RHEUMATOID FACTORS REACT WITH Fab FRAGMENTS OF MONOCLONAL ANTIBODIES TO HERPES SIMPLEX VIRUS TYPES 1 AND 2 Fcγ-BINDING PROTEINS

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Human polyclonal IgM rheumatoid factors (RF) were tested in an enzyme-linked immunosorbent assay with monoclonal antibodies (MAb) (II-481 and B10/A8) to glycoprotein E (gE), the Fcγ-binding protein of herpes simplex virus type 1 (HSV-1), as well as with MAb 88-S to gE of HSV-2. Most of the RF reacted with II-481 and 88-S. Positive reactions were recorded for RF reacting with whole MAb II-481 and 88-S, as well as with their Fab, but not their Fc, fragments. Human monoclonal IgM RF isolated from mixed cryoglobulins showed a similar profile, with reactivity for both whole MAb II-481 and 88-S and for their Fab fragments. Reactivity with MAb to gE was observed regardless of the Gm specificity of the polyclonal RF and the cross-reactive idiotypes (6B6, 17.109, or G6) of the monoclonal RF. No positive reactions were noted between protein A and Fab fragments of any of the anti-gE MAb. These findings indicate that many RF may bear the internal image of the Fcγ-binding regions of 2 different herpesviruses: HSV-1 and HSV-2.

Rheumatoid factors (RF), which represent one of the first autoantibodies ever to be described (1,2), have long been known to react with the Fc portion of IgG (3). A number of studies have characterized the apparent anti-allotypic specificities (4–6) of RF, as well as the interactions of this group of anti–gamma globulins within the serum to form complexes that are possibly involved in the rheumatoid inflammatory process (7–9). Many other human diseases besides rheumatoid arthritis (RA) may be associated with the production of rheumatoid factors, including infective endocarditis, syphilis, schistosomiasis, trypanosomiasis, tuberculosis, and leprosy (10–15). It is possible that RF might be generated as antibodies to autologous antibodies that have been produced against bacterial or viral Fcγ-binding proteins (16,17). Fcγ-binding proteins have been characterized on many strains of streptococci isolated from RF-positive patients with endocarditis, and have been identified on schistosomes as well as on pathogenic, but not nonpathogenic, trypanosomes (18,19).

Numerous attempts to culture bacteria from RA synovial tissues have yielded negative results. At present, it seems unlikely that a primary bacterial etiology for RA can be seriously considered. However, an underlying viral stimulus for the disease remains a distinct possibility. Herpesviruses induce Fcγ-binding proteins on cells following infection (20,21). Moreover, herpes simplex virus type 1 (HSV-1) Fcγ-binding proteins on actual virions have been demonstrated to be transferred to the cell surface by infection with the virus (22). This sort of modula-

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tion of cellular Fcγ-binding protein expression could conceivably favor Fc receptor antigenicity within the infected host. A previous study demonstrated that many human rheumatoid factors isolated from RA patient sera reacted directly with monoclonal antibody (MAb) II-481 (23), a murine IgG2b monoclonal antibody with specificity for the IgG Fcγ-binding site on glycoprotein E (gE), the HSV-1 Fc-binding glycoprotein (24).

In the present report, we demonstrate that many polyclonal, as well as monoclonal, human RF react with the Fab portion of MAb II-481, rather than the Fc fragment. Moreover, using an entirely different MAb (88-S) against the Fcγ-binding protein of HSV-2, we demonstrate the presence of a similar RF specificity. These findings emphasize close antigenic relationships between Fc-binding proteins of two different herpesviruses and may indicate that such antigenically related Fcγ-binding proteins of herpesviruses could possibly be involved as the original antigenic stimulus in the generation of RF in patients with RA or other connective tissue diseases.

MATERIALS AND METHODS

Preparation of RF. RF were prepared, as previously described (24,25), from sera obtained from 16 patients with RA. The RA patient sera were passed over Sepharose 4B columns (Pharmacia, Uppsala, Sweden) coupled with monomeric human IgG (Cohn fraction II; Sigma, St. Louis, MO) and equilibrated with 0.2*M* boric acid, 0.15*M* NaCl, pH 8.8 (borate buffer). The adsorbed RF were eluted with acetate buffer (0.1*M* acetic acid, 0.15*M* NaCl, pH 3.5), dialyzed against phosphate buffered saline (PBS), and concentrated on YM-10 membranes (Amicon, Danvers, MA).

A panel of 13 monoclonal human IgM RF were isolated from the sera of patients with mixed cryoglobulinemia by first preparing the cryoglobulin precipitate. The precipitate, which formed at 4°C, was washed with cold PBS, pH 7.4, at 4°C, and then resolubilized at 37°C in 0.1*M* acetate buffer, pH 3.5. IgM RF were then prepared by Sephadex G-200 separation in acetate buffer, pH 3.5. All monoclonal human RF were typed for the expression of cross-reactive idiotypes 6B6.6 and 17.109 as well as G6 (26–28) by Dr. Ralph Schrohenloher (University of Alabama, Birmingham).

