Fc Receptor(s) Induced by Human Cytomegalovirus Bind Differentially with Human Immunoglobulin G Subclasses

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SUMMARY

The IgG subclass specificity of Fc receptor(s) induced on cells by infection with human cytomegalovirus (HCMV) was studied in a binding assay by using infected cells and purified iodinated IgG of various subclasses from HCMV seronegative healthy adult donors. All four human IgG subclasses bound to HCMV-infected cells, with the following relative magnitudes: IgG1 > IgG4 > IgG2 > IgG3. The IgG subclass specificity of the Fc receptor was further analysed in an inhibition assay by using fragments prepared from purified human IgG by papain digestion, and using unlabelled subclass proteins. Fc but not Fab fragments inhibited the binding of 125I-labelled human IgG to HCMV-infected cells. The biological role of the Fc receptor in HCMV infection is discussed.

Infection with human cytomegalovirus (HCMV) induces the formation of Fc receptors for IgG in the host cell cytoplasm and cell surface (Frey & Einsfelder, 1984; Furukawa et al., 1975; Keller et al., 1976; Rahman et al., 1976; Westmoreland et al., 1976). Infection with herpes simplex virus (HSV) also induces Fc receptors on the infected cell surface (Watkins, 1964; Westmoreland & Watkins, 1974; Yasuda & Milgram, 1968), and their chemical nature, physiological significance and biological function have been investigated (Adler et al., 1978; Baucke & Spear, 1979; Johansson et al., 1984; Para et al., 1982). The Fc receptor induced by HCMV is a 4.2 x 10^4 mol. wt. glycoprotein (Sakuma et al., 1977). However, the nature of HCMV-induced Fc receptors for human IgG subclasses has not been well characterized. In this communication, we have investigated the Fc receptor induced by HCMV with respect to its binding specificity for the four subclasses of human IgG.

MRC-5 human embryo lung fibroblasts obtained from the Wistar Institute, Philadelphia, Pa., U.S.A., were propagated in Eagle’s minimal essential medium (MEM) containing 7.5% foetal calf serum, 0.3 mg L-glutamine per ml, and 50 μg of gentamicin per ml. For use in immunoglobulin-binding studies, the cells were grown in 1 ml of the same medium in wells (Corning no. 25820, diam. 16 mm) to a density of 2 x 10^5 cells per well. The Towne strain of HCMV (Furukawa et al., 1973) was used throughout these experiments. IgG was prepared from sera of HCMV seronegative donors which demonstrated no HCMV antibody by indirect immunofluorescent staining, complement fixation and enzyme-linked immunosorbent assays. The IgG was precipitated by ammonium sulphate precipitation (50% saturation) and further purified by chromatography on Protein A-Sepharose CL-4B (Pharmacia) (Hjelm et al., 1972). The IgG Fc and Fab fragments were prepared by papain digestion of purified IgG by a modification of the method of Porter (1959). The IgG subclasses were purified by affinity chromatography on columns of Sepharose 4B covalently linked to purified anti-human myeloma IgG subclasses (IgG1, IgG2, IgG3 and IgG4) rabbit antibody according to the manufacturer’s instructions. These preparations were homogeneous as judged by SDS-PAGE under reducing conditions using a 7-5 and 10% separation gel (Laemmli, 1970), and by Ouchterlony analysis.
The IgG Fc and IgG subclasses were iodinated by the chloramine-T procedure (Byrt & Ada, 1969). IgG subclasses were $^{125}$I-labelled to a specific activity of about $10^6$ c.p.m./µg. For the protein binding assay, MRC-5 cells incubated in wells with 1 ml of MEM containing 2% bovine serum albumin (BSA-MEM) were either uninfected or infected with HCMV at a multiplicity of 1 p.f.u. per cell. The inoculum was removed after 1 h, and the cells were incubated in BSA-MEM. At 48 h after infection, the medium was removed, the cells were rinsed with phosphate-buffered saline (PBS) and then incubated at 37 °C with about 50 to 150 µg of unlabelled IgG subclasses in 0.2 ml of BSA–MEM or with Fc or medium only. In preliminary studies, these concentrations induced optimal responses. After 2 h, unbound IgG protein was removed by washing with excess PBS. Next, 0.2 ml of BSA–MEM containing about 100 ng of $^{125}$I-labelled IgG subclasses or Fc fragments were added to each culture. After 2 h incubation (predetermined in control experiments to give maximal binding of all four IgG subclasses) at 37 °C, the cells were washed three times with PBS to remove unbound $^{125}$I-labelled IgG, detached from the wells with 1% SDS, and assayed for radioactivity with a gamma counter (Aloka, Auto well Gamma System, ARC-300).

