

Key words: HSV-1/IgG subclasses/immune response

## Murine IgG Subclass Responses to Herpes Simplex Virus Type 1 and Polypeptides

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(Accepted 21 December 1987)

### SUMMARY

The antibody response to herpes simplex virus (HSV) is complex and involves antibody to at least 33 virus-induced polypeptides. Serum IgG contains four isotypes in mice and it is known that the isotypes differ in their biological functions and that individual antigenic proteins may preferentially elicit restricted isotype responses. We therefore examined the anti-polypeptide isotypes induced in immune mouse serum. By ELISA, we found that the total serum virus-specific antibody activity was 51% IgG1, 39% IgG2a, 11% IgG2b and 1% IgG3 in immune ICR strain mice and 51%, 45%, 4% and 0.4% respectively in strain BALB/c mouse immune serum. These proportions are significantly different from those reported for other virus infections. Sepharose-Protein A affinity-purified isotypes were also studied and showed IgG1 > IgG2a ≥ IgG2b > IgG3 activity per µg of isotype, indicating that competition between isotypes present in high concentrations did not significantly alter the results. Immunoblotting studies of the purified isotypes showed that the major immunogenic HSV-1 proteins (VP155, gC, gB, pgB, gD and nucleocapsid proteins 42K and 44K) induced all isotypes. However, the isotype responses were not uniform among the glycoproteins and some other proteins. In addition neutralization assays of the purified isotypes indicated that IgG2a and IgG2b had significantly greater neutralizing capacity than IgG1, suggesting that less of the IgG1 was directed against neutralizing virion epitopes. These data are discussed with respect to the biological implications in host defence.

### INTRODUCTION

In recent years there has been increasing recognition that the IgG isotype response differs with different antigens. Generally polysaccharides such as pneumococcal (Barrett & Ayoub, 1986) or meningococcal type A (Rautonen *et al.*, 1986) antigens when used in vaccines produce an IgG2 response in humans, an IgG2c response in rats (Der Balian *et al.*, 1980) and an IgG3 response in mice (Perlmutter *et al.*, 1978). Meningococcal group A polysaccharide vaccine and many others also produce a significant IgG1 human response (Rautonen *et al.*, 1986; Hammarstrom & Smith, 1986). Some protein antigens such as tetanus toxoid (Yount *et al.*, 1968; Stevens *et al.*, 1983; Barrett & Ayoub, 1986) induce an isotype response restricted to IgG1 in humans. Many other bacterial proteins induce antibody primarily of the human IgG1 class (Hammarstrom & Smith, 1986). Streptococcal M protein, an exception, induces primarily an IgG3 response in humans (Mortimer & Widdowson, 1979). Mouse class I major histocompatibility complex antigens of the H-2K subregion induce both IgG1 and IgG2 isotypes but the H-2D subregion induces primarily IgG2 (Tartakovsky *et al.*, 1983). In mice infected with *Trichinella spiralis* two surface antigens of the organism induce a predominant IgG1 response but a third surface antigen induces primarily IgG2 (Almond & Parkhouse, 1986). Serum antibody to viruses in humans appears to be predominantly in the IgG1 > IgG3 subclass isotypes (Skvaril, 1986). For herpes simplex virus (HSV) one laboratory reports antiviral activity predominantly in the human IgG3 isotype (Beck, 1981); another laboratory, however, reports that IgG1

predominates and IgG3  $\gg$  IgG2 and IgG4 (Linde *et al.*, 1983; Gilljam *et al.*, 1985). Thus there is ample evidence that the isotype response is frequently restricted among both protein and carbohydrate antigens and the nature of the restriction may vary between antigens.

Most studies of the viruses have used ELISA to detect antiviral isotypes and have not related the isotype response to individual virus polypeptides or to subsets of viral proteins, such as those which are the targets of neutralizing antibody. We studied the isotype composition of the antiviral response to HSV-1 antigens, the isotype response to individual polypeptides and the neutralizing activity levels found in purified IgG1, IgG2a and IgG2b subclasses from immune mice. We report that the virus-specific activity per  $\mu$ g of isotype was IgG1 > IgG2a  $\geq$  IgG2b  $\gg$  IgG3 in immune serum from both ICR and BALB/c mice. We have shown by immunoblotting that all IgG isotypes recognized all the major HSV-1 proteins present in lysates from infected cells but many anti-polypeptide responses were not uniform among the isotypes. The IgG2a and IgG2b isotypes had significantly greater neutralizing capacity per  $\mu$ g than did the IgG1 isotype in immune IgG from both ICR and BALB/c mice.

