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Interaction between herpes simplex type 1-induced Fc receptor and human and rabbit immunoglobulin G (IgG) domains

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SUMMARY

Cells infected with herpes simplex virus type 1 (HSV-1) express a cell surface receptor able to bind to the Fc region of immunoglobulin G (IgG). The ability of HSV-1-infected cells to bind ^{125}I-labelled human and rabbit IgG and IgG fragments was studied to localize the site of interaction to the $C_\gamma2$ or $C_\gamma3$ domains of IgG. ^{125}I-labelled IgG and IgG Fc fragments consisting of $C_\gamma2$ and $C_\gamma3$ domains bound strongly to HSV-infected cells and did not bind to uninfected cells. In contrast, ^{125}I-labelled $F(ab')_2$, Facb [consisting of $F(ab')_2$ and $C_\gamma2$ domains] and pFc' (consisting of $C_\gamma3$ domains) fragments did not bind to any of these cells. Unlabelled IgG and IgG Fc fragments inhibited the interaction between ^{125}I-labelled rabbit IgG Fc and the HSV Fc receptor, whereas $F(ab')_2$, Facb and pFc' fragments failed to inhibit this interaction. These data indicate that the HSV Fc receptor requires both the $C_\gamma2$ and $C_\gamma3$ domains for interaction with the IgG molecule analogous to the known interaction of protein A of *Staphylococcus aureus*, the Fc binding proteins of Group A, C and G streptococci, and certain human rheumatoid factors.

INTRODUCTION

Herpes simplex virus (HSV)-infected cells express a receptor able to bind to the Fc portion of immunoglobulin G (IgG) in a non-immune way (Watkins, 1964). The IgG Fc receptor characterized as a glycoprotein (gE) (Baucke & Spear, 1979) is expressed on the surface of herpes-infected cells and on the surface of herpes virions (Para, Baucke & Spear, 1980). Among the five immunoglobulin classes, only IgG expresses the capacity to bind to the receptor on HSV type 1 (HSV-1)-infected cells (Feorino, Shore & Reimer, 1977; Johansson *et al.*, 1984; Wiger & Michaelsen, 1985). Human subclass IgG3 does not bind to HSV-1-infected cells (Johansson *et al.*, 1984; Wiger & Michaelsen 1985), whereas the other subclasses bind with decreasing affinity in the order IgG4 > IgG1 ≥ IgG2 (Johansson *et al.*, 1984). The receptor also displays a specific and rather restricted binding capacity towards different animal IgG and animal IgG subclasses (Johansson, Myhre & Blomberg, 1985).

Protein A from *Staphylococcus aureus* can also combine with the Fc portion of human IgG (Forsgren & Sjöquist, 1966). In similarity with the HSV-1 Fc receptor, protein A reacts with human IgG1, IgG2 and IgG4 subclasses, whereas IgG3 is non-reactive (Kronvall & Williams, 1969). It is known that protein A reacts with IgG in the interface between the $C_\gamma2$ and $C_\gamma3$ domains (Lancet *et al.*, 1978), and the exact location and amino acid side-chains involved have been determined (Deisenhofer, 1981). We have recently shown that IgG rheumatoid factors from patients with rheumatoid arthritis (Nardella *et al.*, 1985) bind to the protein A site of human IgG, and that the IgG Fc receptors on Groups A, C and G streptococci (Schröder *et al.*, 1986) probably also bind to this site. The binding site(s) on the IgG Fc region for HSV Fc receptor is (are) unknown. The similarities to protein A in the affinity for the human IgG subclasses raise the question that the HSV-1 Fc receptor may also bind to the protein A site of IgG. We show here that, like protein A, the HSV-1 Fc receptor does not bind to IgG fragments comprising the $C_\gamma2$ or $C_\gamma3$ domains alone but requires both domains for interaction.

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MATERIALS AND METHODS

Cells

In glass roller tubes (1·5 × 10 cm), African green monkey kidney cells of strain AH1 (GMK AH1) (Günalp, 1965) were grown in Eagle's minimal essential medium (Flow Laboratories, Irvine, Ayrshire, U.K.) supplemented with 10% fetal calf serum (Flow), 0·3 mg of glutamine per ml and 50 μg of gentamicin per ml, to a density of about 5×10^5 cells per tube. The cells were thereafter washed once with tissue culture medium (RPMI-1640; Flow) without serum but supplemented with gentamicin and glutamine.