MAb against herpesvirus glycoproteins and control antibodies. MAb against HSV-1 gE, II-481 (23), was generously provided by Dr. P. G. Spear (Department of Microbiology–Immunology, Northwestern University Medical and Dental Schools, Chicago, IL). MAb BA 10/A8 (29), which also reacts with HSV-1 gE (but not at the site involving actual binding to the Fc of IgG) was kindly provided by Dr. Harvey M. Friedman (Department of Medicine, University of Pennsylvania, Philadelphia). The reactivity of BA 10/A8 with gE had previously been demonstrated by Western blot; its lack of reactivity with the gE site involved in the binding to IgG Fc was established by its failure to inhibit the rosetting of HSV-1–infected cells with IgG-coated erythrocytes. MAb 88-S was generously provided by Dr. Martin Zweig (National Cancer Institute, Frederick, MD). This IgG2b MAb reacts with HSV-2 glycoprotein E and precipitates the same gE component as the 82-S MAb described previously (30). MAb 88-S reacts with the Fcγ-binding region of the HSV-2 gE.

Table 1. Monoclonal antibodies to herpes simplex virus (HSV) and control antibodies used in human rheumatoid factor specificity studies

Monoclonal antibody	IgG subclass	Specificity
II-481	IgG2b	Fc-reactive site or its proximity on HSV-1 glycoprotein E
BA 10/A8	IgG2a	HSV-1 glycoprotein E but not at Fc-reactive site
88-S	IgG2b	Fc-reactive site or its proximity on HSV-2 glycoprotein E
MOPC-141	IgG2b	Control myeloma protein
MOPC-195	IgG2b	Control myeloma protein

Control MAbs included IgG2b proteins MOPC-141 and MOPC-195 (myeloma proteins without known specificity; Sigma). All MAb used in the present studies are shown with their specificity and H chain subgroup in Table 1.

Enzyme-linked immunosorbent assay (ELISA). Preliminary assays using peroxidase-conjugated goat anti-mouse IgG plus anti-mouse light chain as a developing antibody indicated that equal amounts of isolated MOPC-141, II-481, BA 10/A8, and 88-S could coat flat-bottom polyvinylchloride microtiter plates (Dynatech, Chantilly, VA). Binding of purified human RF to murine antibodies was assayed as follows. Microtiter plates were coated with murine antibodies (75 μl/well) at 5 μg/ml in PBS overnight. Control wells received only PBS. Human Fc fragment (Jackson ImmunoResearch, Avondale, PA) was also coated to microtiter plates, in parallel, as a positive control. After washing 3 times with 0.1*M* Tris HCl/0.5*M* NaCl buffer (pH 8.0) containing 1% bovine serum albumin (BSA) and 0.1% Tween 20 (Tris-BSA-Tween), unsaturated protein-binding sites were blocked by incubation with Tris-BSA-Tween (220 μl/well) for 1 hour.

After washing once, 50 μl/well of the human RF was added, at 5 μg/ml in Tris-BSA-Tween, and incubated for 2 hours. After washing 5 times, $F(ab')_2$ fragments of peroxidase-conjugated goat anti-human IgM Fc (Jackson ImmunoResearch), diluted 1:10,000 in Tris-BSA-Tween, were added. After incubation for 1.5 hours, plates were washed 5 times, and substrate solution, consisting of *o*-phenylenediamine (0.4 mg/ml) in phosphate–citrate buffer (pH 5.0) with 0.01% H_2O_2, was added. Absorbance at 490 nm was read in a Bio-Tek (Newton, MA) microplate reader.

The absorbance in the antibody-coated wells was corrected for nonspecific binding by subtracting the absorb-

ance in the uncoated wells that were assayed with each sample. Nonspecific binding was <0.034 optical density (OD) units in all cases. Throughout the assays, buffer with high salt concentration (0.1*M* Tris HCl/0.5*M* NaCl, as noted above) was used to reduce nonspecific protein–protein interactions. As controls, non-RF monoclonal and polyclonal normal human IgM (Calbiochem, La Jolla, CA) were similarly tested in parallel with the various MAb assayed. All assays were done in duplicate, and differences were statistically evaluated by Student's *t*-test.

Preparation of Fab and Fc MAb fragments. Because previous studies had indicated the extreme lability of some IgG2b murine proteins after pepsin digestion, as well as the production of Fab/c fragments that contained long segments of heavy chains (31,32), Fc and Fab fragments were prepared by papain digestion. Production of Fab and Fc papain fragments of MAb employed the initial isolation of the actual MAb from ascites, using hydroxyapatite chromatography (33), reduction in 0.1*M* cysteine and 0.02*M* EDTA, and papain digestion for 5 hours at 37°C, at pH 7.3. Immediately following digestion, the products were alkylated with 0.15*M* iodoacetamide, and the Fab and Fc fragments were separated by DEAE-cellulose chromatography (columns equilibrated with 5 m*M* Tris HCl, pH 7.5) and elution with a linear salt gradient (from 0–0.2*M* NaCl in 5 m*M* Tris, pH 7.5) (31).

