Characterization of IgG Fc Receptors Induced by Human Cytomegalovirus

By XU-BIN, TSUGIYA MURAYAMA, KEIKO ISHIDA AND TORU FURUKAWA*

Department of Microbiology, Kanazawa Medical University, Uchinada, Ishikawa, Japan 920-02

(Accepted 18 November 1988)

SUMMARY

Immunoglobulin G Fc receptors (IgG FcRs) induced by human cytomegalovirus (HCMV) were isolated from solubilized HCMV-infected cell extracts by IgG affinity chromatography and analysed by Western blotting using ¹²⁵I-labelled human IgG and Fc. FcR species of Mr 130K, 65K, 50K and 38K were found to mediate the binding of IgG. The 130K and 65K FcRs were also detected on HCMV virions. All four FcRs were reactive with a murine monoclonal antibody to herpes simplex virus glycoprotein E. Anti-HCMV antibodies markedly inhibited the binding of ¹²⁵I-labelled human IgG Fc to the 130K and 50K species but inhibited to a lesser extent the binding to the 65K FcR.

INTRODUCTION

Receptors for the Fc portion of IgG (IgG FcRs) are present on a variety of cell types including lymphocytes, macrophages, monocytes (Anderson & Abraham, 1980; Unkeless et al., 1981) and some bacteria (Langone, 1982). The IgG FcRs on these cells have been well characterized (Hibbs et al., 1986; Lewis et al., 1986). IgG FcRs can also be induced in cells infected with human herpesviruses such as herpes simplex virus type 1 (HSV-1) (Watkins, 1964; Westmoreland & Watkins, 1974), HSV-2 (Para et al., 1982) and human cytomegalovirus (HCMV) (Frey & Einsfelder, 1984; Furukawa et al., 1975; Keller et al., 1976; Rahman et al., 1976; Westmoreland et al., 1976). The FcR induced by HSV has been identified as the 65K to 85K Mr viral glycoprotein E (gE) (Para et al., 1980). However, that induced by HCMV has not yet been well characterized biochemically, nor is it known whether it is a virus-encoded or cellular product. Recently we have demonstrated that the FcR induced by HCMV has subclass specificity with respect to IgG binding, and that the relative magnitude of binding is IgG1 > IgG4 > IgG2 > IgG3 (Murayama et al., 1986). The present study was undertaken to characterize the FcR induced by HCMV in relation to that induced by HSV.

METHODS

Cells and viruses. MRC-5 human embryo lung fibroblasts (Jacobs et al., 1970) and Vero cells were grown in Eagle's MEM containing 7.5% foetal calf serum (FCS), 0.3 mg l-glutamine/ml and 50 µg of gentamicin. The Towne strain of HCMV (Furukawa et al., 1973) was used throughout the experiments. HCMV was propagated and titrated by plaque assay (Wentworth & French, 1970) on MRC-5 cells. HSV-1 was propagated and titres were determined by plaque assay on Vero cells.

Preparation of cell extracts and purification of HCMV. Monolayers of MRC-5 cells were either mock-infected or infected with HCMV at a multiplicity of 1 p.f.u./cell. After adsorption for 1 h at 37 °C, the inoculum was removed, and the cells were incubated at 37 °C in MEM supplemented with 2% bovine serum albumin (BSA) until 60 to 70% of the cells exhibited c.p.e. Cells were washed with phosphate-buffered saline (PBS), scraped with a rubber policeman, and collected by centrifugation. Cell pellets were suspended in PBS containing 1% NP40, 1% sodium deoxycholate, and kept on ice for 30 min, with occasional mixing. After centrifugation at 10,000 g for 15 min, extracts were stored at −80 °C. HSV-1-infected Vero cell extracts were prepared as described for HCMV-infected cells. For purification of HCMV, the culture supernatant was harvested when complete c.p.e. was observed. Cell debris was removed by centrifugation at 3000 r.p.m. for 30 min, and virus was pelleted by centrifugation at 30,000
Fig. 1. SDS-PAGE of IgG-binding proteins separated on a rabbit IgG column. NP40-solubilized extracts from HCMV-infected or mock-infected cells were applied to an IgG-Sepharose 4B column. The bound material was eluted in 2 M-KSCN and analysed by 7.5% SDS-PAGE and staining with Coomassie Brilliant Blue. IgG-binding proteins of HCMV-infected cells (lane 1) and mock-infected cells (lane 2) after elution from the IgG column. Mr values are indicated to the side of each lane.

r.p.m. for 1 h, then purified by ultracentrifugation through a 10 to 50% (w/w) sucrose gradient (Furukawa et al., 1984) at 22000 r.p.m. for 90 min. Virion fractions were collected.

