Binding of human and animal immunoglobulins to the IgG Fc receptor induced by human cytomegalovirus

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Human cytomegalovirus (HCMV)-infected cells express a virus-encoded receptor that is able to bind the Fc part of IgG. Some basic binding properties of this Fc receptor (FcR) have been examined. The affinity constant (K_a) for human IgG Fc fragment in its interaction with acetone-fixed, HCMV-infected human embryonic lung fibroblasts was estimated to be around 2 × 10^8 M⁻¹ and the number of binding sites was estimated to be around 2 × 10⁶ per cell. Of the human IgG, IgA, IgM and IgD classes, only IgG reacted with the receptor, and all four of the IgG subclasses were reactive. IgG from rabbit, hamster, cat, swine and horse exhibited binding to the HCMV FcR, in contrast to IgG from mouse, rat, guinea pig, dog, sheep, goat, cow and chicken. Immunoglobulins with and without HCMV IgG FcR-binding properties, like IgG from rabbit and mouse, can be of value in revealing the functional importance of the receptor. When the immunoglobulins were tested against herpes simplex virus type 1-induced FcR, both similarities and differences in immunoreactivity were seen relative to the HCMV FcR, which makes it unlikely that the binding sites for these two herpesvirus FcRs on the IgG molecule are identical.

Introduction

Cells infected with human herpesviruses such as human cytomegalovirus (HCMV), herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV) and Epstein–Barr virus (EBV) express specific virus-encoded receptors that are capable of binding the Fc part of IgG (Westmoreland & Watkins, 1974; Furukawa et al., 1975; Keller et al., 1976; Ogata & Shigeta, 1979; Para et al., 1982; Yee et al., 1982). The best characterized of these receptors is that induced by HSV-1. The receptor is composed of two glycoproteins, gE and gI, encoded by the HSV-1 genome (Lee et al., 1982; Para et al., 1982; Johnson et al., 1988). The Fc receptor (FcR) induced by HCMV has not been characterized to the same extent as that of HSV. Furukawa et al. (1975) and Keller et al. (1976) demonstrated that infection of human fibroblasts with HCMV induces expression of a receptor able to bind the Fc part of IgG. The receptor can be detected not only on the cell surface membrane and in the cytoplasm of infected cells (Furukawa et al., 1975; Keller et al., 1976; Rahman et al., 1976; Westmoreland et al., 1976; Sakuma et al., 1977; Frey & Einsfelder, 1984), but also on the HCMV virion (Stannard & Hardie, 1991). Suppressor of HCMV replication like cytosine arabinoside, cycloheximide and phosphonoformate inhibit the formation of the receptor (Furukawa et al., 1975; Wahren & Öberg, 1979). A number of proteins with different molecular masses have been proposed to be the HCMV-induced FcR (Sakuma et al., 1977; Xu-Bin et al., 1989; Stannard & Hardie, 1991), but the specific protein(s) responsible for IgG Fc binding has not yet been identified unequivocally. Neither has the HCMV FcR coding region(s) on the genome been identified.

The immunoreactivity and physico-chemical properties of the HSV-1-induced FcR have been characterized rather well (Johansson et al., 1984, 1985, 1989; Johansson & Blomberg, 1990; Dubin et al., 1992; York & Johnson, 1995; Chapman et al., 1999). This is in contrast to our rather limited knowledge of the HCMV FcR. Here, we describe some binding specificities and other properties of the HCMV FcR and compare these with those known for the HSV-1 FcR in order to reveal a possible relationship between these two virus FcRs. Know-
ledge of binding and non-binding abilities of various immunoglobulins can be of value in revealing the functional importance of the HCMV FcR.

Methods

**Cells.** Human embryonic lung (HEL) fibroblasts and African green monkey kidney (GMK) cells strain AH1 were cultured in glass roller tubes (1.5 x 10 cm) with 1 ml Eagle’s minimal essential medium (MEM) (Flow Laboratories) supplemented with 10% FCS, 0.3 mg/ml glutamine and 50 µg/ml gentamicin. When the cells were confluent, with a density of 10^6–10^8 cells per tube, the medium was changed to 1 ml RPMI 1640 tissue culture medium (Flow Laboratories) supplemented with gentamicin, glutamine and 1% FCS.

