

Production and Characterization of Mouse Monoclonal Antibodies Recognizing Multiple Subclasses of Human IgG

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Abstract

Different IgG subclass profiles are produced in response to different antigenic stimuli in a variety of diseases. IgG subclass levels may reflect disease severity. Quantification of IgG subclasses depends on the availability of specific Monoclonal antibodies (MAbs). In the present study seven hybridoma clones producing MAbs reactive with multiple subclasses of human IgG were established. Splenocytes from Balb/c mice immunized with Fc fractions of human IgG1 or IgG2 myeloma proteins were fused with mouse myeloma cells. Fused cells were selected and cloned by limiting dilution assay. Antibody secreting cells were screened by Enzyme-linked immunosorbent assay (ELISA) and the specificity of secreted MAbs was further analyzed, using a panel of purified human myeloma paraproteins of different IgG subclasses by ELISA and immunoblotting. Cross-reactivity to immunoglobulins (Igs) of other species was studied by indirect ELISA using serum samples collected from 9 animals. The MAbs were found to react with triple IgG subclasses, including IgG1,2,4 (n=4) and IgG1,2,3 (n=3). Immunoblotting studies revealed recognition of linear (n=4) or conformational (n=3) epitopes by these MAbs. The most abundant cross-reactivity (71.4%) was observed with monkey Ig while no cross-reactivity was detected with hen and cat sera. The MAbs mostly displayed a restricted pattern of cross-reactivity and one of them did not bind to any of the animal sera tested. The affinity constant of 3 MAbs was measured by ELISA. Based on the data obtained from this study, mouse MAbs reactive with multiple human IgG subclasses are directed to a variety of immunogenic epitopes, mostly shared with IgG of other species. These MAbs are valuable tools for purification of non-reactive IgG subclasses through negative affinity chromatography. These MAbs could also provide an opportunity for epitope mapping of the Fc region of IgG, as well as serological phylogenetic studies.

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Introduction

IgG, the most abundant Ig present in human serum and extravascular fluids, is subdivided into four subclasses (IgG1-4) based on antigenic differences between their heavy chain constant regions⁽¹⁾. Each subclass has different biological characteristics⁽²⁾ and a particular profile of effector functions⁽³⁾.

Immunoglobulin subclasses are differentially regulated in response to different antigenic stimuli^(4, 5). Thus different patterns of IgG subclasses^(6 - 8) have been reported to be produced in a variety of diseases. Moreover, there is a relationship between IgG subclass levels and severity of diseases^(9, 10). Therefore

IgG subclasses have a great diagnostic and prognostic value⁽¹¹⁾. Analysis of the IgG subclass response to different antigens of a microorganism may reveal the profile of T helper associated cytokine response⁽¹²⁾ and hence is helpful for designing a potential vaccine⁽¹³⁾. Quantification of IgG subclass levels depends on the availability of specific MAbs. Assay sensitivity could be improved by application of high affinity MAbs recognizing appropriate epitopes⁽¹⁴⁾.

Since human IgG subclasses are highly homologous with almost 95% amino acid sequence identity⁽¹⁵⁾, some human IgG subclass restricted mouse MAbs may recognize epitopes shared by multiple IgG subclasses. Such MAbs are valuable tools for epitope mapping of human IgG isotype and may recognize isotypic or isoallotypic markers within IgG subclasses.

The aim of the present study is production and characterization of MAbs with specificity for multiple IgG subclasses.

Materials and Methods

Preparation of purified human IgG subclasses

A panel of 27 different purified human IgG myeloma proteins of known IgG subclasses and light chain types was employed in this study. These myeloma proteins, obtained from patients with multiple myeloma, were either purified by Diethyl amino ethyl (DEAE) cellulose (Whatmann, UK) chromatography or by affinity chromatography using Staphylococcal protein A (SPA) or Streptococcal protein G (SPG) Sepharose 4B (Pharmacia, Sweden). The heavy chain and light chain isotypes and subclasses of myelomas were identified using specific mouse monoclonal antibodies including: AF6 (IgM), 8a4 (IgG), 2D7 (IgA), JA11 (IgD), C4 (λ), 6el (κ), JL512 (IgG1), GOM2 (IgG2), ZG4 (IgG3) and RJ4 (IgG4), kindly provided by Professor R. Jefferis and Dr. M. Goodall (Department of Immunology, University of Birmingham, UK).

Polyclonal IgG was isolated from normal serum with SPG-Sepharose and polyclonal

IgG3 was isolated, as break-through fraction, from polyclonal IgG by SPA-Sepharose column. Fc and Fab and F(ab')₂ fragments were produced from several purified human myeloma proteins of each of the IgG subclasses, by pepsin and papain digestion⁽¹⁶⁾. Digested fragments were isolated by affinity chromatography with SPA-Sepharose column.

Animal sera

Sera from human and nine animals were prepared from their clotted blood. The animal species used in this study were chicken, rabbit, guinea pig, cat, dog, sheep, goat, horse and monkey. The human serum was used as a control.