IgG and IgG fragments. Human IgG was prepared by chromatography of HCMV-seronegative donor serum (as confirmed by indirect immunofluorescence assay and ELISA) on DEAE-cellulose and Protein A–Sepharose CL-4B (Pharmacia). Fc and Fab fragments of human IgG were prepared by papain digestion as described by Murayama et al. (1986). Rabbit and mouse IgG were prepared by the method described above.

Iodination of protein. Human IgG, IgG Fc and IgG Fab were iodinated by the chloramine-T procedure (Byrt & Ada, 1969). IgG, IgG Fc, and IgG Fab were 125I-labelled to a sp. act. of about 10^6 c.p.m./µg.

Immobilized IgG. Rabbit IgG was coupled to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions. An average of 7 mg of rabbit IgG was coupled to 1 ml of packed gel. The gels were then washed extensively with PBS containing 0.5% NP40. Supernatants of HCMV-infected or mock-infected cell extracts were loaded onto the column. Unbound material was washed through the column with PBS containing 0.5% NP40. Bound material was eluted with 2 M-KSCN, precipitated with 5% TCA, washed with ethanol and acetone (Baucke & Spear, 1979) and solubilized for electrophoretic analysis.

Monoclonal antibodies. Murine monoclonal antibody (MAb) II48/18B raised against HSV gE was a gift from Dr P. G. Spear (University of Chicago, Chicago, Ill., U.S.A.).

Electrophoretic analysis. Proteins were analysed by SDS-PAGE (Laemmli, 1970) and gels were stained with Coomassie Brilliant Blue. Ferritin (220K), phosphorylase b (94K), bovine serum albumin (67K) and ovalbumin (43K) (Pharmacia) were used as Mr markers.

Western blotting analysis. Proteins separated by SDS-PAGE were electrophoretically transferred to nitrocellulose membranes (BA85, Schleicher & Schuell) using 20 mM-Tris-HCl, 150 mM-glycine, pH 8.3 in 20%
Characterization of HCMV Fc receptors

Fig. 2. Western blotting analysis of HCMV-infected cell extracts using $^{125}$I-labelled IgG, IgG Fc and IgG Fab. HCMV-infected and mock-infected cell extracts were immunoblotted on nitrocellulose membranes and probed with human IgG probe (lanes 1 and 2), IgG Fc (lanes 3 and 4) and IgG Fab (lanes 5 and 6). Lanes 1, 3 and 5, HCMV-infected cell extracts; lanes 2, 4 and 6, mock-infected cell extracts; lane 7, proteins isolated on the IgG affinity column, after immunoblotting and probing with $^{125}$I-labelled IgG Fc. Mr values are indicated.

methanol (Towbin et al., 1979). Nitrocellulose membranes were then treated overnight with Tris–NaCl buffer (TBS) (10 mM-Tris–HCl, 0.9% NaCl, pH 7.4) containing 2% BSA. The membranes were probed for 2 h in TBS containing $4 \times 10^6$ c.p.m./ml of $^{125}$I-labelled human IgG, IgG Fc or IgG Fab. After repeated washing with TBS containing 0.25% Tween 20 to remove unbound IgG, Fc and Fab, membranes were dried and autoradiographed. In some experiments, membranes were incubated at room temperature for 2 h with purified human IgG, mouse IgG or MAbs against different FcRs as described above. Membranes were then washed with TBS and treated for 1 h with a 1:400 dilution of horseradish peroxidase-conjugated sheep anti-mouse IgG, or goat anti-human IgG (Cappel Laboratories). After washing with TBS, membranes were developed with 0.5 mg/ml 3,3'-diaminobenzidine and 0.05% H$_2$O$_2$ in TBS.

**Blocking assay.** HCMV-infected cell extracts were separated by SDS-PAGE and proteins were transferred to nitrocellulose membranes. Membranes were treated with anti-HCMV human antibodies (1:2) (as confirmed by indirect immunofluorescence assay and Western blotting). After washing, the membranes were probed with $4 \times 10^6$ c.p.m./ml $^{125}$I-labelled human IgG Fc as described above.

**RESULTS**

**Isolation of IgG-binding proteins from HCMV-infected cell extracts**

Solubilized extracts from HCMV-infected cells at 96 h post-infection were applied to a column of rabbit IgG immobilized on Sepharose CL-4B. Bound material was eluted with 2 M-KSCN and analysed by SDS–PAGE and Coomassie Blue staining. Several polypeptides, including species of apparent Mr, 200K, 130K, 85K, 65K, 50K and 38K were detected in the HCMV-infected cell extract but not in the mock-infected extracts (Fig. 1).