**Virus.** Seed stocks of HCMV strain Ad-169 (ATCC VR-538) and HSV-1 strain F were obtained by inoculating virus at low m.o.i. onto HEL fibroblasts and GMK AH1 cells, respectively. At full cytopathic effect, the cells were loosened and homogenized by Dounce homogenization. The suspensions were clarified by centrifugation at 2000 g for 10 min and the supernatant was stored at –80 °C until use.

**Immunoglobulins.** Purified human IgG myeloma proteins IgG1 JRS, IgG1 1-2319, IgG2 19-853, IgG3 Cro, IgG3 Hus, IgG3 Lee and IgG4 15-2522 were a kind gift of Ralph C. Williams, Jr (University of Florida, Gainesville, FL, USA). The purification and characterization of these myeloma proteins have been described previously (Johansson et al., 1994a, b). The allotypic markers for IgG3 Cro and IgG3 Hus myeloma proteins were G3m(sI-1), G0m(b0), G3m(b1) and for IgG3 Lee myeloma protein they were G3m(sI-1), G0m(b0), G3m(b1) and G3m(g4) (Johansson et al., 1994a, b).

Pooled polyclonal human IgM, human serum IgA (kappa) myeloma protein (AG IgA-3015) and human IgD (lambda) myeloma protein (AG IgD-4490) were a kind gift of Anders Grubb (University of Lund, Lund, Sweden) (Johansson et al., 1984). Chromatographically purified human IgG Fc fragment, rabbit IgG, guinea pig IgG, hamster IgG, goat IgG Fc fragment, sheep IgG, bovine IgG, swine IgG and chicken IgG were from Cappel Laboratories and mouse IgG, rat IgG, dog IgG, cat IgG and horse IgG were from Jackson Immuno Research. The suppliers certified the purity of each preparation. The immunoglobulin concentrations were verified by spectrophotometric measurements at 280 nm before they were used in the binding assay.

**Protein iodination.** The lactoperoxidase method was used for 125I labelling of human and animal immunoglobulins and IgG Fc fragments, as described previously (Johansson et al., 1984). The proteins were radiolabelled to a specific activity of 10–20 mCi/mg.

**Infection and fixation of cells.** HEL or GMK cells in glass tubes containing 1 ml RPMI tissue culture medium supplemented with 1% FCS were either kept uninoculated or infected with HCMV at 1 TCID50 per cell or HSV-1 at 5 TCID50 per cell. Triplicate samples of HCMV-infected cells were incubated at 37 °C for 72 h (unless stated otherwise) and triplicate samples of HSV-infected cells were incubated for 18 h, as described previously (Johansson & Blomberg, 1990). The medium was then removed and the cells were fixed with 1 ml acetone for 2 min, unless stated otherwise. The cells were washed once with 4 ml PBS prior to testing in the binding assay.

HCMV-infected cells fixed with ethanol were treated identically, with the exception that acetone was replaced by ethanol (99% v/v). Neither ethanol nor acetone was added to unfixed cells, but these cells otherwise received treatment identical to fixed cells. The number of cells in the glass tubes was determined by counting in a haemocytometer after trypsination of uninfected cells.

**Immunoglobulin-binding assay.** In the binding assays, 10 ng radiolabelled human IgG, IgM, IgD, IgA or animal IgG (3.3 x 10^18 M) or IgG Fc fragment (1.0 x 10^-8 M), in 200 µl PBS with 0.2% ovalbumin (PBS-OVA), pH 7.4, was added to triplicate tubes with infected and uninfected cells. After 4 h rotation in a roller drum at 37 °C, the supernatant was removed and the cells were washed once with 4 ml PBS. The tubes were put in a gamma counter and the cell-bound radioactivity was measured. Binding is expressed as the amount of immunoglobulin retained.