Production and selection of hybridomas

Balb/c mice (8-12 weeks of age) were immunized with four intraperitoneal injections of Fc fragments of human IgG1 or IgG2 myeloma proteins emulsified in Freund's complete adjuvant (Sigma, USA) (first injection) or incomplete adjuvant (Sigma) (other injections) (50 μ g every 2 weeks). Three days after the last injection, spleen cells were fused with SP2/0 myeloma cells (NCBI 129, National Cell Bank of Iran, Pasteur Inst. of Iran, Tehran), using polyethyleneglycol (PEG 1500) (Sigma). Hybridomas were grown in DMEM culture medium (Sigma) containing 20% Fetal Calf Serum (FCS) (Seromed, Germany), penicillin 100 IU/ml and streptomycin (100 μ g/ml) and supplemented with hypoxanthine (1×10^{-4} M), aminopterin (4×10^{-7} M) and thymidine (1.6×10^{-5} M) (HAT) (Sigma).

Ten to fourteen days after fusion, secreting hybrids were identified by analysis of culture supernatants by the ELISA technique described below. Selected antibody producing cultures were cloned by limiting dilution process according to the conventional methods⁽¹⁷⁾. Clones secreting antibody of desired reactivity were expanded in 25 and 75 cm² flasks (Nunc, Denmark), harvested and cryopreserved in 40% FCS, 10% Dimethylsulfoxide (DMSO) (Sigma) and 50% RPMI-1640 culture medium (Sigma).

Detection of specificity of MAbs by indirect enzyme-linked immunosorbent assay (ELISA)

Specificity of MAbs was determined by ELISA technique as described elsewhere ⁽¹⁸⁾. Briefly, microtiter polystyrene plates (Maxisorp, Nunc, Denmark) were coated with 1-10 µg/ml of purified myeloma IgG subclasses or polyclonal IgG in PBS (0.15 M, pH=7.2). Then 0.05 ml of culture supernatant was added. Appropriate dilution of HRP-conjugated sheep anti mouse Ig (prepared in our lab) was then added and the reaction revealed with O-phenylenediamine dihydrochloride (OPD) (Sigma) substrate. Finally, the reaction was stopped with 20% H₂SO₄ and the optical density (OD) measured by a multi-scan ELISA reader (Organon Teknika, Boxtel, Belgium) at 492 nm.

Isotype determination of MAbs by capture ELISA

Goat antimouse IgG1, IgG2a, IgG2b, IgG3, IgA and IgM at 1/1000 dilution, were adsorbed on to the wells of a microtitre ELISA plate (Nunc). Isotypes of MAbs in culture supernatants were determined according to the ELISA technique mentioned above.

Affinity constant (K_{aff}) determination by ELISA

We determined the K_{aff} by ELISA technique as described elsewhere ⁽¹⁹⁾. Briefly, ELISA plates (Nunc) precoated with four different concentrations of human IgG ([Ag], [Ag'] and [Ag'']) were separately incubated with serial concentrations of each MAb. Sigmoid curves were constructed using the OD values obtained for different concentrations of each MAb. Four non-overlapping curves were selected for each MAb to calculate the affinity constant. The half maximum OD (OD-50) was assigned for all selected curves from which the corresponding antibody concentration (([Ab], [Ab'] and [Ab''])) was extrapolated.

Accordingly, [Ab] and [Ab'] are the measurable total Ab concentrations at OD-50 and OD'-50 for plates coated with [Ag] and [Ag'], respectively. The affinity constant was determined using the following equation ⁽²⁰⁾: $K_{aff} = (n-1)/2(n[Ab']t - [Ab]t)$, where $n = [Ag]/[Ag']$

Immunoblotting technique for analysis of specificity of MAbs

Specificity of MAbs was assessed by immunoblotting technique as described elsewhere ⁽²¹⁾. Briefly, affinity purified myeloma IgG subclasses, polyclonal IgG and their fragments were electrophoresed under native and denaturing conditions, in 10% polyacrylamide gel (SDS-PAGE) (Sigma) and then transferred to a nitrocellulose membrane (Schleicher & Schuell, Germany). After blocking with 2.5% skim milk (Merck, Germany), the membrane was incubated with culture supernatants containing the MAbs for 1.5 hr at 37 °C, followed by HRP-conjugated sheep anti-mouse Ig. The bands were finally visualized with Diaminobenzidine tetrahydrochloride (DAB) (Sigma) substrate.

Results

Screening and selection of specific hybridomas

Culture supernatants from growing hybridomas were screened by ELISA using a panel of four IgG myelomas with different subclasses, including their immunogens. Results obtained for these hybridomas with different specificity profiles are illustrated in Table 1.

Characterization of MAbs

Following cloning and subcloning, culture supernatant from the selected hybridomas was further characterized. Five MAbs belonged to IgG1, one MAb was IgG2a and the last one displayed IgG2b isotype (Table 2). Specificity

Table 1. Reactivity of selected MAbs with different human IgG subclasses

MAb	Antigen screening			
	OD (492 nm)			
	IgG1 (MM13)	IgG2 (MM12)	IgG3 (MM98)	IgG4 (MM147)
2F7G8	1.4	1.9	1.7	0.25
1F8G4	1.3	1.5	1.4	0.2
7F14F7	1.1	1.7	1.9	0.2
1F5A8	1.6	1.8	0.3	1.4
6F11E1	1.1	1.2	0.2	1.3
8F9G7	1.5	1.8	0.3	1.7
6F19C11	1.4	1.7	0.2	1.7
1F18A8	0.15	0.2	2.0	0.3
8F9A8	1.8	1.7	1.6	1.4
Anti IgG1(JL512)	1.2	0.27	0.3	0.27
Anti IgG2 (GOM2)	0.4	2.0	0.3	0.25
Anti IgG3(ZG4)	0.3	0.3	1.9	0.3
Anti IgG4(RJ4)	0.3	0.3	0.3	1.6