### METHODS

**Virus.** An HSV isolate previously described and characterized as HSV-1 (McKendall, 1983), was used to prepare a virus stock in human embryonic lung fibroblasts (MRC-5). The virus stock contained  $10^8$  p.f.u./ml and was the eighth passage of the original isolate.

**Mice and immunization.** Strain BALB/c (Simonsen, Gilroy, Ca., U.S.A.) or ICR (Harlan-Sprague, Houston, Tex., U.S.A.) 4-week-old mice were used. Non-immune serum was obtained by pooling serum from 10 sacrificed mice of each strain. Mice were immunized with an initial dose of  $10^5$  p.f.u. HSV-1 by the footpad, followed by  $10^6$  p.f.u. given intraperitoneally (i.p.) 3 weeks later. At age 10 weeks, a third dose of  $10^7$  p.f.u. was given i.p. and 3 weeks later sera from 10 to 20 mice of each strain were pooled.

**Quantification of mouse IgG isotypes by ELISA.** Polyvinylchloride microtitre plates were coated with affinity-purified goat anti-mouse IgG subclass-specific reagents (Southern Biotechnology Associates, Birmingham, Ala., U.S.A.). Purified mouse myeloma subclasses were added at appropriate dilutions to create standard curves for IgG1, IgG2a, IgG2b and IgG3. Sepharose-Protein A column fractions of purified IgG subclasses or unfractionated sera were added to triplicate wells using appropriate serial dilutions. The second antibody was  $\beta$ -galactosidase-labelled goat anti-mouse Ig (Southern Biotechnology Associates) at a 1:150 dilution. The substrate, *o*-nitrophenyl- $\beta$ -D-galactopyranoside, was used at 1.0 mg/ml. Absorbance was read at 405 nm. The concentration of each isotype was determined from the linear portion of the curves for both the specimens and the standards.

**Fractionation of mouse serum into IgG isotypes by Sepharose-Protein A affinity chromatography.** The procedure used the method of Ey *et al.* (1978). Serum samples were delipidated by passage through Sephadex G-25. The serum was then adjusted to pH 8.0 and applied to the column. IgG1 fraction a was first eluted with 0.1 M-citrate buffer pH 6.0. IgG2a and IgG2b (fractions b and c) were eluted with 0.1 M-citrate buffer pH 4.5 and pH 3.5 respectively. In some experiments elutions using citrate buffer at other pHs were employed. Column recoveries of isotypes ranged from 55% to 83%.

**Determination of virus-specific antibody activity in IgG isotypes by ELISA.** Polyvinylchloride plates were coated with HSV-1 antigens obtained by freezing and thawing heavily infected cell cultures. Immune sera or purified Ig subclasses at various dilutions were added to triplicate wells. After washing, affinity-purified goat anti-mouse IgG subclass-specific antisera (Southern Biotechnology Associates) were added. Each subclass-specific antiserum was used at a dilution providing optimum specific binding over the background binding level. Optimum dilutions were 1:142 for anti-IgG1 and anti-IgG3 and 1:100 for anti-IgG2a and anti-IgG2b. After washing, affinity-purified biotinylated donkey anti-goat IgG (Bethesda Research Laboratories) was added to wells at a 1:200 dilution.  $\beta$ -galactosidase-conjugated streptavidin was then added at 1:100 dilution. The substrate employed was *o*-nitrophenyl- $\beta$ -D-galactopyranoside. The absorbance was read at 405 nm. The endpoint was defined as the isotype concentration that produced a mean absorbance of 0.2 above background in triplicate or quadruplicate wells.

**SDS-PAGE and Western blotting of HSV-1 polypeptides.** SDS-PAGE was performed according to the standard procedure of Laemmli (1970). An 8.5% resolving gel cross-linked with diallyltartardiamide and a stacking gel of 3.5% bisacrylamide were employed. HSV-1-infected cell lysate proteins were loaded at 11  $\mu$ g/4 mm lane width in gels 0.75 mm thick and 14 cm long. Proteins were identified on the basis of their SDS-PAGE migration relative to known  $M_r$  markers, and for glycoproteins, by comparison with lanes containing [ $^{14}$ C]glucosamine-labelled infected cell lysate proteins (McKendall *et al.*, 1988a). Proteins were transferred to nitrocellulose membranes as described by Towbin *et al.* (1979), at 25 V, 0.2 A overnight. Optimal time and current were determined as previously reported (McKendall *et al.*, 1988a).