**Period of incubation.** In order to determine the optimal period of incubation, 10 ng radiolabelled human IgG Fc fragment was incubated with HCMV-infected and uninfected cells for 0, 1, 2, 3, 4, 5 and 6 h. Unless stated otherwise, the ligand was incubated for 4 h in all subsequent experiments.

**Inhibition with human and animal immunoglobulins.** In order to verify the Fc-specificity of binding, radiolabelled human and animal IgG molecules and IgG Fc fragments (10 ng in 200 µl PBS–OVA) were incubated with an excess of unlabelled human IgG Fc fragment (10 ng in 200 µl PBS–OVA) in triplicate samples of HCMV- or HSV-1-infected cells. The molar excesses of unlabelled human IgG Fc fragment over radiolabelled human and animal IgG or IgG Fc fragments were respectively 3000- and 1000-fold.

In inhibition experiments with animal IgG, 10 ng radiolabelled human IgG Fc fragment in 200 µl PBS–OVA was incubated with 30 µg unlabelled animal IgG for binding to HCMV-infected and uninfected cells. The molar excess of unlabelled animal IgG over radiolabelled human IgG Fc fragment was 1000-fold. In another inhibition experiment with animal IgG, 10 ng radiolabelled human IgG Fc fragment was incubated with 625, 3125, 15625 and 78125 ng unlabelled animal IgG or with 208, 1042, 5208 and 26042 ng unlabelled animal or human IgG Fc fragment.

**Inhibition with human IgG Fc fragment: Scatchard analysis.** Ten ng radiolabelled human IgG Fc fragment and 0, 10, 20, 40, 80, 160, 320, 640, 1280, 2560, 5120, 10240, 20480, 40960 or 81920 ng unlabelled human IgG Fc fragment in 200 µl PBS–OVA were incubated with HCMV-infected and uninfected cells in the roller drum at 37 °C for 6 h. The cells were treated as described above. The amounts of bound and unbound immunoglobulins were calculated and, after subtracting the uptake by uninfected cells from that by the infected ones, the data were plotted for Scatchard analysis.

Results

**Effect of fixation of HCMV-infected cells.**

Treatment of HCMV-infected cells with acetone or ethanol preserved the Fc-binding properties of the cells. The respective levels of binding of radiolabelled human IgG Fc fragment to unfixed cells and to acetone- and ethanol-fixed cells were: for unfixed cells, 44 ± 7, 47 ± 3 and 47 ± 6 pg; and for HCMV-infected cells, 127 ± 10, 972 ± 77 and 1102 ± 359 pg (means ± 1 SD of triplicates). As the increase in binding on infection was higher for acetone- and ethanol-fixed cells than for unfixed cells, acetone was chosen for fixation of cells in all subsequent experiments.

**Time-course of the appearance of the HCMV Fc-binding protein.**

When 1 TCID50 HCMV per cell was added to cells in 1 ml tissue culture medium and tested every 24 h for 120 h, binding
of radiolabelled human IgG Fc fragment increased until 72 h post-infection. A plateau was then reached (Fig. 1a). In all subsequent experiments with HCMV-infected cells, the binding assay was performed 72 h post-infection.

**Period of incubation**

When radiolabelled human IgG Fc fragment was tested every hour for 6 h for binding to HCMV-infected cells, an increase in binding was seen for the first 3 h. Thereafter, a plateau was reached (Fig. 1b). Unless stated otherwise, radiolabelled immunoglobulin was incubated for 4 h in all subsequent experiments.

**Inhibition of binding of human IgG Fc fragment: Scatchard analysis**

Various amounts of unlabelled human IgG Fc fragment were allowed to inhibit the binding of radiolabelled human IgG Fc fragment to HCMV-infected and uninfected cells. After subtracting the binding of ligand to uninfected cells from that to HCMV-infected cells, the data were analysed and plotted according to Scatchard (1949) (Fig. 2). The data points fell approximately on a straight line, with the exception of a minor deviation from linearity. Extrapolation of the line to the x-axis gave the number of binding sites per cell and the point of intersection on the y-axis gave the association coefficient ($K_a$). Three different experiments gave a mean of $1.5 \times 10^5$ binding sites per cell ($1.7 \times 10^5, 1.7 \times 10^5, 1.2 \times 10^5$) and a mean association constant ($K_a$) of $2.1 \times 10^8$ M$^{-1}$ ($2.5 \times 10^8, 1.7 \times 10^8, 2.1 \times 10^8$).