* Positive reactivity is shown in bold figures. 1F18A8 and 8F9A8 MAbs are IgG3 and Pan-IgG specific MAbs included as controls. Commercial IgG subclass monospecific MAbs, including JL512 (IgG1), GOM2 (IgG2), ZG4 (IgG3) and RJ4 (IgG4), are included as control MAbs for each IgG subclass

Table 2. Determination of the isotype of MAbs by ELISA

MAbs	Isotype					
	IgG1	IgG2a	IgG2b	IgG3	IgA	IgM
2F7G8	0.2	0.7	0.16	0.16	0.14	0.16
1F8G4	1	0.16	0.15	0.11	0.13	0.13
7F14F7	0.7	0.2	0.2	0.2	0.2	0.2
1F5A8	0.6	0.13	0.12	0.11	0.1	0.16
6F11E1	0.2	0.25	1	0.17	0.17	0.17
8F9G7	0.8	0.2	0.2	0.16	0.17	0.19
6F19C11	0.75	0.2	0.2	0.2	0.15	0.17
1F18A8	0.8	0.13	0.1	0.15	0.1	0.1
8F9A8	0.7	0.2	0.2	0.17	0.18	0.2
Control ⁻	0.16	0.14	0.1	0.15	0.1	0.16

The results represent OD values obtained at 492 nm. Control⁻: Culture supernatant from SP2/0 myeloma cells

of these MAbs was determined, using a panel of purified myeloma proteins, including IgG1 (n=9), IgG2 (n=4), IgG3 (n=7) and IgG4 (n=6) subclasses.

According to ELISA and immunoblotting studies, these seven MAbs can be categorized into two groups: 1) Four IgG1, 2, 4 specific MAbs of which three (1F5A8, 8F9G7 and 6F11E1), react with conformational epitopes located on heavy chain of IgG1, 2, 4 (Figure 1) and an unusual IgG3 myeloma protein (Goe) bearing allotypic marker of the Monoclonal populations [G3m(st)] displaying similar specificity to SPA. The fourth MAb (6F19C11) reacts with a linear epitope located on heavy chain of IgG1, 2, 4 subclasses. 2)

Three IgG1, 2, 3 specific MAbs of which two (2F7G8 and 1F8G4) react with linear epitopes and the third one (7F14F7) reacts with conformational epitope located on heavy chain of IgG1, 2, 3 subclasses (Figure 2). Representative immunoblotting results are illustrated in Figures 3 and 4. All the MAbs reacted only with Fc, but not Fab fragments of their immunogens (Figure 5).

Cross-reactivity studies employing whole sera from a range of animal species indicated the most abundant cross-reactivity (71.4%) with monkey Igs, while no cross-reactivity was detected with hen and cat sera. While 4 MAbs displayed a restricted pattern of cross-reactivity, two MAbs [8F9G7 (IgG1, 2, 4 specific) and 7F14F7 (IgG1, 2, 3 specific)] displayed a broad pattern of cross-reactivity and one MAb [2F7G8 (IgG1, 2, 3-specific)], did not bind to any of the animal sera tested (Table 3).

Determination of affinity constant of MAbs

Ascitic fluids of three MAbs were available for purification by affinity chromatography using Streptococcal protein G column. The affinity constant (K_{aff}) of these MAbs was determined by ELISA. Triple serial con-

