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Human Immunoglobulin Class and Subclass Specificity of Fc Receptors Induced by Herpes Simplex Virus Type 1

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Herpes simplex virus is known to induce an immunoglobulin-binding cell surface receptor in infected cells that utilizes a nonimmune mechanism. In the present paper, we report the immunoglobulin class and subclass specificity of this receptor. Of the human immunoglobulins G (IgG), IgA, IgM, and IgD, as well as the structurally related $beta_2$ microglobulin, only IgG and its Fc portion exhibited an increased binding to herpes simplex virus-infected cells versus uninfected control cells. The IgG subclass specificity of the Fc receptor was studied in 37 radioiodinated IgG myeloma proteins representing all four subclasses. We found that IgG3 myeloma proteins did not bind to herpes simplex virus-infected cells to a greater extent than to uninfected cells. On the contrary, proteins belonging to the other subclasses exhibited an increased binding to herpes simplex virus-infected cells of the following relative magnitude: IgG4 > IgG1 ≥ IgG2. This increment of binding could be abolished by addition of a large excess of human IgG Fc fragment. Evidence for the existence of a variable herpes simplex virus-specific binding ability between myeloma proteins belonging to the same IgG subclass was also obtained. Furthermore, we tested two other herpes simplex virus type 1 strains with a limited number of myeloma proteins with very similar results as with the herpes simplex virus type 1 F strain. Several sources of experimental artefacts were controlled, including the state of aggregation of the test proteins, the functional integrity of the Fc portion before and after radioiodination, and the subclass assignments. The implications for the biological role of the Fc receptor of herpes simplex virus are discussed.

Herpes simplex virus (HSV) is a ubiquitous human virus which is usually acquired early and then carried in latent form in sensory ganglia throughout the remaining life. The role of host factors like humoral and cellular immunity in the suppression of viral replication is largely unknown (2, 31). An interaction between the Fc portion of immunoglobulin G (IgG) molecules and protein(s) induced by the virus has been reported (3, 29, 36, 37). A viral glycoprotein of 65,000 to 80,000 molecular weight (gE) has been identified as the receptor (3). Similar IgG Fc receptors have been found for other human viruses belonging to the Herpesviridae (11, 15, 27). The specificity of HSV-induced Fc receptors has previously been investigated with regard to IgG from different species (37, 40) and human IgA and IgM (10). Nakamura et al. (26) could inhibit binding of rabbit antiperoxidase-peroxidase complexes with both human IgG1 lambda and human IgG2 lambda myeloma proteins. However, to our knowledge more extended studies regarding the specificity of HSV-induced Fc receptors towards human immunoglobulin classes and subclasses have not been published.

We have investigated the specificity of the Fc receptor induced by HSV type 1 (HSV-1) with respect to the four subclasses of human IgG. We demonstrate that myeloma proteins belonging to the IgG3 subclass do not bind to this receptor, whereas in the other subclasses the average extents of binding differ according to the sequence IgG4 > IgG1 ≥ IgG2. We also present evidence for differences in the binding ability of individual myeloma proteins within the IgG subclasses. In contrast to the IgG immunoglobulins, we could not find any significant binding to HSV-infected cells of human immunoglobulins belonging to the IgA, IgM, and IgD classes or of the molecularly related human $beta_2$ microglobulin.

MATERIALS AND METHODS

Cells. Green monkey kidney (GMK) cells of the strain AH1 (12) were cultured in the Dulbecco modification of Eagle minimal essential medium (Flow Laboratories, Irvine, United Kingdom) supplemented with 10% fetal calf serum (Flow), 0.3 mg of 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 glass roller tubes (1.5 by 10 cm) to a density of ca. 10^5 cells per tube. After outgrowth (ca. 4 days), the cells were washed once with tissue culture medium (RPMI 1640; Flow) lacking serum but supplemented with gentamicin and glutamine. The tubes were subsequently processed as described below.

Virus. The F strain of HSV-1 (9) was a generous gift from Bernard Roizman, University of Chicago, Chicago, Ill. To obtain seed stock, the virus was inoculated at low multiplicity onto GMK AH1 cells and harvested at the first day of full cytopathic effect, generally after day 3. The cells were loosened by shaking and homogenized by Dounce homogenization, and the suspension was clarified by centrifugation at 200 × *g* for 10 min. A 100-μl portion of the supernatant, containing 10^7 50% tissue culture infective doses, was used for inoculation of GMK AH1 cells in roller tubes. Two additional HSV-1 strains (HSV-1 2715/81, derived from a case of necrotizing HSV encephalitis, and HSV-1 554/82, derived from a case of recurrent herpes facialis) were prepared in the same manner.

Immunoglobulins and related proteins. The purified human

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IgG myeloma proteins used in the study are shown in Table 1. The subclass determinations, made by the three contributors of myeloma proteins, and the purity were controlled with a solid-phase radioimmunoassay, employing labeled monoclonal antibodies (see below). Except for one IgG3 myeloma protein (Table 1), the contamination by nonmyeloma IgG was estimated by the three suppliers to be <1%. Further information regarding the characterization of the myeloma proteins is given in Table 1. A total of 13 IgG1, 9 IgG2, 7 IgG3, and 8 IgG4 myeloma proteins were used in the study. The proteins were kept dissolved in phosphate-buffered saline (PBS) (NaCl, 8; KCl, 0.2; KH_2PO_4, 0.2; $Na_2HPO_4 \cdot 2H_2O$; 1.25; $CaCl_2$, 0.1; $MgCl_2 \cdot 6H_2O$, 0.1 g/liter). The protein concentrations were determined by spectrophotometric measurement at 280 and 310 nm with an immunoglobulin solution of known concentration for standardization and were thereafter stored at −20°C. After iodination, the proteins were stored at 4°C in PBS–10 mM sodium azide–0.2% ovalbumin (Sigma Chemical Co., St. Louis, Mo.).