*Immunoblotting of Sepharose-Protein A-purified mouse IgG subclasses and immune serum to identify antibody to HSV-1 polypeptides.* The Western blots containing HSV-1 proteins were cut into strips and the strips were overlaid with phosphate-buffered saline (PBS) pH 7.22, containing 3% (w/v) bovine serum albumin (BSA), 0.02% (w/v) sodium azide and 0.5% (v/v) Triton X-100 (blocking buffer). After incubation for 2 h at 37 °C blocking buffer was removed and dilutions of immune mouse serum or purified IgG subclasses added to each trough and allowed to react for 1 h at 37 °C. The strips were then washed three times in PBS containing 1% (w/v) BSA, 0.02% (w/v) sodium azide and 0.5% (v/v) Triton X-100. For immunoblotting of purified IgG subclasses, each strip was incubated with 0.045 µCi of <sup>125</sup>I-labelled anti-mouse Ig (approximately 9 mg/ml) for 1 h at 37 °C.

For immunoblotting of unfractionated immune mouse serum, the following sequence of steps was used. First goat anti-mouse IgG subclasses at appropriate dilutions were added and incubated for 1 h at 37 °C. Then strips were washed with wash buffer six times and a 1:1000 dilution of affinity-purified rabbit anti-goat Ig (Zymed, Burlingame, Ca., U.S.A.) was applied. Finally, after 1 h incubation at 37 °C, 0.045 µCi of <sup>125</sup>I-labelled donkey anti-rabbit IgG (Amersham) in blocking buffer was added to each strip and incubated for 1 h at 37 °C. Strips were washed six times with PBS containing 0.5% (v/v) Triton X-100. Subsequently the strips were air-dried and autoradiography was performed by placing the strips against Kodak X-Omat XAR film in a Dupont Cronex cassette with an image intensifying screen. Exposures of 24 h to 12 days were obtained.

*Neutralization assay.* Neutralizing activity was determined with a standard microtitre plaque assay using rabbit skin cell monolayers and 0.5% methylcellulose. The cell sheets were stained with 1% crystal violet. Non-immune and immune serum controls were always included. The endpoint was defined as the concentration of IgG subclass that produced a 50% reduction in mean plaques per well compared to virus controls, in triplicate samples.

## RESULTS

### *Specificity of anti-mouse IgG subclass reagents*

To determine whether each anti-subclass antibody had any cross-reactivity for the heterologous subclasses, we searched for binding of each anti-subclass antibody to mouse myeloma proteins. Microtitre wells were coated with goat anti-IgG1, -IgG2a, -IgG2b or -IgG3. Dilutions of mouse myeloma proteins were then applied at concentrations ranging from 3.12 to 0.10 µg/ml. The myeloma proteins were then detected using a β-galactosidase-conjugated goat anti-mouse Ig. Figure 1 shows that each anti-mouse subclass antibody was specific for its respective isotype. No cross-reactivity with other isotypes was observed.

### *Quantification of IgG subclasses in immune mouse serum and in purified IgG subclasses obtained by Sepharose-Protein A affinity chromatography*

The serum concentration varies considerably among IgG isotypes and their concentrations in mice may vary with a number of factors including age (Natsuume-Sakai *et al.*, 1977; Lee *et al.*, 1986). Therefore we first quantified the serum concentration of each isotype in immune ICR and BALB/c mouse serum as well as in pooled serum from 10 unimmunized littermates (Table 1). IgG1 and IgG2a were the isotypes highest in concentration in both immune ICR and BALB/c serum. In contrast to immune sera, non-immune serum had significantly lower total IgG concentrations, probably reflecting the younger age of the mice as well as their non-immune status. Notably IgG1, which was the dominant subclass in the immune sera, had low concentrations in the non-immune sera at 0.56 mg/ml (ICR mice) and 0.28 mg/ml (BALB/c mice). Overall then, comparing immune and non-immune mice, the higher concentrations observed in the immune mice were due primarily to increases in the IgG1 and IgG2a isotypes. These findings paralleled the prominence of the anti-HSV activity seen in the IgG1 and IgG2a isotypes.