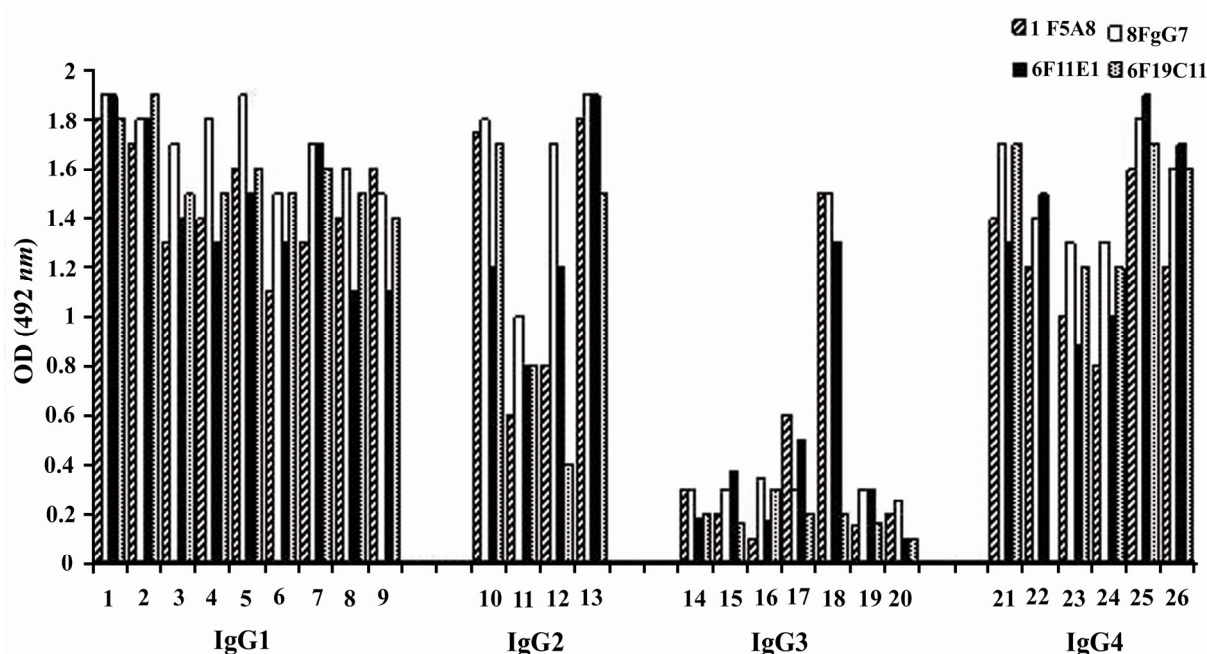


Figure 1. Reactivity of IgG1, 2, 4 specific MAbs with human IgG subclasses
Lanes 1-9, 10-13, 14-20 and 21-26 represent different IgG1, IgG2, IgG3 and IgG4 purified human myeloma proteins, respectively

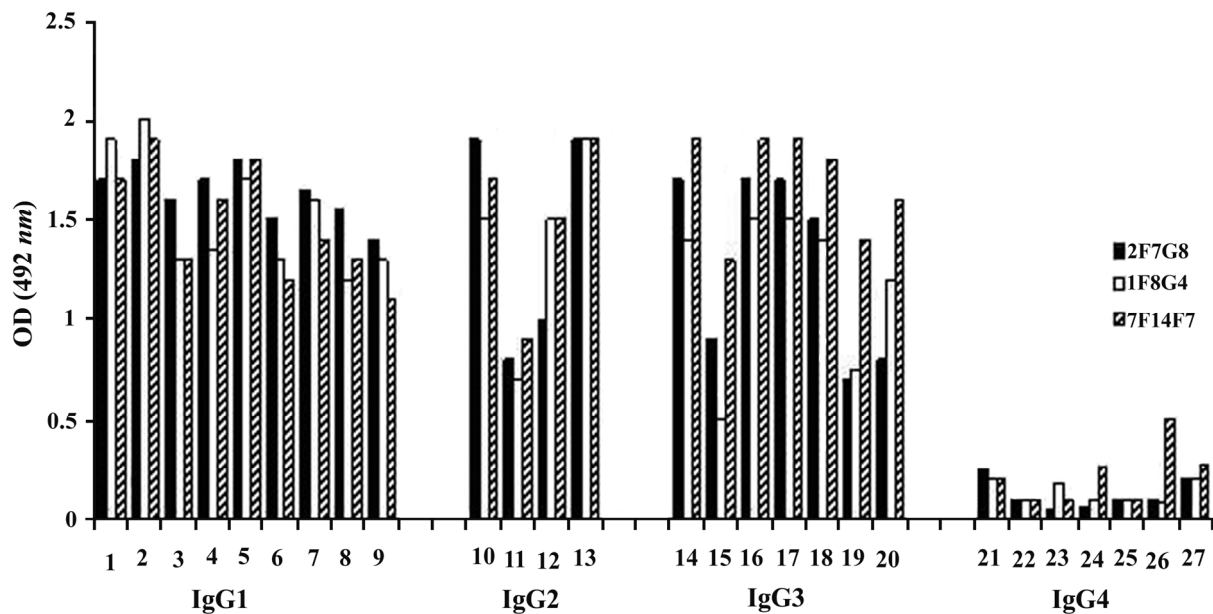


Figure 2. Reactivity of IgG1, 2, 3 specific MAb with human IgG subclasses
Lanes 1-9, 10-13, 14-20 and 21-27 represent different IgG1, IgG2, IgG3 and IgG4 purified human myeloma proteins, respectively

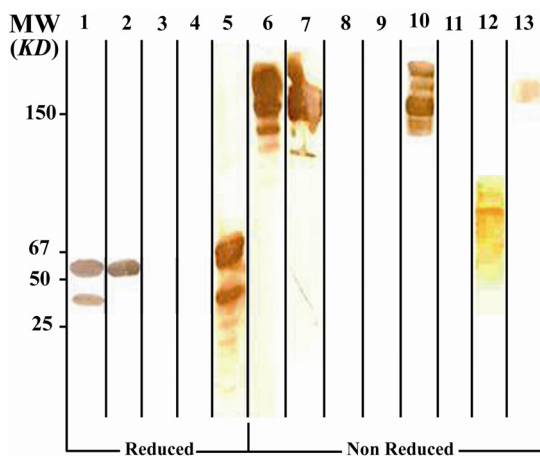


Figure 3. Immunoblot analysis of 6F19C11 MAb (anti-IgG1, 2, 4) reactivity with human IgG subclasses
Lanes 1 to 5 represent reduced forms of IgG1, IgG2, IgG3, IgG3 (Goe) and IgG4 paraproteins, respectively. Lanes 6 to 9 represent non reduced forms of the above mentioned proteins, respectively. Lanes 10 to 12 represent Whole molecule, Fab and Fc fragments of IgG4. Lane 13 represents polyclonal IgG3. * Fc fragments of IgG1 are contaminated with whole IgG molecule because of partial digestion

centrations of the antigens and MAbs were selected to construct the corresponding curves and to extrapolate the K_{aff} values using the formula given in Materials and Methods. The calculated average K_{aff} values are presented in Table 4.