**Binding of different human immunoglobulin classes to HCMV-infected cells**

The binding of radiolabelled human IgM, IgA, IgD and IgG Fc fragment to HCMV-infected and uninfected cells is shown in Table 1. In contrast to radiolabelled human IgG Fc fragment, radiolabelled human IgM, IgA and IgD did not bind to a greater extent to HCMV-infected cells than to uninfected cells and the binding was not affected by addition of an excess of unlabelled human IgG Fc fragment.

**Binding of human IgG subclasses to HCMV-infected cells**

The binding of radiolabelled human IgG1, IgG2, IgG3 and IgG4 myeloma proteins to HCMV-infected and uninfected
Table 1. Binding of radiolabelled human IgM, IgG Fc fragment, IgA and IgD to acetone-fixed, HCMV-infected and uninfected cells

Binding is expressed as pg per assay. Means ± 1 SD of triplicates are shown.

<table>
<thead>
<tr>
<th>Class</th>
<th>Uninfected cells</th>
<th>HCMV-infected cells</th>
<th>Binding inhibition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human IgM</td>
<td>221 ± 21</td>
<td>242 ± 29</td>
<td>198 ± 8</td>
</tr>
<tr>
<td>Human IgG Fc</td>
<td>77 ± 1</td>
<td>948 ± 119</td>
<td>106 ± 11</td>
</tr>
<tr>
<td>Human IgA</td>
<td>59 ± 10</td>
<td>78 ± 4</td>
<td>67 ± 7</td>
</tr>
<tr>
<td>Human IgD</td>
<td>162 ± 6</td>
<td>172 ± 4</td>
<td>175 ± 13</td>
</tr>
</tbody>
</table>

* Inhibition of binding to HCMV-infected cells by an excess of unlabelled human IgG Fc fragment.

Fig. 3. Binding and inhibition of binding of radiolabelled human IgG myeloma proteins to HCMV-infected cells. Bars labelled ‘a’ represent uninfected cells, those labelled ‘b’ represent HCMV-infected cells and those labelled ‘c’ represent inhibition of binding to HCMV-infected cells by an excess of unlabelled human IgG Fc fragment. Means ± 1 SD of triplicates are shown. Binding is expressed as ng protein bound.

The binding of various radiolabelled animal IgGs to HCMV-infected and uninfected cells is shown in Fig. 4. Radiolabelled rabbit IgG exhibited a clear increase in binding to HCMV-infected cells compared with uninfected cells. Also, but to a lesser extent, an increase in binding was seen for hamster IgG, cat IgG, swine IgG and horse IgG. An excess of unlabelled human IgG Fc fragment inhibited the binding, demonstrating the specificity of the interaction for Fc. In contrast, radiolabelled mouse IgG, rat IgG, guinea pig IgG, dog IgG, sheep IgG, goat IgG Fc fragment, bovine IgG and chicken IgG did not bind to a greater extent to infected cells than to uninfected cells and were not affected by addition of unlabelled human IgG Fc fragment.

The inhibition of binding of radiolabelled human IgG Fc fragment to HCMV-infected cells with a 1000-fold molar excess of unlabelled animal IgG or IgG Fc fragment is shown in Fig. 5. Rabbit IgG, hamster IgG, cat IgG, swine IgG and...
Fig. 6. Binding and inhibition of binding of radiolabelled human IgG myeloma proteins to HSV-1 infected cells. Bars labelled ‘a’ represent uninfected cells, those labelled ‘b’ represent HSV-1-infected cells and those labelled ‘c’ represent inhibition of binding to HSV-1 infected cells by an excess of unlabelled human IgG Fc fragment. Means ± 1 SD of triplicates are shown. Binding is expressed as ng protein bound.

horse IgG inhibited the binding of human IgG Fc fragment more extensively than did mouse IgG, rat IgG, guinea pig IgG, dog IgG, sheep IgG, goat IgG Fc fragment, bovine IgG and chicken IgG.