Polyclonal preparations of human serum IgA (lot no. 18447), human secretory IgA (lot no. 18618), human IgG Fc fragment (lot no. 16199), human IgG $F(ab')_2$ fragment (lot no. 25928), and rabbit IgG Fc fragment (lot no. 16513) were obtained from Cappel Laboratories, Cochranville, Pa., whereas a human serum IgA (kappa) myeloma protein (no. AGIgA-3015), a human IgD (lambda) myeloma protein (no. AGIgD-4490), and pooled polyclonal human IgM (no. 801125) originated from one of the authors (A.G.). Iodinated and unlabeled $beta_2$ microglobulins in monomeric and aggregated form were kindly provided by Lars Björck, Department of Medical Microbiology, Lund, Sweden (4).

Solid-phase radioimmunoassay for IgG subclass verification of myeloma proteins. A radioimmunoassay with subclass-specific mouse monoclonal anti-human IgG antibodies was designed for verification of the subclass assignments by an independent method. Of the 37 myeloma proteins in the study, 32 were analyzed. The following commercial anti-IgG monoclonal antibody preparations in ascites form were used: anti-IgG1 (BAM 15, lot no. 3082), anti-IgG3 (BAM 08, lot no. 2901), anti-IgG4 (BAM 11, lot no. 2878), and anti-IgG, directed against all four subclasses (BAM 06, lot no. 2699), all from Seward Laboratories, London, United Kingdom. The anti-IgG2 monoclonal antibody preparation (BAM 10, lot no. 2877) was found to be unsuitable for the test. Mouse ascites fluid containing ca. 10 μg of monoclonal antibody was iodinated by the lactoperoxidase method described below. Myeloma proteins (0.2 μg in 100 μl of PBS, 10 mg of sodium Merthiolate per liter) were adsorbed overnight at 4°C in wells of a negatively charged, flexible, polyvinyl chloride 96-well microtitration plate (Titertek Immuno Assay plate, flat bottom, catalog no. 77-172-05; Flow). After washing three times with PBS–0.05% (wt/vol) Tween 20–10 mM sodium azide (PBS-Tween), 200 μl of a blocking solution (4% [wt/vol] bovine serum albumin [fraction V, Sigma]–0.1% gelatin [Difco Laboratories, Detroit, Mich.]–10 mg of sodium Merthiolate per liter) (5) was added. The plates were then incubated overnight at 4°C and subsequently were washed three times. Finally, 5 ng of radioiodinated monoclonal antibody dissolved in 100 μl of PBS–4% bovine serum albumin–0.1% gelatin–0.05% Tween 20–10 mg of sodium Merthiolate per liter was added to each well. After 4 h at 4°C, the wells were washed five times with 200 μl of PBS-Tween. The wells were cut out and counted individually in a gamma scintillation counter.

Preparation of monomeric and aggregated IgG. Monomeric

TABLE 1. IgG myeloma proteins used in the present study

IgG subclass	Gm allotype	Light chain	Identification no.	Supplying investigator
1		kappa	3584	AG[a]
		lambda	1232	AG
		kappa	3530	AG
	Gm (3)	kappa	Ols	EM[b]
			Ber	EM
	Gm (4)	kappa	112	VO[c]
	Gm (1)	lambda	122	VO
	Gm (1)	kappa	144	VO
	Gm (4)	kappa	152	VO
	Gm (4)	kappa	162	VO
	Gm (4)	lambda	170	VO
	Gm (1)	lambda	PB	VO
	Gm (4)	kappa	GJ	VO
2		lambda	3581	AG
		kappa	3524	AG
	Gm (23)	kappa	Sta	EM
		lambda	Sve	EM
		kappa	Hey	EM
			124	VO
			157	VO
			210	VO
			286	VO
3		lambda	3785	AG
		kappa	2873	AG
		lambda	3498	AG
	Gm (5)	kappa	Blo	EM
			248	VO
			143	VO
			5884	VO
			150	VO[d]
4		lambda	3244	AG
		kappa	3074	AG
		kappa	3798	AG
		lambda	Far	EM
			140	VO
			176	VO
			128	VO
			5764	VO

[a] Obtained from one of the authors (A.G.). The purification and characterization of these IgG myeloma proteins have been described previously (7). In brief, the myeloma protein was isolated by a combination of ammonium sulfate precipitation, ion-exchange and gel chromatography, and preparative gel electrophoresis. Purity was demonstrated by analytical agarose gel electrophoresis, immunoelectrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The subclasses were determined by physicochemical methods.

[b] Kindly provided by Erling Myhre, Department of Medical Microbiology, University of Lund, Lund, Sweden (25). These myeloma proteins were isolated by preparative agarose electrophoresis and purified by gel chromatography on Sephadex G-200 columns. Purity was assessed by immunoelectrophoresis.

[c] Obtained from one of the authors (V.O.). The myeloma proteins were purified by a combination of ammonium sulfate precipitation, DEAE ion-exchange chromatography, Sephadex G-200 gel filtration, and preparative electrophoresis in agarose (28). The subclass determinations and quantitations of the myeloma proteins from contributors E.M. and V.O. were performed by gel precipitation with rabbit antisera (28). Determination of Gm allotypes for the myeloma proteins from contributors E.M. and V.O. was kindly performed by Rune Grubb at the Department of Medical Microbiology, University of Lund, Lund, Sweden.