**Specificity of IgG-binding proteins for IgG Fc**

In preliminary experiments, we found that the proteins eluted from the IgG column were also present in detergent extracts of HCMV-infected cells. Subsequent immunoblotting experiments were carried out using detergent extracts. Western blotting analysis of HCMV-infected or mock-infected cell extracts using $^{125}$I-labelled human IgG, IgG Fc and IgG Fab showed that the
130K, 65K, 50K and 38K polypeptides in the HCMV-infected cell extracts reacted with both the $^{125}$I-labelled human IgG Fc and $^{125}$I-labelled human IgG probes (Fig. 2, lanes 1 and 3). By contrast, the $^{125}$I-labelled IgG Fab probe failed to detect any polypeptide in the extracts (Fig. 2, lane 5). No reactivity with any of the probes was detected in the mock-infected cell extracts. To determine also whether IgG-binding proteins were acting through the Fc portion, proteins isolated on the IgG affinity column were analysed by Western blotting using $^{125}$I-labelled human IgG Fc as a probe. The polypeptides 130K, 65K, 50K and 38K were found to react with $^{125}$I-labelled human IgG Fc (Fig. 2, lane 7). These results indicate that the reactivity of these polypeptides with IgG is mediated through the Fc portion of IgG. They were also reactive with mouse IgG (data not shown). The proteins of $M_r$ 200K and 85K were not reactive with IgG Fc, despite their presence in the eluate from the IgG affinity column.

**Antigenicity of the HCMV-induced FcR**

Experiments were carried out to compare the antigenic relationship of FcR induced by HCMV with other kinds of FcR. MAb II48IB, which is directed against HSV gE, recognized four distinct polypeptides of $M_r$ 130K, 65K, 50K and 38K in HCMV-infected cell extracts (Fig. 3b), i.e. the same size species as bind human IgG (Fig. 3a). Western blotting under the same conditions using HSV-1-infected cell extracts as a control confirmed that MAb II48IB did react with 65K to 80K polypeptides corresponding to gE (Baucke & Spear, 1979) (data not shown). No
Characterization of HCMV Fc receptors

Fig. 4. Reactivity of $^{125}$I-labelled human IgG and IgG Fc with HCMV virion proteins. HCMV virions were purified by sucrose gradient centrifugation of extracellular virus. Virion proteins (15 μg) were solubilized and separated by 7-5% SDS-PAGE, followed by Coomassie Brilliant Blue staining (lane 1) or blotting onto nitrocellulose membranes and probing with $^{125}$I-labelled human IgG (lane 2) or IgG Fc (lane 3). $M_r$ values are indicated.

Fig. 5. FcR shares the antigenic determinants with HCMV-specific antigens. HCMV-infected cell polypeptides were transferred to nitrocellulose membranes after electrophoresis of cell extracts (20 μg) by 7.5% SDS-PAGE. The membranes were either pretreated for 1 h with a 1:2 dilution of anti-HCMV human serum which contained a titre of $> 640$ (estimated by immunofluorescence assay) and antibody against the 130K, 65K, 50K and other HCMV polypeptides (determined by Western blotting) (lane 1), or not treated (lane 2). Both pretreated and non-treated polypeptides on nitrocellulose membranes were probed with $^{125}$I-labelled IgG Fc. $M_r$ values are indicated.

antibody reactivity was detected in mock-infected cell extracts. These experiments suggest that the FcR induced by HCMV carries antigenic determinants that are shared with the FcR induced by HSV.

Detection of HCMV-induced FcRs in virions

To determine whether HCMV-induced FcRs are viral structural proteins, HCMV virions were purified by sucrose gradient centrifugation of extracellular virus. The virion proteins were solubilized, separated by SDS-PAGE, transferred to nitrocellulose membranes, and finally probed with $^{125}$I-labelled human IgG Fc fragment. Both the 130K and 65K polypeptides were detected, although the 130K band was faint. Thus, the HCMV-induced FcRs of $M_r$ 130K and 65K appear to be virion constituents (Fig. 4).
IgG Fc and antiviral antibodies bind to different sites on the FcR

Since the virus-specific polypeptides with the same Mr values of FcRs reported here have been detected in HCMV-infected cells, we examined whether anti-HCMV antibodies block the FcRs. When HCMV-infected cell proteins prepared on nitrocellulose membranes were pretreated with anti-HCMV antibodies, binding of 125I-labelled human IgG Fc to the 130K and 50K FcRs was markedly inhibited; binding to the 65K FcR was also inhibited, but to a lesser extent (Fig. 5).