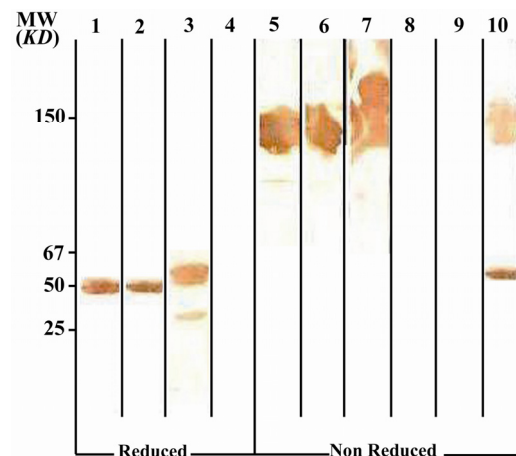


Figure 4. Immunoblot analysis of 2F7G8 MAb (anti-IgG1, 2, 3) reactivity with human IgG subclasses
Lanes 1 to 4 represent reduced forms of IgG1, IgG2, IgG3 and IgG4, respectively. Lanes 5 to 8 represent non reduced forms of the above mentioned proteins, respectively. Lanes 9 and 10 represents Fab and Fc fragments of IgG1, respectively

Discussion

Several anti-human IgG MAbs with specificity for Fc region-dependent epitopes common to three of four human IgG subclasses have been produced in the present study.

One group of our MAbs ($n=4$) was shown to be specific for isotypic epitopes common to IgG1, 2, 4, but not IgG3 subclasses. IgG3 subclass has different amino acid sequences at positions 276 and 291 in CH2 domain and

Table 3. Cross-reactivity of MAbs with animal sera

Animal sera	2F7G8	1F8G4	7F14F7	1F5A8	6F11E1	8F9G7	6F19C11
Monkey	<0.1	<0.1	30	92	40	102	74
Rabbit	<0.1	<0.1	<0.1	<0.1	<0.1	30	<0.1
Guinea Pig	<0.1	<0.1	19	<0.1	<0.1	<0.1	<0.1
Sheep	<0.1	<0.1	19	<0.1	<0.1	19	<0.1
Goat	<0.1	<0.1	27	<0.1	<0.1	17	<0.1
Cat	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Dog	<0.1	81	36	<0.1	<0.1	<0.1	<0.1
Horse	<0.1	<0.1	22	<0.1	<0.1	9	20
Hen	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

% Cross-reactivity is expressed relative to the value obtained for human pooled serum

positions 392, 422 and 435 in CH3 domain in comparison with other subclasses. In IgG1, 2 and 4 subclasses, these amino acids are Asn, Pro, Lys, Val and His, whereas in IgG3 subclass they include Lys, Leu, Asn, Ile and Arg, respectively ⁽²²⁾. We suggest that these four MAbs recognize epitope (s) containing the differentiating amino acids located in CH2 and/ or CH3 domains common to IgG1, 2 and 4 subclasses, but not CH1 domain, because our results showed no reactivity of these MAbs with Fab fragments of IgG1 molecules (Figures 3 and 5). MAbs with similar specificity have already been reported (HP6032, HP6033 ⁽²³⁾; QF1, WC2, WC9 ⁽²⁴⁾ and IG12 ⁽²⁵⁾).

In contrast to HP6032 MAb, HP6033 showed also reactivity with IgG3 myeloma proteins having G3m (g) allotype and with one of two IgG3 myeloma proteins having G3m (b) allotype ⁽²³⁾. These two MAbs were

evaluated only by Immunofluorometric assay (IFMA) ⁽²³⁾ and comparative binding study with SPA has not been conducted.

IG12 MAb reacts with a heat sensitive conformational epitope close to the hinge region of IgG and did not compete with Staphylococcus protein A (SPA) for IgG binding. Furthermore, the affinity constants of the previously produced anti IgG1, 2, 4 MAbs have not been calculated. Three of our anti IgG1, 2, 4 MAbs (1F5A8, 6F11E1 and 8F9G7), recognize conformational epitopes located to heavy chain of IgG1, 2, 4 but not IgG3 subclasses and a protein of IgG3 subclass (Goe protein) (Figure 1), a reactivity pattern similar to SPA ⁽²¹⁾.

The sequence of protein Goe resembled that of other γ_3 molecules, except for the presence of tyrosine at position 296, alanine at position 339 and histidine and tyrosine at positions 435 and 436 ⁽²⁶⁾. The only amino acid which is common between Goe protein and IgG1, 2 and 4 subclasses and is different between Goe protein and other IgG3 molecules, is histidine at position 435. Thus, it appears that histidine 435 plays an important role in binding to 1F5A8, 6F11E1 and 8F9G7 MAbs produced in present study. Reactivity of these monoclonal antibodies with polyclonal IgG subclasses was found to be similar to SPA.