[d] This IgG3 protein was excluded because of a strong IgG1 reactivity, despite further purification efforts.

and aggregated IgG molecules were separated on a Sephacryl S-300 column (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with PBS–0.2% (wt/vol) ovalbumin (Sigma)–10 mM sodium azide. Dextran blue (Pharmacia) and phenol red were included as standards. The column was calibrated by radioiodinated human IgG with a guaranteed minimum content of monomeric IgG of 85% (wt/wt) (Gammaglobulin Kabi 16.5%; Kabi, Stockholm, Sweden), and radioiodinated bovine serum albumin (six times crystallized) (United States Biochemical Co., Cleveland, Ohio).

Protein iodination. The lactoperoxidase method (22, 34) was used. Briefly, 0.5 mCi (19 MBq) of carrier-free $Na^{125}I$ (The Radiochemical Centre, Amersham, England) was added to a mixture of 5 μg of lactoperoxidase (Sigma) and 10 μg of myeloma protein or Fc fragment in 10 to 50 μl of PBS. The reaction was started by addition of 2 μl of 30% hydrogen peroxide diluted 1/20,000 in redistilled water and was stopped one min later by addition of 500 μl of PBS containing 10 mM sodium azide. Unbound iodide was removed by gel chromatography on a PD 10 column (Pharmacia) and eluted with PBS–0.2% (wt/vol) ovalbumin–10 mM sodium azide. A specific activity of 10 to 20 μCi/μg of protein was obtained. Radioiodinated $beta_2$ microglobulin with a specific activity of 0.05 μCi/μg was kindly provided by Lars Björck.

Protein binding and inhibition assay. GMK AH1 cells in roller tubes containing 1 ml of serum-free medium were either kept uninfected or infected with one of the HSV-1 strains at a multiplicity of 100 50% tissue culture infective doses per cell. After 20 h at 37°C with rotation in a roller drum, when a full cytopathic effect was observed, the medium of triplicate infected and uninfected tubes was removed and 100 μl of PBS–0.2% ovalbumin–10 mM sodium azide with or without 13 μg of competing protein was added. After incubation for 1 h at 37°C in a roller drum, 100 μl of PBS–0.2% ovalbumin–10 mM sodium azide containing 5 to 10 ng of radiolabeled immunoglobulin or IgG Fc fragment or 100 ng of $beta_2$ microglobulin was added to each tube. This mixture was incubated for another hour at 37°C in the roller drum. Subsequently, the cells were washed three times with 4 ml of PBS, followed by centrifugation at 200 × *g* for 10 min. The cell-bound radioactivity was measured in a gamma counter (LKB-Wallac; Wallac Oy, Turku, Finland). The HSV-specific binding was calculated by subtraction of the radioactivity bound to uninfected cells from that of HSV-infected cells. The Fc specificity of the binding was always demonstrated by inhibition with unlabeled human IgG Fc fragment in separate tubes. As a control of the cell number, two additional tubes were in some experiments processed identically and after trypsination enumerated with a hemacytometer. It was calculated that with unlabeled immunoglobulins or Fc fragment as competing proteins a 100- to 1,700-fold molar excess versus iodinated Fc was obtained. For unlabeled Fc versus iodinated immunoglobulins, the molar excess was 5,000- to 30,000-fold. Unlabeled $beta_2$ microglobulin versus iodinated Fc reached a molar excess of 7,000-fold, and for unlabeled Fc versus iodinated $beta_2$ microglobulin, the molar excess was 30-fold.

Bacterial Fc receptor binding. As a further parameter describing the capacity of the IgG myeloma proteins to bind to Fc receptors, we used bacterial strains with known Fc receptor specificities, i.e., *Staphylococcus aureus* Cowan 1, human group G streptococcus strain G148, and streptococcus group B strain B1 (kindly provided by E. Myhre). Cultivation procedures, preparation of bacterial suspensions, and the radioligand assay were as previously described (25). Briefly, 10 ng of labeled immunoglobulin in 200 μl of PBS-Tween was added to duplicate tubes containing 2 × 10^8 bacteria per tube. After 1 h at room temperature, 2 ml of PBS–0.02% sodium azide–0.05% Tween 20 was added to each tube, the bacteria were spun down, and the supernatant was removed. The amount of protein bound to bacteria was calculated from the radioactivity in the pellet.

RESULTS

Verification of IgG subclass by solid-phase radioimmunoassay. The subclass assignments were controlled by solid-phase radioimmunoassay (data not shown). In the test, specific binding exceeded nonspecific binding by a factor of 5 to 20. The use of a mouse monoclonal antibody directed against all human IgG subclasses provided a control for the amount of IgG present on the surface in each well of the microtitration plate, thus safeguarding against false-negative results. For IgG2 myeloma proteins, the subclass could only be indirectly determined. Of 32 myeloma proteins tested, 1 was found to be accidentally mislabeled, and 1 (no. IgG3-150) was found to be contaminated. This IgG3 preparation which bound to HSV-infected cells also contained a strong IgG1 reactivity, which could not be removed by further purification. The contaminated protein was excluded from the study, and the mislabeling was corrected.