Table 1 also shows the isotype concentrations found in the eluates from the Sepharose-Protein A column chromatography. Fraction a (eluted at pH 6.0) from both BALB/c and ICR immune serum was 100% IgG1. ICR fraction b (eluted at pH 4.5) was 94% IgG2a and 6% IgG3, and ICR fraction c (eluted at pH 3.5) was 100% IgG2b. BALB/c fraction b was 74% IgG2a with 4% to 15% contamination by other isotypes while fraction c was 70% IgG2b. Thus the ICR fractions for immune IgG1, IgG2a and IgG2b were virtually pure, as was the immune BALB/c IgG1 fraction.

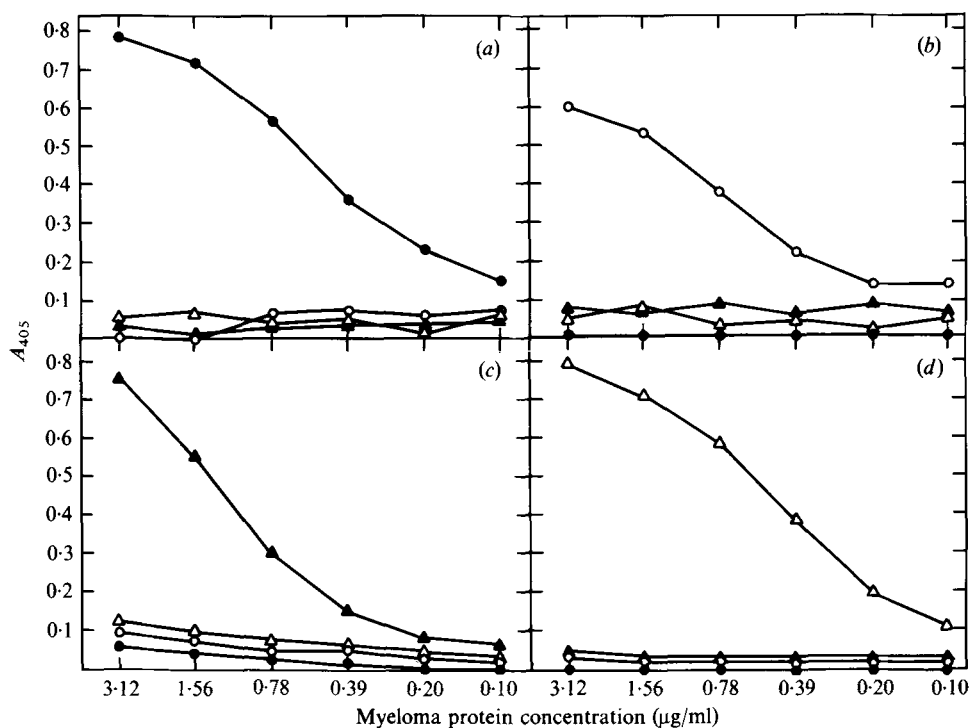


Fig. 1. Determination of specificity of goat anti-mouse IgG subclass reagents. Wells were coated with goat anti-IgG1 (a), anti-IgG2a (b), anti-IgG2b (c) or anti-IgG3 (d) antiserum. Various dilutions of mouse myeloma IgG1 (●), IgG2a (○), IgG2b (▲) or IgG3 (△) were added to each set of wells. To detect binding of the different myelomas,  $\beta$ -galactosidase-conjugated goat Ig raised against mouse heavy and light Ig chains was added. Each myeloma subclass bound only to wells coated with the appropriate goat anti-subclass antibody.

Table 1. Subclass composition in immune and non-immune serum and in column fractions from Sepharose-Protein A affinity-purified immune sera

Specimen	Concentration (mg/ml)*				
	IgG1	IgG2a	IgG2b	IgG3	Total
ICR immune serum	3.60 (41%)	3.20 (37%)	1.60 (18%)	0.30 (3%)	8.70
ICR immune column fraction					
a	4.97 (100%)	0	0	0	4.97
b	0	2.00 (94%)	0	0.13 (6%)	2.13
c	0	0	1.80 (100%)	0	1.80
ICR non-immune serum	0.56 (13%)	2.12 (50%)	1.18 (20%)	0.38 (9%)	4.24
BALB/c immune serum	3.74 (45%)	3.12 (38%)	0.94 (11%)	0.47 (6%)	8.27
BALB/c immune column fraction					
a	1.87 (100%)	0	0	0	1.87
b	0.84 (15%)	4.23 (74%)	0.40 (7%)	0.24 (4%)	5.70
c	0.15 (17%)	0.12 (13%)	0.63 (70%)	0	0.90
BALB/c non-immune serum	0.28 (17%)	0.64 (39%)	0.48 (29%)	0.26 (16%)	1.66

\* Standard deviations were generally 10% or less of the mean and were never greater than 15%. The figures in parentheses represent the isotype concentration expressed as a percentage of the total IgG concentration in each specimen.