Localization of Fab and Fc papain fragments employed an ELISA of serial column fractions coated onto Immunolon II plates (Dynatech), followed by coating with goat anti-mouse $F(ab')_2$ or anti-mouse Fc conjugated with peroxidase. Fractions containing Fab and Fc fragments were also examined by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), with and without reducing conditions. The RF reactivities of separated Fab and Fc fragments of individual MAb were compared with that of whole MAb. The ELISA utilized the technique described above, coating the Immulon plates with Fc and Fab fragments at 5 μg/ml. Equivalent coating of plates by Fc and Fab fragments and whole MAb was always assessed in preliminary ELISA screening, using peroxidase-conjugated goat anti-mouse IgG.

When the RF were tested for their comparative reactivity with whole MAb, such as II-481 or 88-S, in parallel with Fab or Fc fragments of the same, or other, control antibodies, positive reactions were recorded if the means of duplicate assays were at least 2 SD above those of the control myeloma protein MOPC-141 (by Student's *t*-test). All experiments were repeated at least once, and comparative differences in reactivity remained remarkably consistent.

Purification of MAb used in comparative ELISAs with RF. MAb II-481, BA 10/A8, and 88-S were isolated from ascites by protein G affinity chromatography or hydroxyapatite chromatography (31). MAb isolated by either method appeared to show comparable degrees of purity, producing single monoclonal patterns on SDS-PAGE. In the case of control mouse myeloma proteins such as MOPC-141 or MOPC-195, if SDS-PAGE analysis confirmed monoclonal patterns, no further isolation procedure was employed. Where necessary, protein G affinity columns were used to purify preparations for ELISA.

Assays of Gm specificity of RF. Single RF from individual patients were characterized for various anti-Gm allotypic specificities. IgG from RA sera were first typed for their own Gm phenotypes, as follows. IgG was isolated by DEAE chromatography and tested for intrinsic RF activity using Rh-positive human erythrocytes coated with incomplete anti-Rh antibody Ripley (34). If any RF activity was detected, IgG preparations were absorbed, using Cohn fraction II linked to Sepharose 4B, and then assayed for the RA patient's Gm phenotypic profile using standard commercial agglutinators and anti-Rh coats of known Gm specificity in parallel with known positive and negative controls. All RA patient IgG samples were typed for Gm(a), Gm(x), Gm(b), Gm(g), and Gm(f) (35).

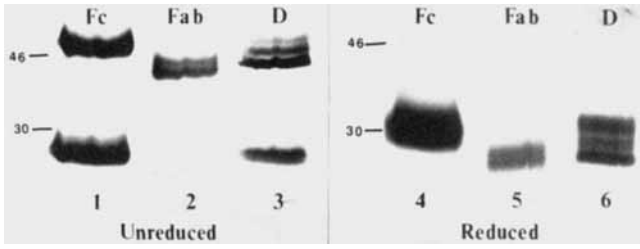

Figure 1. Results of a representative sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of monoclonal antibody II-481 papain-digest components, under reducing and nonreducing conditions. Molecular weight markers are shown at the left. D = whole papain digest.

In parallel, isolated polyclonal RF were tested for their predominant anti-Gm specificity using agglutination of Rh-positive erythrocytes coated with known Gm-specific anti-Rh coats. The highest titers in these reactions were used to assign primary anti-Gm specificities, along with confirmation of anti-Gm(a) or other specificity, using hemagglutination inhibition with isolated normal IgG or myeloma proteins of known Gm specificity (4–6).

Cross-reactivity of II-481 and protein A. Since previous experiments using polyclonal chicken IgG antibody to protein A had demonstrated that human RF bear the internal image of the Fcγ-binding region of staphylococcal protein A (36), it was of interest to examine the protein A reactivity of various mouse MAb to HSV Fcγ-binding glycoproteins. MAb II-481, its Fab and Fc fragments, as well as polyclonal mouse IgG, Fc, and $F(ab')_2$ (Jackson ImmunoResearch) were coated onto Immulon II ELISA plates (5 μg/ml) and, after blocking with 2% BSA-PBS, were developed with peroxidase-conjugated protein A at 5 μg/ml. A second, alternative test protocol employed initial coating of the plates with protein A, 5 μg/ml in PBS, pH 7.4, followed by washing 3 times, blocking with 2% BSA–PBS, and subsequent addition of whole II-481, Fab and Fc fragments of II-481, whole MOPC-141, and polyclonal mouse $F(ab')_2$, Fc, and IgG, all at 5 μg/ml, after washing and development with alkaline phosphatase–conjugated $F(ab')_2$ goat anti-mouse IgG (heavy and light chains).

RESULTS

Enzymatic digestion of MAb. Whole murine MAb were digested with papain, and the resulting Fab

and Fc fragments were separated on DEAE-cellulose columns using a salt gradient. SDS-PAGE analysis confirmed the proper relative sizes of the Fab and Fc fragments prepared (Figure 1).