Virus

Herpes simplex virus type 1 strain F (HSV-1 F) (Ejercito, Kieff

& Roizman, 1968) was inoculated onto GMK AH1 cells. On the first day of full cytopathic effect, the cells were harvested, homogenized and the suspension clarified by centrifugation at 200 *g* for 10 min. One-hundred μl of the supernatant containing 10^7 plaque-forming units (PFU) were used for inoculation of GMK AH1 cells in roller tubes.

Immunoglobulin and immunoglobulin fragments

The preparation of human and rabbit IgG and IgG fragments has been described previously (Schröder *et al.*, 1986). Briefly, human IgG was prepared from human *Cohn* Fraction II (Sigma Chemical Company, St Louis, MO) by ion exchange chromatography on a DEAE Sephacel column (Pharmacia Fine Chemicals, Piscataway, NJ) and monomeric human IgG from gel filtration of the fall-through peak obtained from ion exchange on an S-300 column (Pharmacia). Human Fab and Fc fragments were prepared from the same fall-through materials by papain digestion, DEAE ion exchange chromatography and gel filtration on a G-100 Superfine column (Pharmacia) (Nardella & Teller, 1985). Human $F(ab')_2$ and pFc' fragments were prepared by pepsin digestion of *Cohn* Fraction II by the method of Turner & Bennich (1968). The fragments were gel filtered on an S-300 column and the $F(ab')_2$-containing peak was passed over a Sepharose CL-4B staphylococcal protein A affinity column (Pharmacia) to remove residual intact IgG.

Monomeric rabbit IgG was obtained by S-300 gel filtration of rabbit *Cohn* Fraction II (Sigma), and rabbit Fab and Fc fragments were prepared by papain digestion of *Cohn* Fraction II and CM 52 (Whatman Chemicals, Div. W and R, Balston Ltd, Maidstone, Kent, U.K.) cation exchange chromatography according to the method of Porter (1959). The Fab- and Fc-containing peaks were filtered on a G-100 Superfine column, and $F(ab')_2$ and pFc' fragments were prepared as described for the human fragments. Rabbit Facb fragments were prepared and isolated by a modification (Schröder *et al.*, 1986) of the method of Connell & Porter (1971) from *Cohn* Fraction II. The purity of the obtained fractions was checked on sodium dodecyl sulphate polyacrylamide slab gel electrophoresis (SDS–PAGE). The levels of contaminants in each of the preparations were less than the following: human IgG, 1·4%; human Fc, 1·7%; human $F(ab')_2$, 3·0%; human pFc', 2·0%; rabbit IgG, 2·3%; rabbit Fc, 6·9%; rabbit $F(ab')_2$, 1·4% rabbit Facb, 2·1%; and rabbit pFc', 1·9%.

Protein iodination

^{125}I-labelling of the immunoglobulins and immunoglobulin fragments was performed using the lactoperoxidase method (Marchalonis, 1969; Thorell & Johansson, 1971). Specific activities of 10–20 μCi/μg of protein were obtained.

Protein-binding and inhibition assays

Binding to infected and uninfected GMK AH1 cells in roller tubes was tested as previously described (Johansson *et al.*, 1984). Briefly, cells in triplicate tubes containing 1 ml of serum-free RPMI-1640 medium were either left uninfected or infected with HSV-1 at a multiplicity of 50 PFU per cell. After rotation in a roller drum for 20 hr at 37°, the medium was removed and 100 μl of phosphate-buffered saline (PBS) containing 0·2% (w/vol) ovalbumin with or without competing unlabelled human or rabbit IgG or IgG fragments were added. The quantity per tube of the competing proteins was as follows: for Fc and pFc' fragments, 13 μg; for $F(ab')_2$ fragments, 26 μg; and for IgG and Facb fragments, 39 μg. After rotation for 1 hr at 37°, 100 μl of PBS–ovalbumin containing 5 ng of radiolabelled human or rabbit IgG or IgG fragments (around 200,000 c.p.m.) were added to each tube. Following another 1 hr of rotation at 37°, the cells were washed three times with 4 ml PBS followed by centrifugation at 200 *g* for 10 min. The radioactivity bound to the cells (maximum 20,000 c.p.m.) was measured in a gamma counter. The Fc specificity of the binding was demonstrated by inhibition with unlabelled human IgG Fc fragments in separate triplicate tubes. The cell numbers were enumerated with a haemocytometer after trypsination of two uninfected tubes processed identically. For unlabelled human IgG Fc fragments versus iodinated IgG, Facb, $F(ab')_2$, Fc and pFc' the molar excesses were 7800-, 6500-, 5200-, 2600- and 1300-fold, respectively. The molar excesses for unlabelled IgG, Facb, $F(ab')_2$, Fc, and pFc', versus labelled human IgG Fc fragments were 2600-, 3100-, 2600-, 2600-, and 5200-fold, respectively.