Binding of iodinated IgG Fc fragment and human IgG1 through IgG4 myeloma proteins. The binding of iodinated, pooled human IgG Fc fragment and its inhibition by unlabeled human and rabbit Fc fragment but not by human IgG $F(ab')_2$ fragment was shown first (Fig. 1). With both unlabeled human and rabbit IgG Fc, a dose-dependent inhibition was noted to a concentration of 50 μg/200 μl. We then investigated the interaction of the Fc receptor of HSV-infected cells with iodinated myeloma proteins from the four IgG subclasses. The results of a particular experiment can be seen in Fig. 2, where the increased binding of human IgG Fc fragment to HSV-infected cells is demonstrated. When six iodinated myeloma proteins belonging to all four subclasses

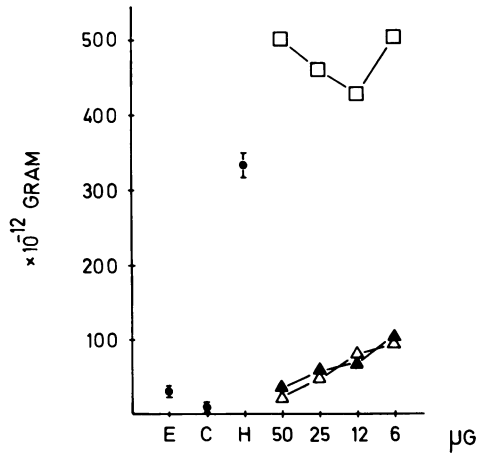

FIG. 1. Binding to HSV-infected GMK AH1 cells of iodinated human IgG Fc fragment. Inhibition of this binding by unlabeled human IgG Fc fragment and rabbit IgG Fc fragment but not by human $F(ab')_2$ fragment. Symbols: E, empty glass tube, no cells; C, control cells, not HSV infected; H, HSV-infected cells. Binding in the presence of 6 to 50 μg of human IgG $F(ab')_2$ fragment (□), human IgG Fc fragment (▲), or rabbit IgG Fc fragment (△). Ordinate, amount of iodinated IgG Fc fragment bound.

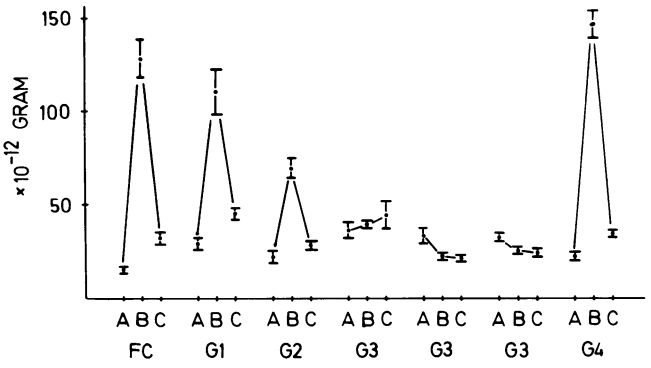

FIG. 2. Binding to HSV-infected cells of iodinated human IgG Fc fragment and six iodinated myeloma proteins from the four subclasses and their inhibition by unlabeled IgG Fc fragment. The figure illustrates the outcome of a typical experiment. Symbols: A, uninfected cells; B, HSV-infected cells; C, inhibition of binding to HSV-infected cells by unlabeled Fc fragment of human IgG.

were analyzed, the IgG1, IgG2, and IgG4 myeloma proteins exhibited an increased binding after HSV infection, which could be inhibited by an excess of unlabeled Fc fragment. On the contrary, the IgG3 myeloma proteins did not bind more to HSV-infected cells than to uninfected cells. The addition of Fc fragment did not affect the limited amount of this binding.

An extended series of binding assays with the same experimental system was then performed (Fig. 3). To compensate for variations between different experiments, the HSV-specific binding of the individual myeloma proteins relative to the HSV-specific binding of iodinated Fc fragment used as a standard (set to 100%) was calculated. Despite equal protein concentrations, six of the seven IgG3 myeloma proteins did not exhibit an increased binding to HSV-infected cells. One of them bound slightly more to HSV-infected cells than to uninfected controls. In contrast, myeloma proteins belonging to the other subclasses all featured a significantly enhanced binding after HSV infection. This corresponded to 0.2 to 4% of the added radiolabeled protein. The group mean of the HSV-specific binding of the 8 IgG4 proteins (171% relative to the Fc control) was significantly higher than that of the 13 IgG1 proteins (72% relative to Fc) ($P = 0.0015$; Wilcoxon rank-sum test). The IgG1 proteins bound somewhat better than the nine IgG2 myeloma proteins (47% relative to Fc) ($P = 0.109$). The comparison between IgG2 and IgG4 myeloma proteins yielded a clear difference ($P = 0.0005$). The HSV-specific binding of the IgG3 myeloma proteins was too close to nil to allow a meaningful statistical evaluation. We conclude that the IgG subclasses differ in their HSV-specific binding ability according to the sequence IgG4 > IgG1 ≥ IgG2 > IgG3.

Intrasubclass variations in binding of myeloma proteins. The precision of the binding values obtained in a single experiment (average of triplicate tubes) generally was 5 to 30% (Fig. 2). The reproducibility of the HSV-specific binding for individual myeloma proteins relative to that of IgG Fc fragment was estimated as the average of the percent deviations from the mean for all repeat experiments (average, 2.6 experiments per myeloma protein) for all myeloma proteins. This variance was 19%, which is compatible with the precision of the values from a single experiment and small compared with the differences in binding within the subclasses. Therefore, we made a statistical comparison of one pair of myeloma proteins within the IgG1 subclass which had been tested four times each and found to bind relatively strongly (myeloma 144) and weakly (myeloma PB), respectively. Protein 144 had an average HSV-specific binding of 61.5 (± 12.8 standard deviation) relative to that of IgG Fc fragment. The same average for protein PR was 24.0 (±5.3 standard deviation). These values differed significantly according to Student's t test ($P = 0.01$) and the Wilcoxon rank-sum test ($P \leq 0.05$). Two other protein pairs within the IgG2 and IgG4 subclasses, respectively, yielded similar differences. Each protein in these two pairs was only iodinated once. However, three other myeloma proteins were analyzed after reiodination and gave essentially the same binding as after the first iodination. Consequently, this source of variation seemed to be minor. We regard this as evidence for the existence of differences in binding within the subclasses.