In the inhibition experiment with various amounts of animal IgG (625, 3125, 15625 or 78125 ng) or IgG Fc fragment (208, 1042, 5208 or 26042 ng), unlabelled rabbit IgG, hamster IgG, cat IgG, swine IgG and horse IgG also exhibited clear inhibition of binding of radiolabelled human IgG Fc fragment, in contrast to mouse IgG, rat IgG, guinea pig IgG, dog IgG, sheep IgG, goat IgG Fc fragment, bovine IgG and chicken IgG, where no inhibition or only minor inhibition was seen (data not shown).

**Discussion**

The protein(s) responsible for the Fc-binding ability of HCMV has not yet been identified unequivocally, nor has the FcR coding region(s) on the HCMV genome been identified. As a consequence, HCMV-infected cells have to be used in order to study the receptor. We utilized fixed HCMV-infected cells to characterize some binding properties of the HCMV-induced IgG FcR. It has been shown for HSV-1-infected cells that fixation does not affect the binding specificity of the receptor (Johansson & Blomberg, 1990). For HCMV-infected cells, a large increase in immunoglobulin binding was seen for cells fixed with acetone or ethanol compared with unfixed cells. An excess of unlabelled IgG Fc fragment inhibited the binding, which verified the specificity of the interaction for Fc.
high binding affinity (Furukawa et al., 1975; Keller et al., 1976; MacCormac & Grundy, 1996). The fact that the HCMV FcR is located mainly in the cell cytoplasm rather than at the cell surface in infected cells makes the availability of the receptors to extracellular IgG limited in vivo. The biological relevance of IgG binding to acetone-fixed cells, in which intracellular receptors become artificially available, is presently not known. It is also not known whether there is another ligand in addition to IgG that participates in binding to the intracellular FcRs.

All binding experiments, including the one used for Scatchard analysis, were performed under optimized binding conditions, that is when expression of the number of receptors on cells had reached a plateau and the ligand–receptor interaction was at equilibrium. When the data obtained from the inhibition experiments with human IgG Fc fragment were plotted according to Scatchard (1949), the data fell on a straight line, with the exception of a minor deviation. The deviation from linearity could implicate molecular heterogeneity at the level of either the receptor or the ligand. The HSV-1 IgG FcR is composed of two glycoproteins, gE and gL, which act together as a high-affinity receptor, while gE alone functions as a low-affinity receptor (Johnson et al., 1988; Dubin et al., 1990; Basu et al., 1997). Whether the HCMV FcR functions in a similar way is not known, as the receptor protein(s) has not yet been identified unequivocally. The ligand used, human IgG, is composed of the four subclasses IgG1, IgG2, IgG3 and IgG4 and different binding affinities for the various IgG subclasses could explain the deviation seen. The different degrees of affinity reported by Murayama et al. (1986) for each of the human IgG subclasses in their reactivity with HCMV FcR (IgG1 > IgG4 > IgG2 > IgG3) support heterogeneity at the ligand level.

The number of Fc-binding sites on the HCMV-infected cell (1.5 × 10⁶) is rather similar to the number of Fc-binding sites reported for the HSV-1-infected cell (3–9 × 10⁶) (Johansson & Blomberg, 1990). These numbers of binding sites are large compared with the numbers of FcRs on other cells, e.g. FcRI on human monocytes and macrophages (1–4 × 10⁴ per cell) (Anderson & Looney, 1986). The large number of binding sites on virus-infected cells may be due to the HCMV- and HSV-directed switch from synthesis of host cell proteins to synthesis of virus proteins and the detection of intracellular virus-binding sites. The number of binding sites does not necessarily mean the same number of receptors, as there may be several binding sites on each receptor molecule, as seen for the bacterial FcRs protein A (Langone, 1982) and protein G (Guss et al., 1986).

