cr (data not shown). However, preincubation in the presence of agg-IgG reduced cytolysis by 25 to 30% (Table 1, lines 7 and 8). These results indicate that the presence of blocking IgG reduces the level of complement-mediated immune destruction of transformed cells also.

Results similar to those described above would also be expected if complement is fixed to the agg-IgG. That is, it may be argued that reduced destruction is not due to blocking of binding sites for immune IgG, but rather to insufficient complement required for immune cytolysis. This possibility was tested by several additional experiments.

KOS-infected B103 cells were incubated first in the presence of anti-HSV serum for 60 min, followed by addition of nonimmune agg-IgG for an additional 60 min before complement was introduced. Under these experimental conditions, no inhibition of complement-mediated cytolysis was found to occur (Table 1, line 9), demonstrating that agg-IgG does not remove sufficient complement to interfere with the observed cytolysis.

The complement-fixing ability of agg-IgG was tested directly. A 1-mg quantity of nonimmune agg-IgG was mixed with infected cells in the presence of complement and incubated at 37°C for 1 h. The tubes were centrifuged at 5,000 × g for 30 min, and the number of residual C'H 50 units in the supernatant was determined. Approximately 25% of the hemolytic complement was fixed, as contrasted with the fixation of 80% of the complement in an assay involving infected cells and immune serum. The supernatant fluid from the agg-IgG mixture was tested for its complement activity in a fresh immune cytolysis assay. The complement remaining in this supernatant was sufficient to cause 98% lysis of infected cells. We conclude that the observed blocking of immune lysis by agg-IgG is not due

TABLE 1. *Effect of blocking antibody on complement-mediated cytolysis*

Test system	^{51}Cr released (±SE)[a]	% Specific ^{51}Cr released
1. B103 (uninfected)	104(±12)	1
2. B103 (KOS infected)	10,437(±213)	100
3. B103 (KOS infected) + 1.0 mg of IgG[b]	6,784(±308)	65
4. B103 (KOS infected) + 2.0 mg of IgG[b]	4,070(±382)	39
5. B103 (KOS infected) + 2.0 mg of F(ab')2[c]	9,915(±124)	95
6. 14-012-8-1	18,115(±250)	90
7. 14-012-8-1 + 1.0 mg of IgG[b]	15,095(±610)	75
8. 14-012-8-1 + 2.0 mg of IgG[b]	13,889(±521)	69
9. B103 (KOS infected) + anti-HSV antibody + 2.0 mg of IgG[b]	10,112(±138)	97

[a] Complement-mediated antibody cytolysis using excess rabbit anti-HSV-1 antibody and complement. Results represent the mean counts per minute released in six independent experiments. SE, Standard error.

[b] Nonimmune rabbit IgG (aggregated at 63°C for 20 min).

[c] Nonimmune rabbit F(ab')2 (aggregated at 63°C for 20 min).

to depletion of complement.

Cytolysis by sensitized effector cells. Immune destruction of cells expressing HSV antigens may also be demonstrated by incubation of the infected cells with peripheral blood lymphocytes from rabbits sensitized to the virus (15). Complement plays no role in this system. Incubation of infected B103 cells in the presence of sensitized lymphoyctes resulted in the release of approximately 50% of the chromium (Table 2, line 2). Preincubation of the cells with nonimmune agg-IgG was found to reduce the level of immune destruction by as much as 67% (Table 2, line 4). These results suggest that blocking effects of agg-IgG may also be demonstrated under these conditions of complement-independent immune destruction.

Immunofluorescence by fluorescein isothiocyanate-agg-IgG. Infected cells were incubated in the presence of fluorescein isothiocyanate–agg-IgG and observed for the distribution of fluorescence. Fluorescence appeared to be randomly distributed over the surface of the cells, with no evidence of cap formation.

DISCUSSION

Receptors for the Fc portion of the nonimmune IgG have been demonstrated on cells infected or transformed by HSV (22, 23). In this report, FcR induced by HSV infection were quantitated by binding ^{125}I-labeled Fc fragments to the cell surface. Uninfected cells failed to bind either nonimmune IgG or Fc components. Because the ability of infected cells to bind Fc fragments dramatically increases in the latter stages of the infection cycle (8 to 10 h postinfection), a time during which host cell macromolecular synthesis has been largely turned off, FcR are thought to be virally encoded proteins. The viral origin of these FcR is also suggested by the ability of only specific immune F(ab')2 fragments to interfere with binding of radiolabeled Fc components. Whether this represents direct competition for the same binding site, or, as has been reported for FcR and Ia antigens on lymphocytes (4), the FcR are located in very close association with viral cell-surface antigens, is unknown. In either case, however, the binding of Fc fragments or immune F(ab')2 components are mutually exclusive.