Bacterial Fc receptor binding

Staphylococcus aureus Cowan I (NCIC 8530) was grown, suspended and tested for immunoglobulin binding as previously described (Kronvall, 1973; Christensen & Oxelius, 1974; Johansson *et al.*, 1984). Briefly, 5 ng of radiolabelled IgG or IgG fragments in 200 μl of PBS with 0·02% sodium azide and 0·05% Tween 20 were added to duplicate tubes containing 2×10^8 bacteria per tube of either *Staphylococcus aureus* strain Cowan I, known to carry protein A, or human *Streptococcus* Group A type M6, which did not express IgG Fc receptor activity (negative control strain). After 1 hr at room temperature, 2 ml PBS with 0·02% sodium azide and 0·05% Tween 20 were added to each tube, the bacteria spun down and the supernatants removed. The binding was expressed as the radioactivity of the bacterial pellet in the percentage of labelled immunoglobulin originally added to the test tube.

RESULTS

Binding of radiolabelled human and rabbit IgG and IgG fragments to HSV-1-infected and non-infected cells

The binding of the radiolabelled human and rabbit IgG and IgG fragments to HSV-infected cells is shown in Fig. 1. Varying results were obtained with the different IgG fragments. Radiolabelled human IgG and Fc fragments bound effectively to infected but not to uninfected cells. Radiolabelled human $F(ab')_2$ and pFc' fragments did not bind to any of these cells. The same increase in binding after infection of the cells was seen for radiolabelled rabbit IgG and Fc fragments, whereas radiolabelled rabbit Facb, $F(ab')_2$ and pFc' were non-reactive. In addition, unlabelled human Fc fragments strongly inhibited the binding of human and rabbit IgG and Fc to the infected cells. The results show the Fc-specific nature of the binding and demonstrate that the binding site requires intact $C_\gamma 2$ and $C_\gamma 3$ domains. The number of receptors in the test system (around 5×10^5) cells/tube) greatly exceeds the number of labelled ligands (data not shown).

Binding of radiolabelled rabbit IgG Fc fragment and its inhibition by unlabelled human and rabbit IgG and IgG fragments

In order to exclude damage to the various IgG fragments as a

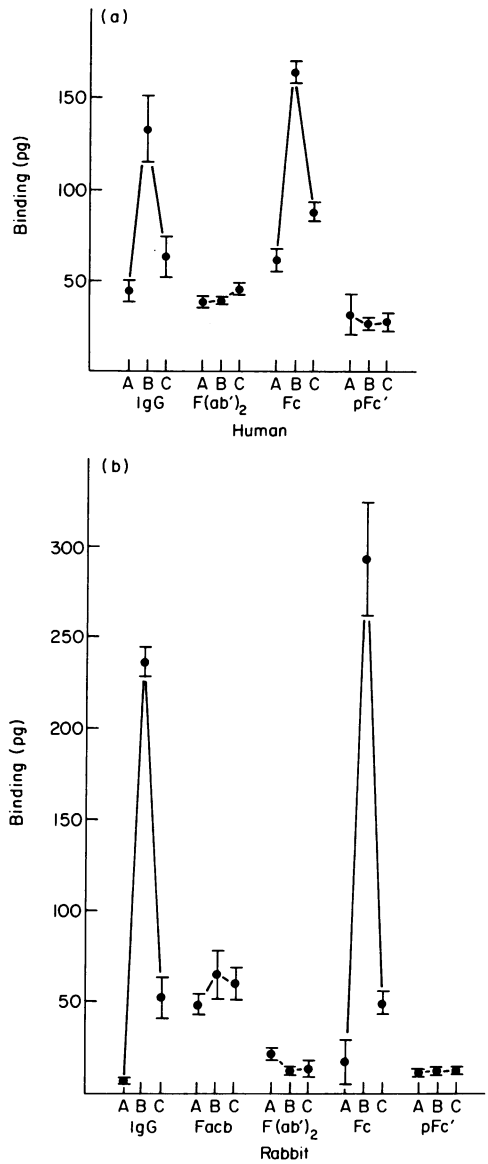

Figure 1. Binding of iodinated (a) human and (b) rabbit IgG and IgG fragments to HSV-infected cells and its inhibition by unlabelled human IgG Fc fragment. Means of triplicates with one standard deviation are shown. The binding is expressed in picograms (pg): (A) uninfected cells; (B) HSV-1-infected cells; (C) inhibition of binding to HSV-1-infected cells by unlabelled human IgG Fc fragment.