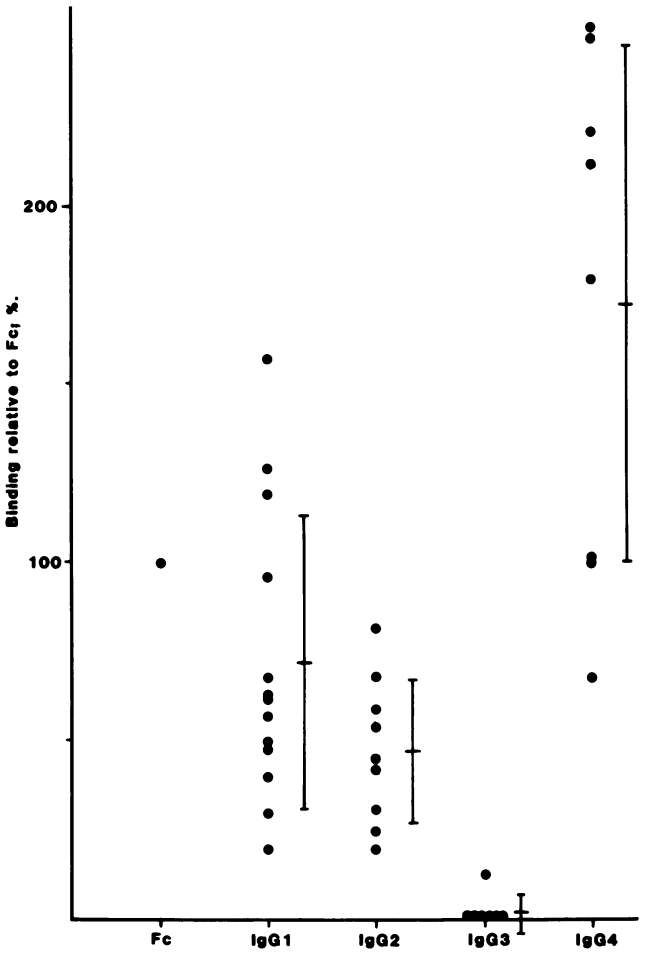

FIG. 3. Binding of iodinated polyclonal IgG Fc fragment and of 37 purified iodinated IgG myeloma proteins to GMK AH1 cells infected with HSV-1. The increment in binding of Fc fragment after HSV infection (corresponding to 110 ± 32 standard deviation, pg/10^5 cells) was set to 100%. The HSV-specific binding of individual myeloma proteins was calculated in relation to that of the Fc fragment and plotted versus IgG subclass. Each point represents the average of several (average, 2.6) experiments. The group means of the binding for each subclass and their standard deviations are depicted to the right of the experimental points.

Information regarding Gm allotype was available for nine of the IgG1 proteins (Table 1). No consistent pattern of covariation between HSV-specific binding and Gm allotype was apparent (data not shown).

Fc binding induced by different HSV-1 strains. Of the iodinated myeloma proteins, 17 (9 IgG1, 4 IgG2, 1 IgG3, and 3 IgG4) were tested against cells infected with each of two additional HSV-1 strains. No differences in binding properties were seen between any of the three strains (data not shown).

Inhibition of binding of iodinated Fc fragment or iodinated myeloma protein by unlabeled myeloma protein. To exclude the possibility of a selective inactivation of the IgG3 myeloma proteins by the radioiodination procedure, unlabeled myeloma proteins belonging to different IgG subclasses were tested for their ability to inhibit the binding of radiolabeled human Fc fragment (Fig. 4) at different concentrations. When 11 purified myeloma proteins were tested, none of the 3 tested IgG3 myelomas were able to inhibit iodinated IgG Fc fragment binding, whereas a dose-dependent inhibition was found with the other subclasses. Thus, even before iodination, the same pattern of affinity as described above for the iodinated myeloma proteins was observed.

The specificity of the binding of the radioiodinated myeloma proteins was further ascertained by demonstrating the inhibition given by a 1,000-fold excess of unlabeled homologous myeloma protein (data not shown). The binding of IgG1, IgG2, and IgG4 was inhibited by an excess of unlabeled homologous protein, whereas the low binding of the three IgG3 myelomas was unaffected. We take this as further evidence of the nonsaturable and therefore unspecific nature of the weak binding of IgG3 proteins to uninfected and infected cells.

Control of some parameters of the immunoglobulin binding system. The choice of an incubation time of 1 h was based on a control experiment where incubation times from 0.5 to 4 h were tested. The binding was close to equilibrium within 1 h (data not shown).

The possible interference in the test by heat-labile components in the fetal serum used for cultivation of the GMK AH1 cells was controlled by an experiment with five radiolabeled myeloma proteins (1 IgG1, 2 IgG2, and 2 IgG3) and uninfected or HSV-1-infected cells grown with either non-inactivated serum or heat-inactivated (56°C for 30 min) serum. No difference in binding was discernible. Nor were the results altered by inclusion or exclusion of 10 mM sodium azide during the incubation of cells with radiolabeled protein (data not shown).