The affinity constant (Kₐ) for human IgG Fc fragment in its interaction with acetone-treated, HCMV-infected HEL fibroblasts was estimated to be around 2–1 × 10⁸ M⁻¹. This is in the range of the Kₐ of the human FcR in its interaction with human IgG (1–3 × 10⁸ M⁻¹; Unkeless et al., 1988). The affinity constant of the HSV-1 FcR in its interaction with rabbit IgG was determined to be around 2.3 × 10⁷ M⁻¹ (Johansson & Blomberg, 1990). That the affinity constant of the HCMV FcR is higher than that of the HSV FcR is in agreement with the report that, in twofold serial dilutions, HCMV-infected cells bound human and rabbit IgG Fc fragments at lower concentrations than did HSV-1-infected cells (MacCormac & Grundy, 1996). We conclude that the number of Fc-binding sites on HCMV-infected cells is approximately equal to that on HSV-1-infected cells and that the affinity constant of the HCMV receptor in its interaction with human IgG Fc fragment is higher than that of the HSV-1 receptor in its interaction with rabbit IgG.

The receptor activity induced in HCMV-infected cells is immunoglobulin class-specific. No evidence was found for specific interactions between the FcR of HCMV and human IgM, IgA or IgD, which supports earlier results regarding human IgA and IgM (Keller et al., 1976; Rahman et al., 1976; Mackowiak & Marling-Cason, 1987). We also found that all four human IgG subclasses bound HCMV-infected cells (Murayama et al., 1986; Mackowiak & Marling-Cason, 1987), in contrast to the HSV-1 FcR, which is non-reactive to the IgG3 subclass (Johansson et al., 1984). Variations in binding were seen both within and between the various human IgG subclasses. It has been shown recently that the HSV-1 FcR discriminates between various IgG allotypes (Atherton et al., 2000) and whether the same is true for the HCMV FcR is presently not known. However, this cannot explain the discrepancy in binding seen for the IgG3 Cro and IgG3 Hus myeloma proteins, for example, which have identical allotypic phenotypes. The binding of the IgG3 myeloma proteins was performed at only one concentration and it cannot be excluded that other binding patterns might be seen at other concentrations.

When we looked at the ability of the HCMV-induced IgG FcR to bind different animal IgG molecules, only a few species exhibited clear reactivity. These species were rabbit, hamster, cat, swine and horse. In contrast, no reactivity or only minor reactivity were seen for IgG from mouse, rat, guinea pig, dog, sheep, goat, cow and chicken. These results are in agreement with earlier reports that monkey, rabbit and hamster IgG were reactive (Furukawa et al., 1975; Keller et al., 1976; Rahman et al., 1976; Westmoreland et al., 1976; MacCormac & Grundy, 1996) in contrast to rat and mouse IgG (Keller et al., 1976). Guinea pig IgG has been reported to be both reactive (Furukawa et al., 1975) and non-reactive (Keller et al., 1976). However, it cannot be excluded that there exist IgG subclasses for the apparently non-reactive animal IgG that exhibit non-immune binding to the HCMV FcR. In support of this suggestion, MacCormac & Grundy (1996) reported that, of the four mouse IgG subclasses, three were non-reactive, while only IgG3 was reactive.

A modest discrepancy in binding was seen in some instances between radiolabelled and unlabelled immunoglobulins. For example, radiolabelled cat and swine IgG bound
moderately well to HCMV-infected cells, in contrast to the distinct binding seen for the same unlabelled proteins. This discrepancy is probably due to the detrimental effect of the iodination procedure on the immunoglobulin molecule, which diminished its ability to bind the FcR. With these exceptions, similar patterns of binding were seen for radiolabelled and unlabelled animal IgG in most other cases, demonstrating the functionality of the radiolabelled immunoglobulins. Additional support for the functionality of the radiolabelled IgG molecules is that some of the same radiolabelled animal IgGs that were non-reactive when tested for binding with HCMV were reactive in the binding assay with HSV-1.