Papain digestion of all other anti-HSV gE MAb and control myelomas resulting in Fab and Fc fragments was complete, and no whole IgG remained, as indicated by analysis of digestion products under reducing conditions using SDS-PAGE. Every monoclonal antibody required slightly different conditions and duration of digestion to produce optimal yields of Fab and Fc fragments.

Comparative reactivity of RF with whole MAb and their Fc and Fab fragments. A series of polyclonal IgM human RF were tested in an ELISA for their relative reactivity with whole MAb II-481, its Fab and Fc fragments, whole MOPC-141, and whole BA10/A8. Thirteen of the 16 polyclonal RF tested showed significantly stronger reactivity with whole MAb II-481 and its Fab fragment than with its Fc fragment. No significant reaction of most of these RF was noted with IgG2b control MOPC-141 or with BA 10/A8, although some RF showed weak reactions with MOPC-141 or BA 10/A8. No reactions with any of the MAb preparations were noted when normal polyclonal human IgM or monoclonal human IgM preparations without RF activity were used.

As shown in Figure 2 and Table 2, the level of reactivity of the individual RF for MAb II-481 varied, but equal or equivalent reactivity was noted when one RF was tested in parallel with whole MAb II-481 and its Fab fragment. Only 3 of the 16 polyclonal RF tested showed reactions which differed from those observed in other RF samples. One showed no reaction with either whole MAb II-481 or its Fab and Fc fragments. One RF (McC) showed strong but equal reactivity with whole II-481, Fab II-481, Fc II-481, and MOPC-141. Another RF (Abr) reacted with whole II-481, the Fab fragment of II-481, and whole MOPC-141, but not with the Fc fragment of II-481.

The specificity of the reactivity of RF McC with the Fc and Fab fragments of MAb II-481 was compared by coating Fab fragments of II-481 onto ELISA plates at 5 μg/ml. Aliquots of McC RF were preincubated for 3 hours at room temperature with decreasing concentrations (from 500 μg to 0.1–1.0 ng) of Fc II-481, Fab II-481, whole MOPC-141, and the Fc fragment of MOPC-195, before adding the McC RF to the ELISA plate coated with Fab II-481. After washing, the detecting antibody for the measurement of RF binding (peroxidase-conjugated $F[ab']_2$ goat anti-Fc of human IgM) was added, and the ELISA was completed.

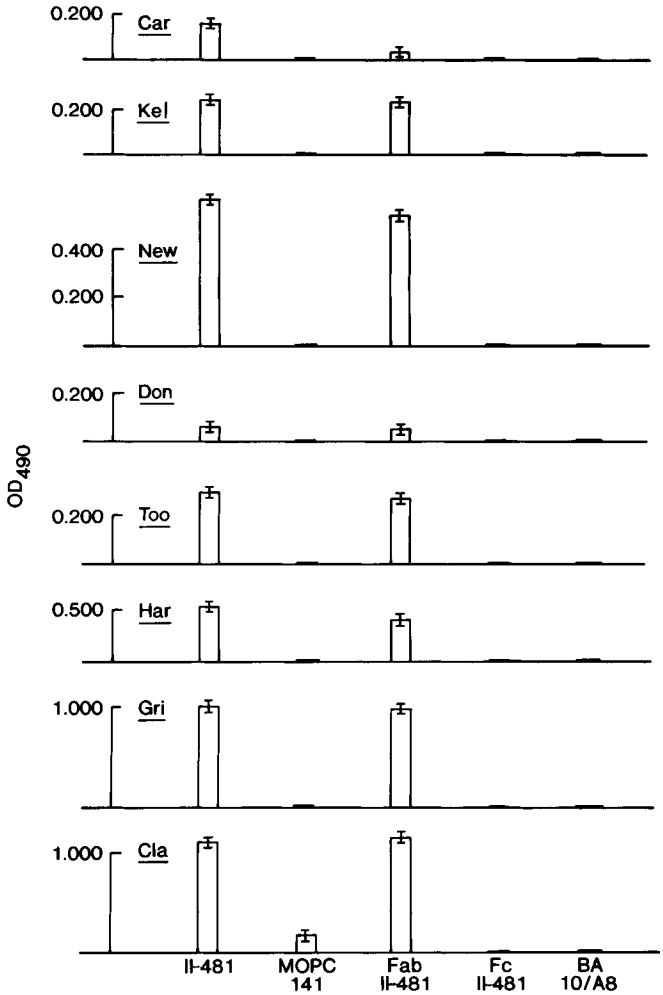

Figure 2. Reactivity of IgM rheumatoid factors (RF) isolated from rheumatoid arthritis patient sera in an enzyme-linked immunosorbent assay (ELISA) of whole IgG2b monoclonal antibody (MAb) II-481, control IgG2b MAb MOPC-141, papain-digested Fab and Fc fragments of MAb II-481, and control MAb BA 10/A8 (coated onto ELISA plates at 5 μg/ml). Plates were developed with peroxidase-conjugated $F(ab')_2$ goat anti-human Fc fragment of IgM. Negative reactions were recorded using polyclonal normal human IgM or monoclonal human IgM without RF activity. Bars show the mean ± SD. OD = optical density; patients' names (abbreviations) are underlined.