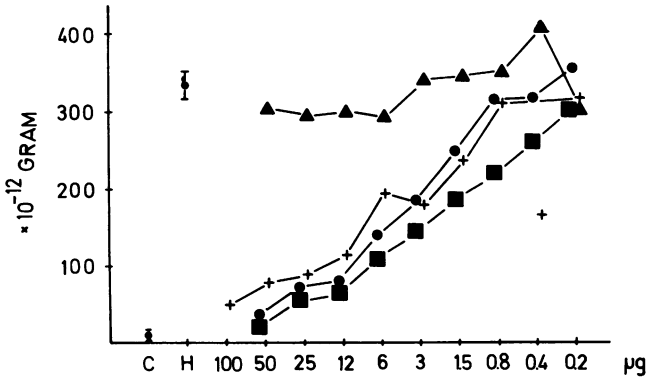

FIG. 4. Inhibition of binding of iodinated human IgG Fc fragment to HSV-infected cells by 100 to 0.2 μg of unlabeled human myeloma proteins. Symbols: C, uninfected GMK AH1 cells and H, HSV-infected cells. Myeloma protein identification numbers are as follows: IgG1, 3584 (■); IgG2, 3524 (+); IgG3, 3785 (▲); and IgG4, 3244 (●). Ordinate, amount of iodinated IgG Fc fragment bound.

Influence of state of aggregation. Immunoglobulins, especially purified preparations, have a propensity to aggregate. This tendency is especially strong for the IgG3 myeloma proteins (6). To exclude that the differences in HSV-specific binding were due to variations in aggregation between the myeloma proteins, two myeloma proteins within each subclass exhibiting especially strong or weak binding ability were fractionated by gel chromatography. Two major peaks, representing monomeric and aggregated IgG, were observed. The proportion of aggregated protein relative to monomeric protein varied between 0.5 and 1.2 (data not shown). No correlation between the amount of protein in these peaks and the binding ability of the myeloma protein was observed, either within or between subclasses. The binding ability of the monomeric and polymeric IgG fractions is depicted in Fig. 5. Both polymeric and monomeric fractions of the IgG4 myeloma protein were able to bind to the HSV-infected cells, but the polymeric fraction exhibited a smaller increment of binding than did the monomeric fraction. This was due to a more pronounced binding of the polymeric fraction to the uninfected control cells. Essentially the same results were obtained for two additional myeloma proteins (one IgG4 and one IgG2). On the contrary, neither the monomeric nor polymeric fractions of the IgG3 myeloma protein exhibited specific binding to HSV-infected cells. Thus, the state of aggregation was not a likely explanation of the variations in binding between and within subclasses. Nor could this variation be explained by differences in binding to uninfected control cells.

Monomeric and polymeric fractions from a previous gel chromatography were rechromatographed on the same column after 2 and 3 days of storage at 4°C (data not shown). Two percent of the polymeric fraction appeared in the monomeric fraction, and 1% of the monomeric fraction appeared in the polymeric fraction. These results make it less probable that re- or disaggregation would have influenced our binding experiments.

Binding to bacterial Fc receptors. These experiments were performed as further controls of the functional integrity of the Fc portion of the iodinated myeloma proteins. Bacteria known to bind the IgG molecule via an Fc receptor were used. The G148 streptococcus binds all four IgG subclasses, the Cowan 1 staphylococcus binds all subclasses except IgG3, and the B1 streptococcus lacks Fc receptors and served as a negative control. The eight myeloma proteins studied demonstrated the expected binding pattern to these bacterial strains. Thus, the iodinated IgG3 myeloma proteins retained the binding capacity for the G148 strain, indicating that at least the part of the IgG3 molecule that binds to these bacteria was functionally intact (Table 2). Furthermore, the amount of protein bound to uninfected and HSV-infected cells was not related to the amount bound to bacteria, even for strongly and weakly binding myeloma proteins belonging to the same subclass.

Binding of IgA, IgM, IgD, and beta$_2$ microglobulin. We also tested iodinated polyclonal secretory IgA, polyclonal serum IgA, and monoclonal IgA, including a monomeric fraction of this myeloma protein. No specific binding to HSV-infected cells nor any inhibition by an excess of the IgG Fc fragment or an excess of the different IgA preparations was discernible. Polyclonal IgM and monoclonal IgD