The reactivity of radiolabelled IgG on HSV-1-infected cells verifies earlier results regarding the binding specificity of the HSV-1 FcR (Johansson et al., 1984, 1985). This demonstrated the functionality of the radiolabelled IgG, and experiments with unlabelled immunoglobulins on HSV-1-infected cells were therefore not necessary. Some differences and some similarities were seen when the binding specificities of the HCMV and HSV-1 FcRs with various animal IgGs were compared. Differences in binding were seen not only for the human IgG subclasses but also for various animal IgGs. It is unlikely that the differences seen for the HCMV and HSV-1 FcRs are due to the fact that they were expressed on different cell lines, since we have shown previously that the cell line is not important for the specificity of the receptor (Johansson & Blomberg, 1987).

A binding site for the HSV-1 FcR is proposed to be located on the IgG molecule at the interface region between the CH2 and CH3 domains (Johansson et al., 1986, 1989; Chapman et al., 1999). This binding site is very close, although not identical, to that which interacts with the bacterial FcRs protein A (Deisenhofer, 1981) and protein G (Stone et al., 1989). There have been suggestions that the site on the Fc portion of IgG that binds to HCMV FcR either is (Stannard & Hardie, 1991) or is not (Mackowiak & Marling-Cason, 1987) located close to the attachment site for protein A. Even if the HCMV FcR interacts with the same region on IgG as the above proteins, it is unlikely that the HCMV FcR-binding site is identical to that of HSV-1, for example, as these herpesviruses possess different binding patterns for some human IgG subclasses and animal IgGs.

A comparison of the binding properties and nucleotide sequences of the various IgG FcRs could allow conclusions to be reached about their possible relationships. HSV-1, HSV-2 and VZV encode homologous Fc-binding proteins (Para et al., 1982; Dubin et al., 1990; Litwin et al., 1992) and similarity has been observed between an HSV-1 gE domain and mammalian FcR sequences (Dubin et al., 1994). In contrast, HCMV does not contain genes with sequence similarity to the FcR genes of HSV or VZV or to the FcR gene identified in murine CMV (Thale et al., 1994). The HCMV IgG FcR also did not react with a panel of mouse monoclonal antibodies directed against human FcγRI, FcγRII and FcγRIII (MacCormac & Grundy, 1996). This indicates that convergent evolution has taken place in developing IgG-binding properties. IgG FcRs are expressed by a number of herpesviruses, supporting the view that these receptors serve important functions for the viruses.

Discussion of the functional role of the HSV-1 FcR has focused on protection from immunological defence mechanisms (Dubin et al., 1992; York & Johnson, 1995; Nagashunmugam et al., 1998) and its importance in efficient transfer of virus across cellular junctions (Dingwell et al., 1994). The domain on the HSV-1 gE molecule that participates in IgG binding is distinct from the domain that mediates cell-to-cell spread (Weeks et al., 1997). For HSV-1, a process called ‘antibody bipolar bridging’ of anti-HSV antibodies has been proposed to take place (Frank & Friedman, 1989; van Vliet et al., 1992). This ‘antibody bipolar bridging’ occurs when the Fc domain of an anti-HSV IgG molecule that is in contact with an HSV antigen by its antigen-binding Fab part binds to the HSV FcR. This protects HSV-1 from antibody- and complement-mediated neutralization and antibody-dependent cellular cytotoxicity by blocking fixation of complement and recognition by FcR on cytotoxic cells (Frank & Friedman, 1989; Dubin et al., 1991; van Vliet et al., 1992). IgG with or without FcR-binding properties has been used in order to clarify the functional importance of the HSV-1 FcR (Frank & Friedman, 1989; Dubin et al., 1991; Nagashunmugam et al., 1998). Similar experiments with binding- and non-binding immunoglobulins can now be performed in order to reveal the functional role of the HCMV FcR. The finding that mouse IgG, for example, binds weakly or not at all to the HCMV receptor, whereas rabbit and human IgG bind well, can be valuable in such experiments.

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