As shown in Table 3, the binding of McC RF to II-481 Fab was strongly inhibited at Fab II-481 concentrations as low as 0.1 ng/ml. However, high concentrations (100 μg) of Fc II-481 were not as effective at absorption or inhibition as were concentrations in the range of 10 μg/ml to 1 ng/ml.

Table 2. Profile of reactivity of polyclonal human rheumatoid factors (RF) with MAb to herpes simplex virus types 1 and 2 glycoprotein E*

Polyclonal RF (patient/Gm phenotype/ anti-Gm specificity	Mouse MAb or MAb fragment tested						
	II-481	II-481 Fab	II-481 Fc	88-S	88-S Fab	88-S Fc	MOPC-141 (control)
Car/a−,b+,g−,f+/Gm(a)	1+	0	0	1+	1+	0	0
Kel/a−,b+,g−,f+/Gm(a)	1+	1+	0	1+	1+	0	0
Hub/a−,b+,g−,f+ Gm(a)	1+	1+	0	1+	1+	0	0
Tom/a−,b+,g−,f+/Gm(a)	1+	1+	0	1+	1+	0	0
Gri/a−,b+,g−,f+/Gm(a)	3+	3+	0	3+	3+	0	0
Ske/a−,b+,g−,f+/Gm(a)	0	0	0	1+	1+	0	0
McC/a−,b+,g−,f+/Gm(a)	4+	4+	4+	0	0	0	4+
Low/a−,b+,g−,f+/Gm(a)	2+	2+	0	2+	2+	0	0
Abr/a−,b+,g−,f+/Gm(g)	3+	3+	0	0	0	0	4+
Str/a−,b−†,g−,f+/Gm(g)	2+	2+	1+	2+	2+	0	0
New/a+,x+,b+,g+,f+/Gm(a)	2+	2+	0	2+	2+	1+	0
Har/a+,x−,b+,g+,f−†/Gm(a)	2+	2+	0	2+	1+	1+	0
Cla/a+,x+,b+,g+,f+/Gm(a)	3+	3+	0	3+	2+	1+	1+
Mor/a+,x+,b+,g−†,f−†/Gm(a)	2+	2+	0	2+	2+	0	0
Don/a+,x+,b−†,g−†,f+†/Gm(f)	2+	1+	0	2+	1+	0	0
Too/a+,x+,b+,g+,f+/Gm(g)	1+	1+	0	1+	1+	1+	0

* Values are optical density (OD) units at 490 nm (0 = 0–0.1 OD, 1+ = 0.1–0.5 OD, 2+ = 0.5–1.0 OD, 3+ = 1.0–2.0 OD, and 4+ = >2.0 OD). Monoclonal antibodies (MAb) II-481, 88-S, and MOPC-141 are IgG2b.

† Unusual phenotypes: Gm(b) and Gm(f) usually show similar results, and most patients in this population who were Gm(a) positive were also Gm(g) positive.

Parallel controls using Fc fragments of MOPC-195 or whole MOPC-141 showed equivalent inhibition of binding of McC RF to Fab II-481. Similarly, it was demonstrated that absorption or preincubation of polyclonal RF New, which reacted with Fab II-481, was inhibited by 95–98% at a similarly broad range of concentrations of Fc MOPC-195, as well as of whole human IgG and whole MOPC-195. In like manner, polyclonal RF Gri, showing positive reaction with Fab II-481, was 95–97% inhibited at a broad range of concentrations of Fc MOPC-195 as well as of whole MOPC-195. Fab fragments of II-481, again, completely inhibited this reaction, but Fab of MOPC-195 showed only 10% inhibition.

Table 3. Effects of absorption (preincubation) of rheumatoid factors on subsequent binding to Fab fragments of II-481 coated onto microtiter plates*

Concentration of inhibitor	Fab II-481	Fc II-481	Fc MOPC-195	Whole MOPC-141
100 μg/ml	4.0 ± 0.4	85.5 ± 1.9	3.2 ± 0.07	12.2 ± 1.1
10 μg/ml	3.0 ± 0.4	9.3 ± 1.0	3.2 ± 0.07	9.0 ± 0.1
1.0 μg/ml	3.5 ± 0.28	4.2 ± 0.9	3.5 ± 0.16	4.3 ± 0.2
100 ng/ml	2.9 ± 0.1	2.4 ± 0.4	3.9 ± 0.35	3.9 ± 0.1
10 ng/ml	3.0 ± 0.1	1.2 ± 0.1	3.5 ± 0.42	5.6 ± 0.5
1.0 ng/ml	3.0 ± 0.02	2.1 ± 0.1	4.2 ± 0.98	5.2 ± 0.2
0.1 ng/ml	3.0 ± 0.4	–	3.9 ± 0.28	–
None	100 ± 5.8	100 ± 2.2	100 ± 5.8	100 ± 2.2

* McC polyclonal IgM rheumatoid factors were preincubated with the fragments or whole antibody shown, added to the plates coated with Fab II-481, and analyzed by enzyme-linked immunosorbent assay (see Materials and Methods for further details). Values are the mean ± SD percentage of binding.