It was previously shown that histidine 435 has a major role in binding to SPA ⁽²⁷⁾. In the crystal structure of the complex between

Table 4. Affinity constants of human IgG subclass-restricted MAbs determined by ELISA

<i>MAbs</i>	[Ag] (<i>ng/ml</i>)	OD-50 [*]	[Ab] at OD-50 (<i>ng/ml</i>)	K _{aff} (<i>M⁻¹</i>)	Average K _{aff} (<i>M⁻¹</i>)± SD
1F5A8					
	5000	1	760	3.1 × 10 ⁹	2.8 × 10 ⁸
	2500	0.9	860	3.2 × 10 ⁹	
	1250	0.7	900	2 × 10 ⁸	
	625	0.55	1300		
6F11E1					
	20000	0.65	480	4.69 × 10 ⁸	4.64 × 10 ⁸
	10000	0.6	560	4.54 × 10 ⁸	
	5000	0.46	600	4.69 × 10 ⁸	
	2500	0.28	620		
7F14F7					
	2000	0.97	46	4.5 × 10 ⁹	2 × 10 ⁹
	1000	0.9	56	0.94 × 10 ⁹	
	500	0.75	190	0.75 × 10 ⁹	
	250	0.5	300		

*OD-50 represents the half maximum optical density obtained for a given concentration of h-IgG ([Ag]) and the corresponding MAb ([Ab]). The affinity constant (K_{aff}) for each selected concentration of Ag and Ab was determined using the formula described in the Materials and Methods

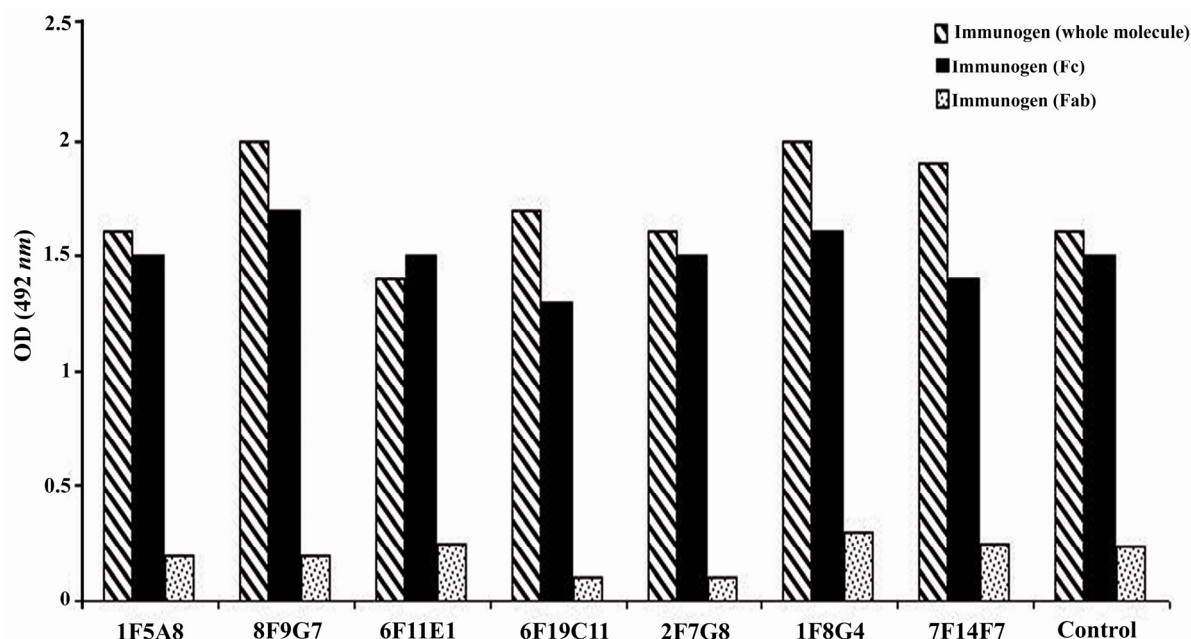


Figure 5. Reactivity of IgG1, 2, 4 and IgG1, 2, 3 specific MAbs with enzymatic fragments of human IgG

* Control represents a mouse MAb (8a4) with pan-IgG specificity

IgG1-Fc and domain B of SPA⁽²⁸⁾, it was revealed that His 435 is situated at the CH2-CH3 domain interface and is in contact with SPA. In IgG3 proteins from Caucasian populations the protein A contact residue is replaced by Arg. This lengthy side chain prevents the formation of favorable IgG-SPA contact, so that such IgG3 proteins do not bind protein A⁽²⁹⁾.

Since three of our MAbs (1F5A8, 6F11E1 and 8F9G7) displayed similar binding activity to SPA with regard to interaction with purified human myeloma proteins of IgG1, 2 and 4 and the IgG3 myeloma protein Goe, as well as human polyclonal IgG1, 2 and 4, but not IgG3 subclasses (Figure 1), the specificity of these MAbs resembled that of SPA. Our proposition is further supported by the fact that our MAbs only reacted with the unreduced form of IgG1, 2 and 4 subclasses and the Goe protein by immunoblotting (Figure 3). These results indicate that the epitopes recognized by these MAbs are conformational, depending on covalent interaction of the two heavy chains of IgG molecule.