Table 2. *Virus-specific antibody activity in immune serum and in purified subclasses*

	Concentration of IgG isotype at ELISA endpoint ( $\mu\text{g/ml}$ )*			
	IgG1	IgG2a	IgG2b	IgG3
ICR immune serum	$0.28 \pm 0.03^\dagger$	$0.32 \pm 0.08$	$0.56 \pm 0.09$	$1.26 \pm 0.25$
purified ICR subclasses	$0.25 \pm 0.03$	$0.58 \pm 0.19$	$0.61 \pm 0.08$	$1.09 \pm 0.16$
BALB/c immune serum	$0.85 \pm 0.08$	$0.81 \pm 0.08$	$2.80 \pm 0.36$	$13.8 \pm 2.1$
purified BALB/c subclasses	$0.51 \pm 0.08$	$1.00 \pm 0.08$	$1.12 \pm 0.22$	$< 75.0 \pm 11.2$

\* Endpoint concentration of each IgG subclass ( $\mu\text{g/ml}$ ) was defined as the concentration at which the specimen dilution curve intersected the absorbance line of 0.2 above background.

† Mean in triplicate samples  $\pm$  standard deviation.

*Virus-specific antibody activity detected by ELISA in IgG subclasses of immune ICR and BALB/c serum and in purified IgG subclasses*

The goat anti-mouse subclass reagents were assumed to have different binding affinities for their respective isotypes. Therefore to compare the virus-specific antibody activity in the isotypes of immune serum, it was first necessary to correct for these affinity differences. To do this we coated wells with myeloma isotypes at various dilutions. Each goat anti-mouse IgG subclass reagent was then added at a dilution that optimized the signal to background ratio (see Methods). Then biotin-labelled donkey anti-goat IgG was added, followed by  $\beta$ -galactosidase-streptavidin conjugate. The concentrations of myeloma protein that produced an absorbance of 0.2 were 150 ng/ml (IgG2a), 115 ng/ml (IgG2b) and 62 ng/ml (IgG1 and IgG3). These differences reflect the different potencies of each goat anti-subclass reagent for each respective isotype. Therefore, the apparent IgG2a and IgG2b endpoints for the virus-specific antibody ELISA were adjusted by factors of 2.4 and 1.85, respectively, to compensate for their lower affinity compared to the IgG1 and IgG3 reagents. In the HSV-specific ELISA all endpoint concentrations were read at absorbances of 0.2.

To determine the virus-specific antibody activity in each isotype of immune serum, wells were coated with HSV antigen. Dilutions of immune serum were added in order to study each isotope at concentrations ranging from 100 to 0.075  $\mu\text{g/ml}$  in serial twofold dilutions. The endpoint was defined as the lowest IgG isotype concentration that produced an absorbance of 0.2 above background. Table 2 shows that IgG1 and IgG2a had the most virus-specific activity, requiring only 0.28 and 0.32  $\mu\text{g/ml}$  to be detectable in ICR serum. ICR IgG2b and especially IgG3 had significantly less virus-specific activity, requiring 0.56 and 1.26  $\mu\text{g/ml}$  to be detectable. The same pattern of activity was seen in BALB/c immune serum, although all isotypes had less virus-specific activity than the respective isotypes in the ICR serum.

The presence of multiple subclasses in unfractionated immune serum could lead to competition for antigenic sites (Djurup & Weeke, 1986). This competition could influence the apparent titres of the scarcer subclasses, particularly IgG2b and IgG3. To circumvent this, we studied the virus-specific antibody activity of purified subclasses obtained from Sepharose Protein A-chromatography. A similar pattern of results was seen (Table 2) with the activity greatest in IgG1, intermediate in IgG2a and IgG2b isotypes and least in the IgG3 isotype. For the ICR specimens there was excellent agreement between the values obtained for whole serum and purified subclasses. For the BALB/c IgG2b column fraction (from fraction c, Table 1) the endpoint was detected at  $1.12 \pm 0.22$   $\mu\text{g/ml}$  compared with  $2.80 \pm 0.36$   $\mu\text{g/ml}$  in the serum. For IgG2b therefore the serum measurements may have somewhat underestimated the antiviral activity. There was also more IgG3 antiviral activity ( $P < 0.05$ ) in the BALB/c serum compared to the BALB/c fraction b (from Table 1). The basis of this is not clear since the column fraction was not significantly enriched for IgG3 compared with the serum. Possibly the purification caused some denaturation of the isotype, but no such denaturation was seen in the ICR IgG3.