Similar experiments were conducted using a panel of monoclonal IgM human RF isolated from 13 patients with mixed cryoglobulinemia. Representative results are shown in Figure 3. In 10 of the 13 monoclonal IgM RF studied, significantly stronger reactivity was noted with both whole MAb II-481 and its Fab fragment than with MOPC-141, the Fc fragment of II-481, or with whole BA 10/A8 (Table 4).

Using 3 other monoclonal RF (Wag, Lew, and Cor) a different pattern of reactivity was observed. Equivalent reactions were noted with whole II-481, control IgG2b MOPC-141, and both the Fab and Fc fragments of II-481, as well as with whole BA 10/A8. This pattern was similar to that seen with the small proportion of polyclonal RF shown in Table 2.

Preincubation or inhibition experiments similar to those shown in Table 3 demonstrated that the reaction of monoclonal RF Cor with Fab II-481 was virtually completely inhibited (95%) by Fab of II-481, as well as by a similarly broad concentration range (100 μg/ml to 0.1 ng/ml) of Fc of MOPC-195, whole

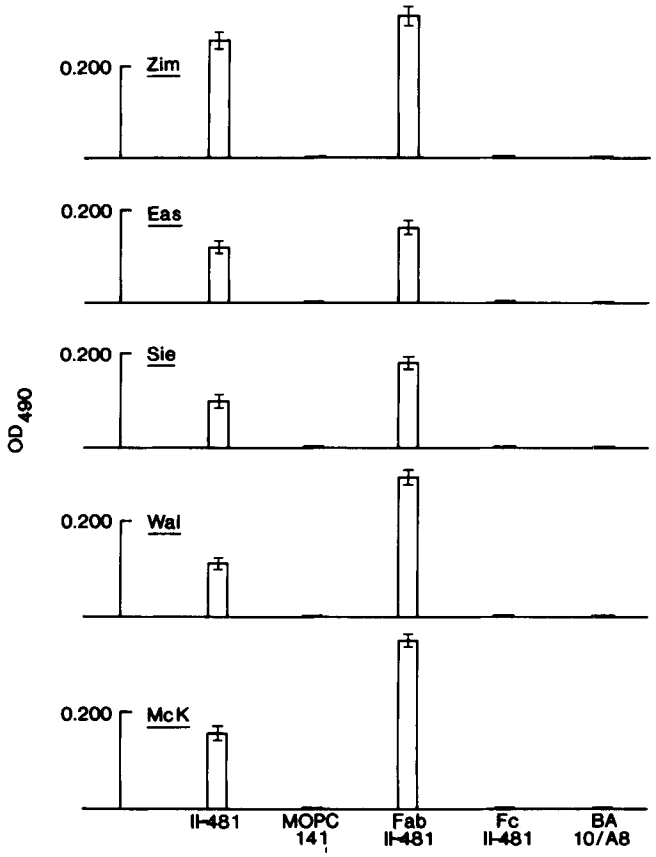

Figure 3. ELISA reactions using whole MAb II-481, MOPC-141, Fab and Fc fragments of II-481, and control anti–glycoprotein E BA 10/A8 with 5 monoclonal IgM RF (Zim, Eas, Sie, Wal, and McK; see Figure 2 for details and for explanations of abbreviations).

MOPC-195, and human IgG. Similar experiments using monoclonal RF Sie, which reacted with Fab of II-481, showed 99% inhibition of binding by whole MOPC-195, Fc of MOPC-195, and Fab of II-481, but only 20% inhibition with Fab of MOPC-195.

Reaction of RF with MAb to HSV-2 gE. The IgG2b MAb 88-S and its papain-digested Fab and Fc fragments, which react with gE of HSV-2, were next studied in the same ELISA system. Controls included MAb against HSV-1 Fcγ-binding protein, whole MAb II-481, and MOPC-141 (also an IgG2b). Results of these experiments are shown in Tables 2 and 4. It can be seen that 12 of 16 polyclonal RF showed stronger reactions with both the whole 88-S and its Fab fragment than with its Fc fragment or with MOPC-141. Two polyclonal RF, McC and Abr, showed no reaction with 88-S. Two other polyclonal RF, Har and Too, showed positive reactions with whole 88-S, Fab 88-S, Fc 88-S, and whole II-481, but no positive reaction with MOPC-141 (Table 2). A similar pattern of reactivity for whole 88-S, Fab of 88-S, and whole II-481 was also recorded for monoclonal RF (Table 4). Negative ELISA reactions were recorded when non–RF-containing polyclonal IgM or monoclonal IgM were tested with the 88-S and control substrates. These results indicated that an entirely different MAb to the HSV-2 gE Fcγ-binding glycoprotein reacted with RF, yielding a profile similar to that previously documented for II-481, the MAb against HSV-1 gE, at its actual IgG binding site.