TABLE 2. *^{51}Cr release from infected cells by sensitized lymphocytes*

Test system	^{51}Cr released (±SE)[a]	% Lysis
1. B103 (uninfected)	117(±51)	1
2. B103 (KOS infected)	7,538(±417)	48
3. B103 (KOS infected) + 1.0 mg of nonimmune agg-IgG	5,653(±698)	36
4. B103 (KOS infected) + 2.0 mg of nonimmune agg-IgG	2,512(±134)	16

[a] Results are expressed as counts per minute from four experiments.

The experiments reported here demonstrated that FcR can play a role in escape from immune destruction in vitro. Preincubation of infected cells with heat-aggregated nonimmune IgG reduced complement-mediated cytolysis as much as 60% and cell-mediated lysis by approximately 67%, presumably by preventing access to the cell surface of either antiviral antibody or immune effector cells. In some experiments, as much as 90% blocking of immune lysis was achieved.

A number of phenomena can be ruled out as contributing to the blocking of immune cytolysis by agg-IgG. First, the addition of agg-Fc fragments or agg-IgG might cause redistribution of FcR on the cell and result in clustering or capping. This might render the cells resistant to immune destruction if the HSV antigens capped along with the FcR. A direct examination with fluorescein isothiocyanate–agg-IgG-treated infected cells failed to demonstrate any evidence for capping. Fluorescence was randomly distributed on the surface of the infected cells. Second, reduced cytolysis would be expected in the complement-mediated test if bound agg-IgG removed large amounts of complement. The blocking phenomenon, however, cannot be attributed to complement depletion for several reasons. (i) Addition of excess complement has no effect on the level of cytolysis (data not shown). (ii) Under the experimental conditions utilized here, immune serum fixed 80% of the available complement in the presence of infected cells. agg-IgG plus infected cells fixed only 25% of the complement. In a separate assay, the remaining complement was sufficient to kill over 98% of infected cells exposed to cytolytic antibody. (iii) Cytolysis assays in which the immune antibody was mixed with infected cells before the addition of agg-IgG and complement resulted in 97% destruction of the cells. That is, in a direct test, agg-IgG did not interfere with complement-mediated cytolysis.

The mechanism by which agg-IgG blocks cell-mediated immune lysis is not clear. The simplest explanation might be that the binding of the aggregated complexes to target cell surface FcR blocks access to immune effector cells. In addition, agg-IgG could block by binding to FcR on the activated lymphocytes. The relative roles of these two mechanisms for blocking cell-mediated cytolysis remains to be explored.

The failure of agg-IgG to protect 100% of the cells from cytolysis in vitro is not understood.

Several explanations, however, are possible. (i) FcR on HSV-infected cells have been shown to turn over at the cell surface (23), and thus not all cells may be simultaneously expressing sufficient FcR activity to afford protection from immune lysis. (ii) IgG may transiently bind to FcR (20), or FcR may shed from the cell surface upon binding with agg-IgG, as has been shown to be the case with FcR on certain murine leukemia cells (2), exposing HSV antigenic sites for attachment. (iii) The kinetics of binding between immune F(ab')2 and Fc components may favor immune complexing, and consequently, a portion of cells will have immune antibody bound to the cell surface when complement is added and thus be susceptible to lysis. It is possible that in vivo conditions favor increased protection. Even if the level of protection in vivo is no more than we find in vitro, this could provide for the selection of increased numbers of FcR-bearing cells and increased resistance to immune destruction.

Numerous studies have demonstrated that immune complexes adhere to cells bearing FcR via the Fc portion of IgG (10). Evidence suggests that immune adherence can mediate or induce a wide variety of immunological effects, including tolerance (10), feedback suppression of immune functioning (11), and immunological enhancement of tumor development (10). Initially, it was thought that immunological enhancement of tumor development is a process whereby certain antitumor cell-mediated reactions are inhibited by the production of tumor-specific antibodies which combine with tumor cell surface antigens (7). As a consequence, target cell antigens become "masked" by antibody so as to prevent recognition by killer or aggressor lymphocytes. This mechanism does not explain why complement-dependent or antibody-dependent cell-mediated immune lysis would not occur. It is now speculated that adherence of immune complexes to tumor cell FcR would mask the presence of specific antigens by sterically blocking the attachment of specific antibody or cytotoxic immune effector cells (1, 10). This mechanism predicts that FcR and tumor-specific cell surface antigens are located in close proximity on the cell surface, or that the tumor antigen itself has Fc binding activity. The plausibility of such a blocking mechanism is supported by the vast array of tumor cells, both of lymphoreticular and non-lymphoreticular cell origin, which possess FcR (12, 21). Although this mechanism remains to be tested in a tumor-bearing host, the present report, in which physiological concentrations of blocking molecular complexes were employed, represents a demonstration that such an escape mechanism is functional in vitro. Whether FcR play a role, in vivo, during a herpetic infection or in herpesvirus-induced latency or oncogenesis, remains to be tested.

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