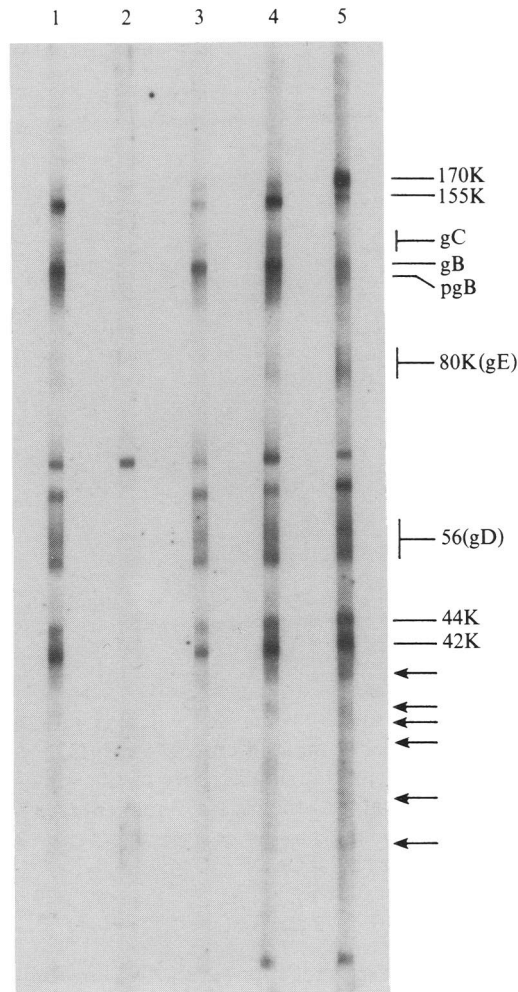


Fig. 2. Autoradiograph of immunoblot strips cut from a Western blot containing HSV-1 polypeptides. Strips were probed with affinity-purified IgG1 (lane 3), IgG2a (lane 4) and IgG2b (lane 5) from immune ICR mouse serum. Major bands of reactivity are designated by name or  $M_r$ . Minor bands are indicated by arrows (see text). Controls included unfractionated immune mouse serum (lane 1) and non-immune mouse serum (lane 2). The second antibody was  $^{125}\text{I}$ -labelled sheep anti-mouse Ig.

#### *Determination of HSV-1 polypeptides recognized by purified immune IgG subclasses*

To determine which viral polypeptides were recognized by each isotype and whether the antibody response to some viral polypeptides was restricted in isotype expression, we studied the ICR purified subclasses by immunoblotting. Nitrocellulose strips containing the separated HSV-1 polypeptides were incubated with each ICR immune isotype at 100  $\mu\text{g}/\text{ml}$  and were then probed with  $^{125}\text{I}$ -labelled sheep anti-mouse Ig. Fig. 2 shows that all three subclasses detected the major capsid protein VP155, gC, pgB, gB, gE and gD. Proteins were identified by apparent  $M_r$  and by [ $^{14}\text{C}$ ]glucosamine incorporation as previously described (McKendall *et al.*, 1988*a*). The assignment for gB is tentative since another glycoprotein, gH, migrates closely with pgB and gB at 110K to 115K (Buckmaster *et al.*, 1984). Two other major bands were the 42K and 44K proteins which are probably part of the nucleocapsid protein complex (Heilman *et al.*, 1981; Eberle *et al.*, 1985; McKendall *et al.*, 1988*a, b*). All isotypes also reacted with a high  $M_r$  band of about 170K. For IgG2b the 170K band was more intense than the VP155 band. For IgG2a the

Table 3. *Neutralizing activity compared with total antiviral antibody detected by ELISA in purified subclasses and immune serum*

	IgG concentration required for neutralization*	IgG concentration required for positive ELISA†	Neutralization/ELISA ratio
ICR IMS‡	25 µg/ml	ND§	—
ICR subclasses			
IgG1 (fraction a)	100 µg/ml	0.25 ± 0.03	400
IgG2a (fraction b)	25 µg/ml	0.58 ± 0.19	43
IgG2b (fraction c)	25 µg/ml	0.61 ± 0.08	41
BALB/c IMS	3 µg/ml	ND	ND
BALB/c subclasses			
IgG1 (fraction a)	6.13 µg/ml	0.51 ± 0.08	12
IgG2a (fraction b)	1.50 µg/ml	1.00 ± 0.08	1.5
IgG2b (fraction c)	3.00 µg/ml	1.12 ± 0.22	2.7
Non-immune serum	No neutralization	No activity	—

\* IgG concentration (µg/ml) required for 50% reduction in plaques.