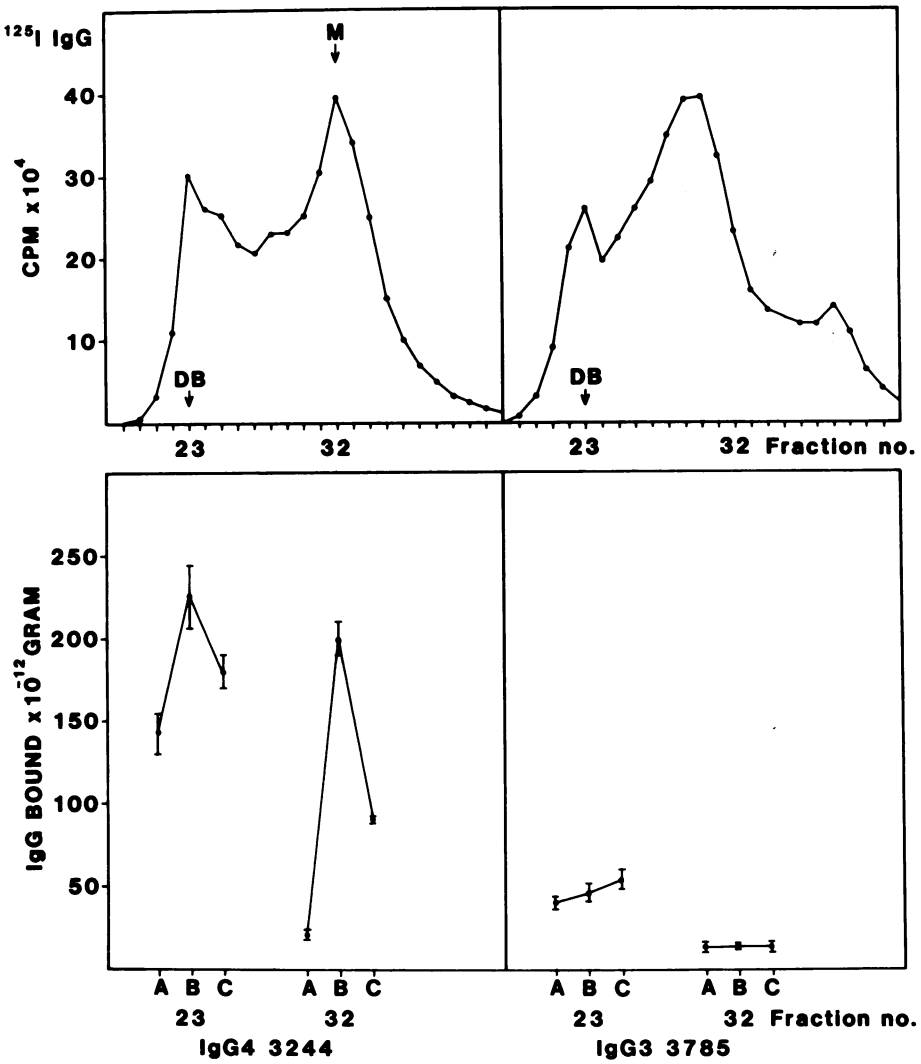

FIG. 5. Analysis of degree of aggregation of two iodinated IgG myeloma proteins by gel exclusion chromatography, and its influence on the binding to HSV-infected cells. A 4-μg portion of radioiodinated myeloma protein was applied to a 90-cm column. Fractions (total no. collected, 60) corresponding to monomeric IgG (fraction 32, labeled M) and high molecular weight aggregates (void volume, fraction 23, dextran blue [DB]) were used in the standard binding assay with uninfected cells (A), HSV-infected cells (B), and HSV-infected cells in the presence of a large excess of unlabeled Fc fragment (C). Results obtained with proteins no. 3244 (IgG4) and 3785 (IgG3) are shown. The binding assay was performed within 24 h after the separation of the fractions.

were tested with the same negative result. Iodinated monomeric or aggregated $beta_2$ microglobulins did not bind to a greater extent to HSV-infected than to uninfected cells. Neither was any inhibition of the binding of this labeled molecule with a 30-fold molar excess of IgG Fc fragment or with a 100-fold excess of monomeric or aggregated $beta_2$ microglobulins observed. In addition, neither monomeric nor aggregated $beta_2$ microglobulins in 7,000-fold molar excess were able to inhibit the binding of iodinated IgG Fc fragment.

DISCUSSION

Human IgG subclass specificity of the Fc receptor induced by HSV-1. In this paper, we report a subclass specificity in the IgG binding capacity of the Fc receptor of HSV-1. Myeloma proteins belonging to the IgG3 subclass did not bind. The magnitudes of the HSV-specific binding of the remaining subclasses varied in the decreasing order IgG4 > IgG1 ≥ IgG2. This intersubclass variation could be due to either differences in affinity for a single Fc receptor or, less plausibly, the existence of several receptors with differences in affinity for the subclasses. The subclass restriction of IgG binding of the HSV-induced Fc receptor resembles that of protein A (17; however cf. reference 14). On the other hand, there are several examples of IgG Fc binding proteins with other subclass preferences. In most studies of Fc receptors on normal human mononuclear blood cells, IgG1 and IgG3 seem to bind most avidly (35). The restriction in IgG3 binding has been found in some (17) but not other (16) bacterial Fc receptor systems. Interestingly, human complement factor C1q binds in the following order: IgG3 > IgG1 > IgG2 > IgG4 (32), which approximately is the inverse of the HSV Fc receptor.

Intrasubclass variations in the binding of IgG myeloma proteins to the HSV Fc receptor. In some cases, the precision

TABLE 2. Binding of selected radioiodinated myeloma IgG proteins to HSV-infected cells and to bacteria[a]

Myeloma IgG subclass and protein id. no.	Binding (pg) to HSV-1-infected GMK AH1 cells[b]			Binding (pg) to bacterial strains[c]		
	A	B	B-A	Cowan 1	G148	B1
1 122	46	150	104	2,740	3,750	500
1 GJ	26	78	52	2,820	4,980	320
2 210	20	69	49	3,160	3,740	420
2 124	18	39	21	2,160	4,150	330
3 248	28	20	<0	410	3,730	400
3 3785	34	22	<0	120	3,150	80
4 176	24	292	268	3,240	3,150	430
4 3244	22	146	124	4,140	5,400	750

[a] Each figure was obtained in one experiment. Representatives exhibiting strong and weak HSV-specific binding within each subclass (except for IgG3) were selected.

[b] The figures represent the average of triplicate determinations. Binding is expressed in picograms per 10^5 cells. The standard deviations varied from 5 to 30% of the average. Symbols: A, radioiodinated myeloma protein binding to uninfected GMK AH1 cells; B, radioiodinated myeloma protein binding to HSV-1-infected GMK AH1 cells; B-A, increment of binding after HSV infection.

[c] The figures represent the average of duplicates. Binding is expressed as picograms of myeloma protein bound per tube. The values have been rounded to the nearest tenth digit. The deviations from the averages varied from 0 to 10%.

of the assay system and the number of repeat experiments permitted a statistical comparison between myeloma proteins belonging to the same subclass. A significant difference in binding was demonstrated for several pairs. This may be taken as evidence for the involvement of specificities other than subclass specificities in the binding site. However, neither the type of light chain nor the Gm allotypic specificity seemed (in a limited number of proteins) to be related to the intrasubclass variation that we observed.

The observation that the extent of binding to bacterial Fc receptors was unrelated to that of HSV-infected cells (Table 2) also suggests that the intrasubclass variation is due to a specificity intrinsic to the HSV Fc receptor. However, the limited amount of data per myeloma protein makes it difficult to completely exclude that this variation was due to imperfections in the test system that we are not aware of. A more definite demonstration of a variable binding ability between myeloma proteins within the IgG subclasses requires further experimentation.