Table 4. Reaction of monoclonal IgM rheumatoid factors (RF) with MAb to herpes simplex virus glycoprotein E Fcγ-binding proteins*

		Mouse MAb or MAb fragment tested						
Monoclonal RF	CRI	II-481	II-481 Fab	II-481 Fc	88-S	88-S Fab	88-S Fc	MOPC-141 (control)
Eas	G6, 17.109	1+	1+	0	1+	1+	0	0
Sie	17.109	1+	1+	0	1+	1+	0	0
Wag	17.109	1+	1+	1+	1+	1+	0	1+
Wal	G6	1+	1+	0	1+	1+	0	0
McK		1+	1+	0	1+	1+	0	0
Sal		2+	1+	0	2+	1+	0	0
Sen		2+	1+	0	2+	1+	0	0
Sier		1+	1+	0	1+	1+	0	0
Tub	6B6	2+	1+	0	2+	2+	0	0
Zim	6B6	1+	1+	0	1+	1+	0	0
Hun	6B6	2+	1+	0	1+	1+	0	0
Lew	6B6	1+	1+	1+	1+	1+	0	1+
Cor	6B6	2+	2+	2+	4+	4+	4+	1+

* Values are optical density (OD) units at 490 nm (0 = 0.0–0.1 OD, 1+ = 0.1–0.5 OD, 2+ = 0.5–1.0 OD, 3+ = 1.0–2.0 OD, and 4+ = >2.0 OD). Monoclonal antibodies (MAb) II-481, 88-S, and MOPC-141 are IgG2b. Cross-reactive idiotype (CRI) expression was determined as described elsewhere (26–28). Proteins McK, Sal, Sier, Tub, Wal, and Sen have been typed as κIIIb.

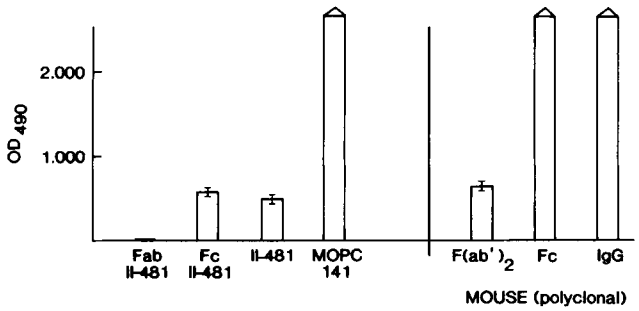

Figure 4. Results of ELISA using protein A–coated (5 μg/ml) plates with Fab and Fc fragments of MAb II-481 in parallel with whole undigested MAb II-481 and control mouse IgG2b MOPC-141 (5 μg/ml). Bound antibodies were detected by peroxidase-conjugated $F(ab')_2$ goat anti-mouse IgG. No reactivity between protein A and Fab of II-481 was noted; however, Fc and whole II-481 showed moderate positive reactivity, and whole control MOPC-141 showed strong reactivity. Results of parallel studies using polyclonal mouse $F(ab')_2$, Fc, and IgG reacting with protein A are shown to the right (see Figure 2 for details and for explanations of abbreviations). Bars show the mean ± SD; triangles atop bars show OD readings higher than scale.

Gm specificity and cross-reactive idiotype (CRI) analysis. Comparisons of the relationship to Gm phenotype of the RA serum donor, the principal anti-Gm specificity of the polyclonal RF studied, and the profile of reactivities of the 16 different polyclonal RF with MAb to HSV-1 and HSV-2 gE are shown in Table 2. No correlation could be made with the IgG Gm phenotype profile of the RF donors, nor with the primary anti-Gm specificity.

Analysis of the profile of monoclonal human RF reactions with MAb to HSV-1 and HSV-2 gE is shown in Table 4. No clear-cut pattern of RF reaction specificity could be identified with 6B6, 17.109, or G6 CRI expression of the monoclonal RF.

Reactivity of II-481 with protein A. It was pertinent to test the reactivity of MAb II-481 and its fragments with protein A directly, since protein A is the vintage example of a bacterial cell surface Fcγ-binding protein. Results of a direct binding experiment are shown in Figure 4. Whole MAb II-481, as well as its Fc fragment, but not its Fab fragment, reacted with protein A. Strong positive reactivity between protein A and MOPC-141, as well as both whole pooled mouse IgG and its Fc fragment, was seen. An alternative experimental protocol employing ELISA plates coated with the same reactants followed by application of peroxidase-labeled protein A produced similar results. These experiments indicated that the paratope of MAb II-481 did not react directly with epitopes constituting the IgG binding site on protein A. Similar experiments using whole MAb 88-S and another IgG2b control (MOPC-195) in parallel with their Fab and Fc fragments showed that protein A reacted only with the whole IgG or with its papain-digested Fc fragments, but not with the Fab fragments of any of the antiherpes gE MAb or control MAb.