result of iodination, the capacity of the various immunoglobulin and immunoglobulin fragments to inhibit the binding of radiolabelled rabbit IgG Fc fragment was examined (Fig. 2). An excess of unlabelled human $F(ab')_2$ or pFc' or unlabelled rabbit Facb, $F(ab')_2$ and pFc' fragments did not inhibit the binding of iodinated rabbit IgG Fc fragment to HSV-infected cells (Fig. 2). In contrast, a significant inhibition was seen with unlabelled human and rabbit IgG and IgG Fc fragments, indicating that they bound to the HSV-induced Fc receptor. The results corroborate the Fc-specific nature of the binding and also show that intact $C_\gamma 2$ and $C_\gamma 3$ domains are required for binding to the Fc receptor.

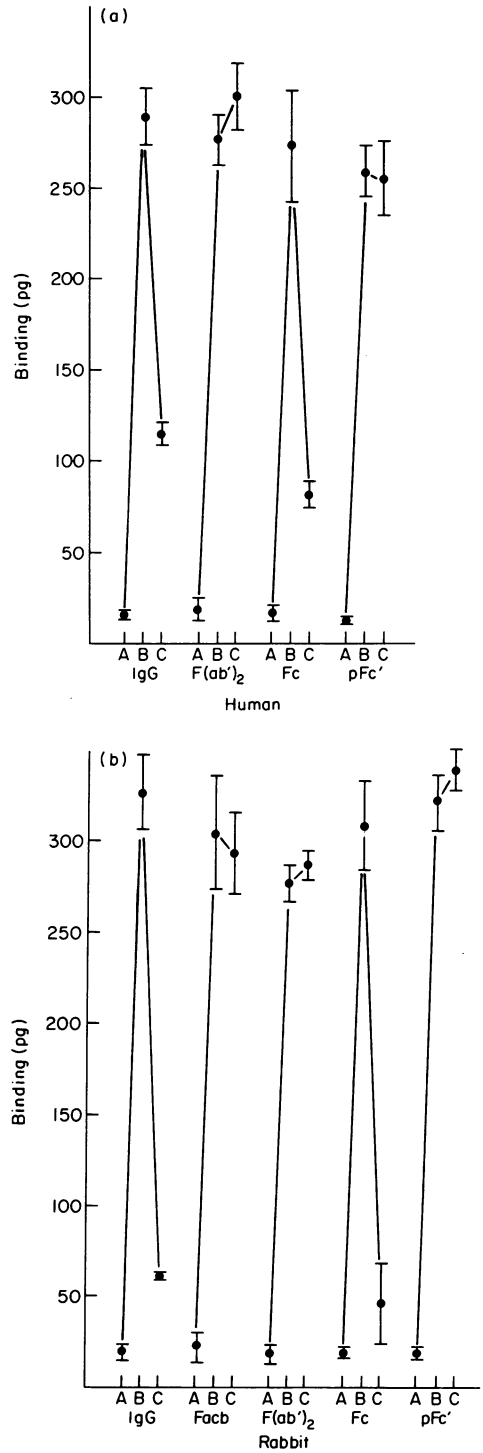

Figure 2. Inhibition of binding of iodinated rabbit IgG Fc fragment to HSV-infected cells by unlabelled (a) human and (b) rabbit IgG and IgG fragments. Means of triplicates with one standard deviation. The binding is expressed in picograms (pg): (A) uninfected cells; (B) HSV-infected cells; (C) inhibition of binding of HSV-infected cells by unlabelled human and rabbit IgG and IgG fragments.

Binding of iodinated human and rabbit IgG and IgG fragments to bacteria

Tests for the binding of radiolabelled human and rabbit IgG and

Table 1. Test of binding of radiolabelled human and rabbit IgG and IgG fragments to *Staphylococcus aureus* Cowan I and to *Streptoccocus* Group A type M6

	Staphyloccocus aureus	*Streptococcus* Group A, type M6
Human IgG	++	−
$F(ab')_2$	+	−
Fc	++	−
pFc′	−	−
Rabbit IgG	+++	−
Facb	−	−
$F(ab')_2$	−	−
Fc	++	−
pFc′	−	−

The binding is expressed as a percentage of the total amount of IgG and IgG fragments added to the test tube: (−) <10%; (+) 10–39%; (++) 40–69%; (+++) >70%.