We examined the binding of iodinated human IgG subclasses to the HCMV-infected cells (1 p.f.u./cell) at various times after infection. Binding analysis of human IgG subclasses to HCMV-infected MRC-5 cells demonstrated the expression of specific cell surface receptors for each IgG subclass. Fig. 1 shows the binding levels measured for each subclass at intervals after infection with HCMV. Significant binding in each case was first observed at 12 h post-infection, somewhat earlier than the onset of virus DNA synthesis (Stinski, 1978), and reached a plateau at 24 to 48 h post-infection. The binding levels of IgG1 and IgG4 were about threefold higher than those of IgG2 and IgG3. The results were qualitatively similar at protein concentrations of 10 to 1000 ng per 0.2 ml for all four IgG subclasses. To exclude the possibility that IgG2 and IgG3 subclass proteins were selectively inactivated by the radioiodination procedure, unlabelled IgG subclass proteins were tested for their ability to inhibit the binding of radiolabelled human Fc. Each subclass protein used at a 1500-fold excess was able to inhibit the binding of iodinated IgG Fc, although the lowest inhibition was found with IgG3 proteins (data not shown).
We then investigated the interaction of the Fc receptor of HCMV-infected cells with iodinated protein from the four IgG subclasses, using the infected cells at 48 h post-infection. The results of a representative experiment are shown in Table 1. Binding of iodinated IgG1 and IgG4 proteins was completely inhibited by pretreatment with unlabelled Fc, whereas binding of the IgG2 and IgG3 proteins was inhibited by 37% and 54%, respectively, by the addition of Fc. Addition of Fab did not inhibit binding of any of the four IgG subclasses. The results indicate that the relative HCMV-specific binding ability of the IgG subclasses could be ranked as: IgG1 > IgG4 > IgG2 > IgG3. We also tested the binding of mouse iodinated IgG subclasses to HCMV-infected MRC-5 cells. The binding levels of IgG2a and IgG2b proteins were higher after HCMV infection compared with uninfected controls, but no such increase was seen with the IgG1 and IgG3 proteins after HCMV infection (data not shown).

In the present study, we have demonstrated a subclass specificity with respect to the IgG binding capacity of the Fc receptor induced by HCMV infection. The magnitude of the HCMV-induced binding of the IgG subclasses varied, having the order IgG1 > IgG4 > IgG2 > IgG3. This variation among IgG subclasses could be due to the existence of several receptors with different binding affinities for the subclasses. The subclass restriction of IgG binding of the HCMV-induced Fc receptor resembles that of the HSV-induced Fc receptor; IgG myeloma protein subclasses exhibited increased binding to HSV-infected cells with the following relative magnitude: IgG4 > IgG1 > IgG2, whereas IgG3 myeloma protein did not bind to HSV-infected cells (Johansson et al., 1984). In most studies of Fc receptors on normal human mononuclear blood cells, IgG1 and IgG3 seem to bind with the highest avidity (Unkeless et al., 1981) and the Fc receptor on a human leukaemic cell line also demonstrates this result (Anderson & Abraham, 1980). Interestingly, human complement factor Clq binds the subclasses in the following order: IgG3 > IgG1 > IgG2 > IgG4 (Schumaker et al., 1976).

The physiological role of the HCMV-induced Fc receptor on infected cells is unclear at present. Adler et al. (1978) proposed that the Fc receptor might exert a protective effect on the infected cells by interfering with viricidal or cytocidal effectors of the immune system. Antiviral immunoglobulins must be considered major factors in the interaction between a virus and the infected host. Although there is evidence that deficiencies in immunoglobulin synthesis may affect susceptibility to viral infections (Linnemann et al., 1973; Wright et al., 1977; Wyatt, 1973), the relative importance of various classes and subclasses of human immunoglobulins in recovery from viral infections is still not well understood. Recent findings indicate that IgG3 is the first subclass to appear after HCMV, HSV, varicella-zoster, rubella and polio virus infections, and IgG3 shows a higher ratio of IgG concentration to antibody titre than the IgG1, IgG2 and IgG4 subclasses as determined in neutralization and haemagglutination inhibition tests (Beck, 1981; Linde et al., 1983). These observations suggest the possibility that IgG3 proteins in particular assume a major role in immunological defence mechanisms. Whether this kinetic difference in IgG3 production has any connection with the specificity of the Fc receptor demonstrated here remains to be determined.
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REFERENCES


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