DISCUSSION

The findings presented herein confirm that many human rheumatoid factors react with the paratope of monoclonal antibodies to herpes IgG Fcγ-binding glycoproteins (24). In previous investigations, we documented the reactivity between many polyclonal human RF from RA patients and MAb II-481, which showed specificity for sites close to or on the HSV-1 gE Fcγ-binding protein, actually binding to the Fc fragment of IgG (24). Results of reactions of RF with papain-digested fragments of MAb II-481, 88-S, and control IgG2b monoclonal proteins indicated that many RF react primarily with the Fab, rather than the Fc, fragment of MAb II-481 and 88-S. This principal specificity against MAb II-481 and 88-S and their Fab fragments confirmed the RF reactivity with the paratope region of the MAb, as strongly suggested by inhibition assays conducted in the previous study (24). The findings of our current study are very similar to those previously reported by Oppliger et al (36), who utilized polyclonal affinity-purified IgG chicken antibodies reacting with protein A to study reactions with rheumatoid factors. Our results strongly suggest that the paratope of II-481 that reacts with the IgG Fc-binding site on HSV-1 gE represents a common target for many RF tested, regardless of anti-Gm specificity or CRI expression.

The results obtained using protein A and whole MAb II-481 and its papain fragments were surprising, since we saw no reactivity between Fab II-481 and protein A. Actually, protein A showed positive reactions with whole II-481 and its Fc fragment, as well as with whole polyclonal mouse IgG and its Fc fragment, which was expected. The lack of a direct reaction of Fab II-481 with protein A suggests that the actual antigenic epitopes on protein A and HSV-1 gE Fcγ-binding protein may not be identical, although the Fcγ-binding sites on HSV-1 gE and protein A were reported to bind to similar epitopes on human IgG Fc (37). A comparative parallel analysis of the reactivities of individual RF with chicken antibodies to protein A and II-481 might elucidate whether internal image

specificities of individual RF are similar or diverse with respect to antiherpes Fcγ-binding protein and anti–protein A reactivity.

A small proportion of the polyclonal RF studied showed more universal reactions than with only the whole II-481 and its Fab fragment. This was also found with ~20% of the monoclonal IgM RF studied. In these instances, positive reactions were also recorded with control IgG2b myeloma protein MOPC-141. From the inhibition studies, in which RF were preincubated with various MAb fragments, including Fab II-481, Fc II-481, as well as Fc MOPC-195, whole MOPC-195, or MOPC-141, it seems likely that these positively reacting RF shared similar binding capacity for the paratope on Fab fragments of II-481, along with Fc epitopes on whole mouse myeloma proteins. Such cross-reactivity was confirmed by cross-inhibition studies between II-481, Fab of II-481, and Fc fragments of reactive control myeloma protein (Table 3).

One of the most interesting findings in this study is the similarity in RF reactivities using the Fab fragments of 2 different MAb to HSV gE proteins, which reacted with regions of the molecule involved in IgG binding: II-481 and 88-S. In many instances, the patterns of specificity were quite similar, but in others, they were quite different. This may be related to slight differences in the primary binding sites of the MAb to gE. However, the fact that 2 different MAb to gE showed similar or overlapping profiles of reactivity with the same panel of RF could also suggest that the gE HSV glycoproteins may exhibit a major immunodominant epitope. Precise definition of such an epitope should now be feasible.

The results of the studies reported here provide a more extended view of the possible role of viral Fcγ-binding proteins in the generation of human RF. The positive reactions between human RF and MAb 88-S, an entirely different monoclonal with specificity for HSV-2 gE Fcγ-binding protein, represent an interesting new observation. For some time, it has been known that there may occur cross-reactions between viral glycoproteins of HSV-1 and HSV-2 (38–41). Moreover, MAb II-481 has been shown to immunoprecipitate HSV-2 gE (42). However, our finding that many RF appear to show the same sort of reactivity with MAb 88-S and its Fab fragment as with MAb II-481 is an observation of particular interest, since it could suggest that Fcγ-binding glycoprotein antigens might exist in a relatively conserved manner within a number of members of the herpesvirus family. Further emphasizing the cross-reactivity of antigens related to herpesvirus Fcγ-binding proteins is the report by Xu-Bin et al (43) that MAb II-481 also cross-reacts with the cytomegalovirus Fcγ-binding glycoprotein.

In view of these observations, it is important to attempt to explore which other herpesvirus, or even other non-herpesvirus, Fcγ-binding proteins might cross-react with MAb such as II-481 or 88-S, since RA might conceivably be a disorder caused by aberrant immune response of genetically predisposed individuals to heterogeneous viruses possessing Fc-binding proteins. If such a hypothesis is correct, the V region sequences of RF, especially those from patients with RA, should reflect the stimulation by anti–Fcγ-binding protein antibody (Abl). In support of this, a recent report clearly demonstrated that expression of CRI such as 6B6 or 17.109 is limited to a small proportion of polyclonal RF, which suggests the importance of other germline V_L genes or the presence of extensive somatic mutations in the generation of RF (44). Such a viewpoint may provide new insight for future research regarding the pathogenesis of the disease.

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