The fourth MAb (6F19C11) in this category is completely different from the others as it does not recognize any protein of IgG3 subclass and it recognizes a linear epitope located to IgG1, 2, 4 subclasses (Figure 3).

The second group of our MAbs (three MAbs) was shown to be specific for isotypic epitopes common to IgG1, 2, 3, but not IgG4 subclasses (Figure 2). IgG4 subclass has different amino acid sequences at positions 268, 330 and 331 in CH2 domain and positions 355, 409, 419 and 445 in CH3 domain. In IgG1, 2 and 3 subclasses, these amino acids are His, Ala, Pro, Arg, Lys, Gln and Pro, whereas in IgG4 subclasses they include Gln, Ser, Ser, Gln, Arg, Glu and Leu, respectively⁽²³⁾. It seems that these three MAbs, recognize epitope (s) containing the differentiating amino acids located in CH2 and/ or CH3 domains common to IgG1, 2 and 3 subclasses, because they did not bind to the Fab fragment of IgG (Figure 5). It was shown by immunoblotting that two of these MAbs (2F7G8 and 1F8G4) recognize linear epitopes and the third one (7F14F7) recognizes a conformational epitope located to IgG1, 2, 3, but not IgG4 subclasses. Representative data is shown in Figure 4.

A number of MAbs [(HP6034 and HP6058)⁽²³⁾, (Iq1, X3a8 and 3e 10)⁽²⁴⁾ and (IG14 and IG15)]⁽²⁵⁾, have been produced with specificity similar to our MAbs. HP6034 and HP6058 recognize epitopes located to hinge⁽²³⁾ and CH2 domains of IgG-Fc, respectively⁽³⁰⁾. The affinity constant of HP6058 has been

reported to be about $2 \times 10^8 M^{-1}$ (30). IG14 and IG15 recognize conformational epitope (s) located to Fc region of IgG1, 2, 3 subclasses (25). The reported MAbs belonged to IgG1 subclasses. Two of our IgG1, 2, 3 specific MAbs (1F8G4 and 7F14F7) belonged to IgG1 and one of them (2F7G8) belonged to IgG2a subclass. The affinity constant of one of our MAbs (7F14F7) was found to be $2 \times 10^9 M^{-1}$.

The MAbs produced in this study displayed different profiles of cross-reactivity with a variety of animal sera. The most abundant cross-reactivity (71.4%) was observed with monkey Ig, while no cross-reactivity was detected with hen and cat sera. Cross-reactivity of anti human IgG MAbs have been reported by other investigators (23, 25). Most of our MAbs displayed a restricted pattern of cross-reactivity and one of them (2F7G8) did not bind to any of the animal sera tested. This is in agreement with previous reports (23), which showed anti IgG isotype specific MAbs cross-react more commonly with IgG of other animal species, due to the amino acid sequence homology between IgG molecules of human and other animals.

In conclusion, we produced and characterized seven MAbs each recognizing triple subclasses of human IgG. These MAbs are valuable tools for purification of the non-reactive IgG subclass by negative affinity chromatography. Combination of such MAbs with different specificities may improve the sensitivity of immunoassays, as they complement each other via reactivity to different epitopes located to the same IgG. These MAbs could also be used for epitope mapping of the Fc region of IgG and for serological phylogenetic studies.

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References

1. Hajighasemi F, Khoshnoodi J, Shokri F. Development of two murine monoclonal antibodies recognizing human nG1m(a)-like isoallotypic markers. *Hybridoma* 2008;27(6):473-479.
2. Hussain A, Pankhurst T, Goodall M, Colman R, Jefferis R, Savage CO, et al. Chimeric IgG4 PR3-ANCA induces selective inflammatory responses from neutrophils through engagement of Fcγ receptors. *Immunology* 2009;128:236-244.
3. Bartel G, Wahrmann M, Exner M, Regele H, Schillinger M, Horl WH, et al. Determinants of the complement-fixing ability of recipient presensitization against HLA antigens. *Transplantation* 2007;83(6):727-733.
4. Rynnel-dagoo B, Freud A, Hammarstrom L, Oxelius V, Persson MA, Smith CI. Pneumococcal antibodies of different immunoglobulin subclasses in normal and IgG subclass deficient individuals of various ages. *Acta Otolaryngol* 1986;101(1-2):146-151.
5. Soderstrom T, Enskog A, Samuelsson BE, Cedergren B. Immunoglobulin subclass (IgG3) restriction of anti-P and anti-Pk antibodies in patients of the rare p blood group. *J Immunol* 1985;134(1):1-3.
6. Saha S, Mazumdar T, Anam K, Ravindran R, Bairagi B, Saha B, et al. Leishmania promastigote membrane antigen-based enzyme-linked immunosorbent assay and immunoblotting for differential diagnosis of Indian post-kala-azar dermal leishmaniasis. *J Clin Microbiol* 2005;43(3):1269-1277.
7. Beenhouwer DO, Yoo EM, Lai CW, Rocha MA, Morrison SL. Human immunoglobulin G2 (IgG2) and IgG4, but not IgG1 or IgG3, protect mice against *Cryptococcus neoformans* infection. *Infect Immun* 2007;75(3):1424-1435.
8. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 2006;313(5787):670-673.
9. Abd El-Aal AA, El-Arousy MH, El-Gendy AM, Tunkul Ael-K, Ismail SA, El-Badry AA. Early post-treatment immunoglobulin profile in human schistosomiasis. *J Egypt Soc Parasitol* 2005;35(1):167-180.
10. Tangteerawatana P, Montgomery SM, Perlmann H, Looareesuwan S, Troye-Blomberg M, Khusmith S. Differential regulation of IgG subclasses and IgE antimalarial antibody responses in complicated and uncomplicated *Plasmodium falciparum* malaria. *Parasite Immunol* 2007;29:475-483.
11. Suresh L, Kumar V. Significance of IgG4 in the diagnosis of mucous membrane pemphigoid. *Oral*

- Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104(3):359-362.
12. Windle HJ, Ang YS, Athie-Morales V, McManus R, Kelleher D. Human peripheral and gastric lymphocyte responses to *Helicobacter pylori* NapA and AphC differ in infected and uninfected individuals. *Gut* 2005;54(1):25-32.
 13. Morais CG, Soares IS, Carvalho LH, Fontes CJ, Krettli AU, Braga EM. IgG isotype to C-terminal 19 kDa of *Plasmodium vivax* merozoite surface protein 1 among subjects with different levels of exposure to malaria in Brazil. *Parasitol Res* 2005; 95(6):420-426.
 14. Chevrier MC, Chateaneuf I, Guerin M, Lemieux R. Sensitive detection of human IgG in ELISA using a monoclonal anti-IgG-peroxidase conjugate. *Hybrid Hybridomics* 2004;23(6):362-367.
 15. Burton DR, Gregory L, Jefferis R. Aspects of the molecular structure of IgG subclasses. *Monogr Allergy* 1986;19:7-35.
 16. Hajighasemi F, Shokri F. Generation and characterization of mouse hybridomas secreting monoclonal antibodies specific for human IgG3. *AJMB* 2009;1(1):19-26.
 17. Loirat MJ, Gourbil A, Frioux Y, Muller JY, Blanchard D. A murine monoclonal antibody directed against the Gerbich 3 blood group antigen. *Vox Sang* 1992;62(1):45-48.
 18. Hajighasemi F, Gharagozlou S, Ghods R, Khoshnoodi J, Shokri F. Private idiotypes located on light and heavy chains of human myeloma proteins characterized by monoclonal antibodies. *Hybridoma* 2006;25(6):329-335.
 19. Hajighasemi F, Saboor-Yaraghi AA, Shokri F. Measurement of affinity constant of anti-human IgG monoclonal antibodies by an Elisa-based method. *Iranian J Immunol* 2004;1(3):154-161.
 20. Beatty JD, Beatty BG, Vlahos WG. Measurement of monoclonal antibody affinity by noncompetitive enzyme immunoassay. *J Immunol Meth* 1987;100 (1-2):173-179.
 21. Hajighasemi F, Gharagozlou S, Ghods R, Roohi A, Khoshnoodi J, Shokri F. Generation and characterization of a mouse monoclonal antibody with specificity similar to staphylococcal protein A (SPA). *Hybrid Hybridomics* 2003;22(1):33-39.
 22. Jefferis R, Lowe J, Ling NR, Porter P, Senior S. Immunogenic and antigenic epitopes of immunoglobulins. I. Cross-reactivity of murine monoclonal antibodies to human IgG with the immunoglobulins of certain animal species. *Immunology* 1982;45(1):71-77.
 23. Reimer CB, Phillips DJ, Aloisio CH, Moore DD, Galland GG, Wells TW, et al. Evaluation of thirty-one mouse monoclonal antibodies to human IgG epitopes. *Hybridoma* 1984;3(3):263-275.
 24. Lowe J, Bird P, Hardie D, Jefferis R, Ling NR. Monoclonal antibodies (McAbs) to determinants on human gamma chains: Properties of antibodies showing subclass restriction or subclass specificity. *Immunology* 1982;47(2):329-336.
 25. Girkontaite I, Leckiene M, Mauricas M. Immunochemical study of human immunoglobulin G Fc region. *Cancer Biother Radiopharm* 1996;11(1): 87-96.
 26. Recht B, Frangione B, Franklin E, Van LE. Structural studies of a human $\gamma 3$ myeloma protein (Goe) that binds Staphylococcal protein A. *J Immunol* 1981;127(3):917-923.
 27. Boyle MDP. Bacterial immunoglobulin-binding proteins (Vol 1). San Diego: Academic press; 1990,17-28.
 28. Eliasson M, Olsson A, Palmcrantz E, Wiberg K, Inganäs M, Guss B, et al. Chimeric IgG-binding receptors engineered from staphylococcal protein A and streptococcal protein G. *J Biol Chem* 1988; 263:4323-4327.
 29. Burton DR, Gregory L, Jefferis R. Aspects of the molecular structure of IgG subclasses. *Monogr Allergy* 1986;19:7-35.
 30. Hamilton RG, Morrison SL. Epitope mapping of human immunoglobulin-specific murine monoclonal antibodies with domain-switched, deleted and point-mutated chimeric antibodies. *J Immunol Meth* 1993;158(1):107-122.