DISCUSSION

In this report, we have analysed IgG FcRs induced by the Towne strain of HCMV by Western blotting. Proteins of Mr 130K, 65K, 50K and 38K were identified as IgG FcRs based on the isolation of these proteins from detergent extracts of HCMV-infected cells through their capacity to bind to an immobilized rabbit IgG column, and their ability to bind 125I-labelled human IgG through its Fc region, as indicated by the specific interaction of these four proteins with 125I-labelled human IgG Fc but not with 125I-labelled human IgG Fab. Although both human and mouse IgGs were reactive with other candidate FcRs, the 200K and 85K polypeptides, 125I-labelled IgG Fc failed to react with them. The inconsistent reactivity of these FcRs with 125I-labelled IgG Fc compared to unlabelled IgG might reflect a partial loss of reactivity of IgG Fc that might have occurred during the labelling process and/or a decrease in the antigenicity during extraction. Thus far, few studies have been carried out to determine whether structural differences exist between virus-induced FcRs, blood cell FcRs and bacterial FcRs. In this study, we analysed HCMV-induced FcRs with a MAb raised against the FcRs of HSV. The results suggest that the HCMV-induced FcR cross-reacts with other antibodies raised against different species of FcR. As in the report by Balachandran et al. (1987), who showed that HCMV encodes proteins that are cross-reactive with HSV gE, the MAb to HSV gE we used was reactive with the 130K, 65K, 50K and 38K FcRs, suggesting that the HCMV-induced FcR is antigenically related to those of HSV. MAb KuFc79 is reactive with the human IgG FcRs on monocytes, B lymphocytes and granulocytes, and it has been proposed, therefore, that these human IgG FcRs may exhibit structural similarities (Vaughn et al., 1985). We have shown that the 130K and 65K FcRs are constituents of the virion. The failure to detect the 50K and 38K FcRs in virions might be a consequence of the small size of these polypeptides, or it may be that they are soluble products of virus-infected cells. Grundy et al. (1987) have recently demonstrated that HCMV strain AD169 can bind β₂ microglobulin (β₂m) which has structural similarities to the Fc domain of IgG (Peterson et al., 1972; Smithies & Poulik, 1972). One of the β₂m-binding proteins (β₂m BP2), like one of the HCMV Towne strain-induced FcRs, has an Mr of 65K. As both FcR and β₂m BP2 are localized on the same polypeptide of the HCMV virion, and Fc and β₂m have structural similarities, it is possible that the FcR shares some binding sites with β₂m BP2. The relationship, if any, between the FcR and β₂m BP2 requires further genetic and biochemical analysis. The Mr of the HCMV-induced FcR polypeptide described here differs from those reported by Sakuma et al. (1977), who used anti-human IgG to immunoprecipitate a 42K HCMV-IgG FcR-IgG complex from trypsinized HCMV-infected cells without detergent treatment. However, our methodology differs substantially from that used by Sakuma et al.

The role of the HCMV-induced FcR is unclear. Previous studies have reported that FcR might protect HSV-infected cells from cell-mediated complement-dependent lysis in vitro by binding the immune complex to HSV-induced FcR (Adler et al., 1978). Dowler & Veltri (1984) showed that preincubation of non-immune IgG and Fc fragments with HSV virions significantly protected against neutralization by specific anti-HSV F(ab')2. They proposed that IgG provides a physical barrier that limits access of the anti-viral antibodies to the virion. McTaggart et al. (1978) also reported that anti-HSV F(ab')2 fragments competed with the binding of radioiodinated IgG to HSV-infected cells by 85%, and anti-HSV Fab' inhibited the binding of radioiodinated Fc. The binding inhibition assay used in the present study directly demonstrated that anti-HCMV antibodies can substantially inhibit binding of 125I-labelled Fc to the 130K and 50K FcR and, to a lesser extent, the 65K FcR. Thus antiviral antibodies might...
bind and sterically hinder binding of \(^{125}I\)-labelled Fc. In vitro, the binding of non-immune IgG to the HCMV virion or to infected cells through FcR might lead to protection of the virus from immune mechanisms. It remains to be seen whether persistent HCMV infection relies, in part, on such an FcR-determined means of escape from the host immune system.

We thank Drs P. Spear, M. Boyle and Y. Matsuo for providing the antibodies. We are also grateful to Dr T. Takegami for many helpful discussions. The technical assistance of W. Yoneda and Y. Funamoto is thankfully appreciated. This work was supported in part by the grants from the Kitasato Foundation and by a Grant-in-Aid from the Ministry of Education, Science and Culture.

REFERENCES


PARA, M. F., BAUCKE, R. B. & SPEAR, P. G. (1980). Immunoglobulin G (Fc)-binding receptors on virus of herpes simplex virus type 1 and transfer of these receptors to the cell surface by infection. *Journal of Virology* 34, 512–520.


(Received 12 August 1988)