IgG fragments to bacterial strains with and without Fc receptors were performed in order to verify the test system independently. The results were in agreement with previous reports (Lancet *et al.*, 1978; Schröder *et al.*, 1986), i.e. human and rabbit IgG and IgG Fc fragments were taken up by *Staphylococcus aureus* Cowan I, whereas pFc′ was non-reactive (Table 1). The human $F(ab')_2$ expressed some affinity for protein A via its alternative binding site, whereas no reactivity was detected for rabbit $F(ab')_2$ and Facb. The type M6 Group A streptococcal strain without Fc receptors was non-reactive with all IgG and IgG fragments (Table 1).

DISCUSSION

The functional role of HSV Fc receptor-mediated IgG binding to herpes virions and to herpes-infected cells is unknown. The binding site on the IgG molecule may be of help to elucidate the importance of the receptor. The present data demonstrate that the HSV-induced Fc receptor bound radiolabelled human and rabbit IgG and IgG Fc fragments, whereas no affinity was found for radiolabelled human and rabbit $F(ab')_2$ or pFc′ fragments. The pFc′ fragments, which are composed of $C_\gamma3$ domains (Turner & Bennich, 1968), has previously been found to be non-reactive (Wiger & Michaelsen, 1985). No significant binding of radiolabelled rabbit Facb fragments, consisting of all the IgG domains except $C_\gamma3$, to HSV-infected cells was seen. Possible damage to the various IgG fragments during the iodination procedure was ruled out by evaluating the capacity of the various unlabelled IgG and IgG fragments to inhibit the binding of iodinated rabbit IgG Fc fragments to HSV Fc receptor. It can therefore be concluded that intact IgG Fc fragments, consisting of both $C_\gamma2$ and $C_\gamma3$ domains, are necessary for the non-immune interaction with the HSV Fc receptor.

The binding of radiolabelled human IgG and IgG Fc fragments, and rabbit IgG and IgG Fc fragments, respectively, to HSV-infected cells was approximately the same. As the number of receptors on the cells in the test system greatly exceeds the number of labelled ligands, no saturation was obtained. It can, therefore, not be concluded that IgG Fc molecules bind more effectively than whole IgG molecules.

The fact that both $C_\gamma2$ and $C_\gamma3$ domains were obligate for the interaction with the HSV Fc receptor does not necessarily imply that the binding was situated in the interface between them. It has been proposed that one of the major functions of the $C_\gamma3$ domain is to stabilize the rather flexible $C_\gamma2$ domain (Burton, 1985). The removal of the $C_\gamma3$ domain could, therefore, make a possible binding site on the $C_\gamma2$ domain non-functional. Another less likely possibility was that the enzymes used in the preparation of the fragments acted on the binding site within one of the domains. Nevertheless, it seems likely that the binding site on the IgG molecule for the HSV Fc receptor is situated in the interface of the $C_\gamma2$ and $C_\gamma3$ domains. Protein A also expresses the same binding pattern for the IgG Fc fragments (Lancet, 1978; Schröder *et al.*, 1986) as the HSV Fc receptor, and the binding site on human IgG molecules has been established as the interface between the $C_\gamma2$ and $C_\gamma3$ domains (Deisenhofer 1981). As there are minor differences in binding ability between the HSV Fc receptor and protein A for some human IgG3 myeloma proteins (Wiger & Michaelsen, 1985) and some animal IgG and IgG subclasses (Johansson *et al.*, 1985), it is less likely that they possess exactly the same binding site. IgG rheumatoid factors from patients with rheumatoid arthritis also interact with amino acids in the area of the protein A binding site on IgG (Nardella *et al.*, 1985). The same site on IgG probably interacts with the Fc binding proteins of Groups A, C and G streptococci (Schröder *et al.*, 1986). The biological relevance of these associations remains to be elucidated.

The study of the interaction of IgG with the receptors on human cells such as monocytes and granulocytes and with the receptors induced by herpes simplex may answer some important questions. For example, it has been suggested that HSV Fc receptor is involved in the protection of herpes-infected cells against cell-mediated and complement-dependent immune lysis (Adler *et al.*, 1978). Furthermore, the relationship between different receptors will perhaps clarify the origin of the viral genes that lead to the synthesis of the HSV Fc receptor.

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