† IgG concentration (µg/ml) required to produce  $A_{405}$  of 0.2 above background.

‡ IMS, immune serum unfractionated.

§ ND, Not done.

intensity pattern was reversed, thus IgG2b contained relatively more anti-170K and relatively less anti-VP155 antibody than IgG2a. In addition IgG2b appeared to react with several lower  $M_r$  proteins below 42K.

For the glycoprotein regions, some differences in isotype reactivity were notable. For IgG2a, the pgB/gB/gC region had more intense reactivity whereas IgG2b was least intense in that region. However gE and gD appeared most intense in the IgG2b strip than the others. These differences led us to examine the neutralizing activity of the subclasses. IgG3 was studied in unfractionated serum using the goat anti-mouse IgG3 reagent (see Methods). All polypeptides were less intense than the respective bands in the other isotype, as expected since the IgG3 serum concentration was low. Nonetheless the 170K protein, VP155, pgB, gC, gE and gD bands were easily visualized (data not shown). Thus, overall no anti-polypeptide response was completely restricted to one isotype but qualitative differences in intensities were observed.

#### *Neutralizing activity in purified IgG subclass*

The availability of the purified subclasses and the glycoprotein reactivity seen on immunoblotting prompted us to determine the relative neutralizing activity. Table 3 shows the isotype concentration required to produce a 50% reduction in plaques. In both the ICR and BALB/c specimens the IgG1 subclass (fraction a from Table 1) had the weakest neutralizing activity. The IgG2a and IgG2b subclasses had comparable activity. The weak IgG1 neutralizing activity is surprising because the ELISA activity in that isotype was the highest of all the subclasses. This suggests that relatively more of the IgG1 antibody may be directed at non-neutralizing epitopes or that the avidity of the IgG1 for epitopes involved in neutralization is less than that of other subclasses.

Table 3 also indicates that all purified BALB/c subclasses exhibited greater neutralizing activities than the ICR immune subclasses. This could be either because there is more virus-specific antibody in the BALB/c subclasses or because there is a larger neutralizing fraction of the antiviral antibody in each subclass. To distinguish which of these postulates was correct, we compared the ratio of virus-specific antibody activity as determined by ELISA with that determined by neutralization assay. For the ICR IgG1, the concentration required for neutralization was 400 times that required for a positive result by ELISA. For the BALB/c IgG1 the ratio was only 12 (Table 3). Thus neutralization is achieved with less virus-specific antibody from the BALB/c IgG1 than from the ICR IgG1, indicating that more of the virus-specific IgG1 antibody in the BALB/c is neutralizing. Similar results were found in the ratios of the IgG2a and IgG2b isotypes.

## DISCUSSION

Two previous reports have observed that in humans virus-specific antibody occurs most frequently in the IgG1 isotype (Gilljam *et al.*, 1985; Coleman *et al.*, 1985). These studies did not contain controls for differences in the serum concentrations of each isotype. Thus it is not surprising that the IgG1 isotype, which is usually 60 to 80% of the total serum IgG (Linde *et al.*, 1983; Lee *et al.*, 1986), would most frequently contain virus-specific antibody. Our study clearly shows that IgG1 and IgG2a contain the greatest proportion of the virus-specific antibody activity detected by ELISA. Using the serum isotype concentration at endpoint in the HSV-1 antibody ELISA and the serum isotype concentration, we calculate that the serum virus-specific antibody activity is 50% IgG1, 39% IgG2a, 11% IgG2b and 1% IgG3 in the ICR immune serum. For the BALB/c immune serum, the respective proportions are 51%, 45%, 4% and 0.4%. Studies of the purified subclasses confirm these findings. These data suggest that there is a preferential IgG heavy chain isotype switch and that the apparent preference is IgG1 > IgG2a > IgG2b > IgG3.

The low level of IgG3 is typical of murine antibody responses for many viruses (Coutelier *et al.*, 1987) and is expected since IgG3 tends to be preferentially induced by carbohydrate- and T-independent antigens (Yount *et al.*, 1968; Radbruch *et al.*, 1986). Since purified IgG3 was not available, we cannot exclude the possibility that the virus-specific IgG3 activity may have been underestimated due to competition for antigen by the presence of other isotypes. In one virus infection, murine influenza, the isotype preference is IgG3 > IgG2a = IgG2b > IgG1 (Reale *et al.*, 1985).