Specificity of Fc receptors induced by different HSV-1 strains. A pronounced intratypic variability of HSV strains with respect to the restriction endonuclease cleavage pattern has been reported (13, 20). However, within the accuracy of our experimentation, we could not find any differences in immunoglobulin-binding spectrum between our three HSV-1 strains.

Influence from contaminating proteins. The low relative amount of myeloma protein that bound (0.2 to 4%) to HSV-infected cells even in the case of the most strongly binding myeloma proteins may cause concern. This could simply be due to a relatively low affinity or a low number of receptor sites. Alternatively, one must consider the possibility that a small amount of a contaminant with very strong binding ability to HSV-infected cells was present in some myeloma protein preparations. However, besides the exhaustive purification sequence that many of the myeloma proteins had undergone, we judge this as improbable for the following reasons. (i) Of all myeloma proteins, only the IgG3 representatives should not contain the contaminant in question, which is unlikely. (ii) A total or nearly total inhibition of the binding of the myeloma proteins was observed with Fc fragment pure by immunoelectrophoretic criteria (data from Cappel). Thus, the contaminant should copurify not only with myeloma proteins but also with the Fc fragment. (iii) Gel chromatographic fractions from several myeloma proteins eluting at the position of monomeric IgG gave the same percentage of binding as the unfractionated myeloma proteins. (iv) If the putative contaminant was other IgG molecules, the most strongly binding IgG subclass, IgG4, is also present in serum in the lowest concentration (3% of total IgG). Even a relatively strong contamination with polyclonal IgG should not contain enough IgG4 to influence our results. The most likely contaminant would be polyclonal IgG1. However, the average binding of IgG1 myeloma proteins was only 1.5 times higher than that of IgG2, and 2.4 times lower than that of IgG4 (Fig. 3). Thus, a very substantial admixture of IgG1 in the IgG2 myeloma proteins would have been required to give a false impression that IgG2 proteins bind whereas in reality they had no binding capacity at all. Such a substantial contamination was excluded by purity controls made by the supplying investigators and by us by means of the solid-phase radioimmunoassay.

Functionality of myeloma proteins. Controls of the adequacy of the test system included binding experiments with monomeric and polymeric fractions and bacterial radioligand assays. It could be argued that the differences in immunoglobulin binding to Fc receptors between and within subclasses were due to a variation in the aggregation of the myeloma proteins. Immunoglobulin aggregates would be expected to bind more strongly than monomeric proteins due to their multivalent nature, but we found that the HSV-induced increment of binding was smaller for aggregated than for monomeric IgG. The functional integrity of the Fc portion of the myeloma proteins was ascertained when both a pan-IgG streptococcal Fc receptor and protein A on Cowan 1 staphylococci exhibited the expected binding behavior.

Binding of IgG-like molecules. IgG binding could represent only a special case of a broad reactivity towards molecules with Fc-like structure. However, we found no evidence for specific interactions between the Fc receptor of HSV-1-infected cells and human IgA, IgM, IgD, and beta$_2$ microglobulin. This confirms the results of Feorino et al. (10) regarding human IgA and human IgM.

Implications for the biological role of the Fc receptor of HSV. The IgG binding glycoprotein gE, which belongs to the early group of viral proteins, has been identified in both HSV-1- and HSV-2-infected cells. In both cases, the gene coding for this protein has been localized in the unique part of the S portion of the HSV genome (18, 30). Consequently, the nonimmune IgG binding ability induced during HSV infection is a virally coded property, which should have a considerable survival value as it is present in many representatives of the Herpesviridae (11, 15, 27). Hypotheses concerning the functional role of Fc receptor-mediated IgG binding to virions and infected cells have aimed at either protection from immunological defense mechanisms or regulation of latency.

Antiviral immunoglobulins must be considered as major factors in the interaction between a virus and the infected animal. Viruses belonging to the Herpesviridae must experience a strong selection pressure for mechanisms of escape from antibody-mediated lysis of virus or virus-infected cells

due to the seemingly lifelong interplay between host and virus. The Fc receptor could take part in such mechanisms by interfering with viricidal or cytocidal effectors of the immune system (cf. reference 1). The subclass preferences of complement-mediated lysis (32, 33) (see above) are of interest in this context. Although there is evidence that deficiencies in immunoglobulin synthesis may affect the susceptibility to virus infections (21, 23, 24, 38, 39), the relative importance of the various classes and subclasses of human immunoglobulins in recovery from viral infections still is not well known. However, recent findings (19; V.-A. Sundqvist, A. Linde, and B. Wahren, *in* V. A. Sundqvist, M.S. thesis, University of Stockholm, Stockholm, Sweden, 1983) indicate that IgG3 is the first subclass to appear after HSV, varicella-zoster virus, and cytomegalovirus infections. Whether this kinetic difference has any connection with the specificity of the Fc receptor demonstrated here remains to be determined.

On the other hand, polyclonal immunoglobulins lacking antiviral specificity have been suggested as modulators of HSV expression. The results obtained by Costa et al. (8) do suggest that the Fc receptor of HSV can suppress virus production. Thus, release of HSV was inhibited in the presence of the polyclonal IgG Fc fragment. Although such a mechanism may have advantages to the virus that are hard to appreciate at this time, it seems inappropriate that the concentration of certain IgG subclasses without regard to viral antibody specificity would control the production of virus. Further work is clearly needed. However, knowledge concerning the subclass specificity of the IgG Fc receptor on HSV-infected cells should facilitate the understanding of the role of the Fc receptor in the relationship between HSV and host.

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