The prominence of the virus-specific IgG1 is an important finding which appears to distinguish HSV-1 infection from many other virus infections. For lymphocytic choriomeningitis virus, lactate dehydrogenase-elevating virus (LDV) and most other viruses studied in mice, the IgG1 antiviral activity is low and IgG2a is the dominant isotype produced (McDonald *et al.*, 1983; Coutelier & Van Snick, 1985; Thomsen *et al.*, 1985; Coutelier *et al.*, 1986, 1987). The high levels of virus-specific IgG1 probably reflect an influence of B cell differentiation factor, a recently described lymphokine which *in vitro* causes a preferential production of IgG1 (Sideras *et al.*, 1985; Isakson *et al.*, 1982; Isakson, 1986; Radbruch *et al.*, 1986; Mongini *et al.*, 1982; Tesch *et al.*, 1986).

At least two other contributing mechanisms can be envisioned. First, virus-induced production of isotype-restricted virus-specific and non-specific antibody could result from direct B cell activation by virus. For example, in LDV infection, the predominant antiviral isotype is IgG2a and it is accompanied by a large IgG2a-restricted production of antibody which is not virus-specific (McDonald *et al.*, 1983) and which has been attributed to polyclonal activation of B cells (Coutelier & Van Snick, 1985; Michaelides & Simms, 1977). Possibly HSV-1 causes a similar B cell activation, however a role in isotype restriction by a subset of T helper cells cannot be excluded (Teale & Abraham, 1987).

Second, we noted a large overall increase in total serum IgG1 and IgG2a levels during the period of immunization. Some of the increase was due to production of antibodies which were non-viral, since the virus-specific isotype endpoints by ELISA were 280 to 13900 ng/ml, yet the assay was capable of detecting concentrations of 62 to 150 ng/ml. These increased total serum isotype levels could be the results of a general isotype switch pattern which presumably results from concomitant stimulation by other antigens to which the mice are naturally exposed during the several weeks of the experiments. This is consistent with the known age-dependent increases in IgG isotypes, particularly IgG1, observed in BALB/c and other mice (Natsuume-Sakai *et al.*, 1977) over several months after birth. Thus the predominance of virus-specific antibody to IgG1 (and IgG2a) may be non-specifically promoted by factors controlling the age-dependent isotype switch pattern. Studies to investigate these possibilities are in progress.

Several important biological implications are raised by our data. The dominance of serum antiviral IgG1, which does not bind complement (Klaus *et al.*, 1979) and is relatively poor in neutralization efficiency, can be viewed as potentially detrimental to the control of infection. It may act to compete with and block neutralization by the more effective IgG2a and IgG2b antibodies and also hinder antibody-dependent cell-mediated cytotoxicity (ADCC). More



efficient ADCC killing by IgG2a than by other isotypes has been reported from several laboratories recently (Kipps *et al.*, 1985; Herlyn *et al.*, 1985; Matsui *et al.*, 1986; Langlois *et al.*, 1985; Steplewski *et al.*, 1985; Johnson *et al.*, 1986). In addition it has been shown that some IgG2a antibodies, despite specificity for target cell membranes, do not accomplish lysis with appropriate ADCC effectors (Christiaansen & Sears, 1984). Thus both the antibody isotype and the specific target protein (or epitopes) seem to influence the efficiency of ADCC, possibly because antibody orientation on the target cell surface may be critical for interaction with the effector cell Fc receptor (Christiaansen & Sears, 1984).

Our study indicates that the antibody response to the glycoproteins is not uniform in isotype composition on the basis of differences observed in both the Western blot assay and neutralization assay. In addition we have recently observed that the antibody response to the viral glycoproteins in individual mice displays limited diversity and differs between mice (R. R. McKendall, unpublished data). Therefore it is possible that restricted diversity in anti-polypeptide antibody responses and associated isotype restrictions to those responses could combine to have an important effect on antibody-mediated antiviral defence. Whether these factors have a significant impact *in vivo* remains to be determined in further studies.

We thank Drs William G. Stroop and G. John Stanton for critical review of the manuscript. The excellent assistance of Lyn Schilling in the preparation of the manuscript is gratefully acknowledged. This work is supported by a grant from the Forman Foundation and NIH Grant no. RR-05427.

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(Received 28 May 1987)