

Virus-Specific Immunoglobulin G Subclasses in Herpes Simplex and Varicella-Zoster Virus Infections

VIVI-ANNE SUNDQVIST,* ANNIKA LINDE, AND BRITTA WAHREN

Department of Virology, National Bacteriological Laboratory, and Department of Virology, Karolinska Institute, Stockholm, Sweden

Received 27 December 1983/Accepted 4 April 1984

The subclass specificities of antiviral immunoglobulin G (IgG) produced in response to herpes simplex and varicella-zoster virus infections were investigated. IgG1 and IgG3 with anti-herpes simplex virus activity were seen in patients with primary and reactivated disease, as well as in healthy seropositive subjects and in immunoglobulin preparations. IgG4 was occasionally seen alone or together with IgG1 and IgG3 in patients. In varicella, IgG3-specific antiviral antibodies were predominant, whereas in zoster, IgG1 was the dominant subclass.

Four subclasses of human immunoglobulin G (IgG) are known. These are termed IgG1 (60.9%), IgG2 (29.6%), IgG3 (5.3%), and IgG4 (4.2%) (11; for a review, see reference 18). The subclasses differ in the heavy chains of the IgG molecule, in electrophoretic mobility, and in reactivity with staphylococcal protein A, as well as in biological functions, such as complement fixation and binding to mononuclear cells. A subclass-restricted response to bacterial antigens has been demonstrated (12, 14, 15), but very little is known about the subclasses that react in viral infections.

With protein A fractionation, Beck (1) found antibody activity (as measured by hemagglutination or neutralization) to rubella, polio virus types 1-3, and herpes simplex type 1 (HSV-1) predominantly in IgG3, but only one serum was studied for each viral antibody. IgG subclasses differing in their electrophoretic mobilities were described by Leonard et al. (6), who studied the IgG response in primary and reactivated varicella-zoster virus (VZV) infections by using neutralization tests.

With monoclonal antibodies to different subclasses of human IgG (8), an enzyme-linked immunosorbent assay (ELISA) has been developed for subclass IgG antiviral antibody (7). In the present study, we employed this subclass-specific ELISA to demonstrate the specific antiviral IgG subclass pattern in serum samples from patients with primary and reactivated infections with HSV or VZV.

MATERIALS AND METHODS

Patient sera and gamma globulins. Ten serum samples with an ELISA titer to HSV of <50, and 15 sera negative to VZV in immunofluorescence (IF) were used as negative controls. Ten serum samples had both HSV and VZV antibodies, as determined by complement fixation (CF), IF, ELISA, or all three.

Paired acute- and convalescent-phase sera were obtained from six patients with a zoster infection, six with a varicella infection, and six with a primary HSV-1 or -2 infection, as judged by site of lesions and significant titer rises measured by CF and ELISA (16). Sera collected over an extended period from five other patients with primary HSV infections were kindly donated by S. Gronowitz, Department of Medical Virology, Uppsala University, Uppsala, Sweden. Gamma globulin, 16.5%, was from Kabi (Stock-

holm, Sweden), and zoster immunoglobulin was from the National Bacteriological Laboratory (Stockholm, Sweden).

Antigen preparations. (i) **HSV-1 and HSV-2 nuclear antigens.** These antigens were prepared as previously described for cytomegalovirus (CMV) antigen (17). Vero cells in roller bottles were infected with either HSV-1 strain F9004 or HSV-2 strain D64, both propagated in our laboratory. After 48 h, the cells were suspended in 0.4% KCl by shaking, centrifuged, and resuspended in 0.4% KCl with 1% Nonidet P-40 and homogenized in a Dounce homogenizer. The homogenate was layered on a cushion of 0.25 M sucrose in 0.4% KCl and centrifuged at $700 \times g$ for 5 min. The pellet containing nuclei was suspended in 5 ml of 10 mM Tris (pH 7.5)-1.5 mM $MgCl_2$ -10 mM KCl, sonicated, and stored at $-70^\circ C$.

(ii) **VZV antigen.** The method of preparation was described previously (16). VZV-infected human lung fibroblasts were pelleted by centrifugation, suspended in 0.01 M Tris buffer with 0.15 M NaCl and 0.002 M EDTA (pH 9.0), sonicated at 20 KHz for 5×10 s. The cell lysate was clarified by centrifugation, and the supernatant fluid was used as the antigen.

(iii) **Antigens for CF.** HSV antigen for CF was prepared by suspending HSV-1-infected green monkey kidney cells in distilled water. After ultrasonication (at 20 KHz for 30 s), the suspension was clarified by low-speed centrifugation. The supernatant fluid constituted the antigen. Varicella-infected human lung fibroblast cells were harvested in saline, frozen and thawed, and sonicated.

(iv) **Control antigens.** Control antigens were prepared from uninfected human lung fibroblast or green monkey kidney cells treated in the same way as described for nuclear or CF antigens.

Monoclonal antibodies. Monoclonal antibodies (Seward Laboratory, London, England) directed to human IgG subclasses 1-4 were used at dilutions of 1:100 (IgG1, clone BAM 9), 1:400 (IgG2, clone BAM 10), 1:1,600 (IgG3, clone BAM 8), and 1:800 (IgG4, clone BAM 11). Standardization of the test was performed as previously described for CMV (7).

Treatment of sera with protein A. Sera were adsorbed by mixing 100 μ l of serum with 400 μ l of 20% suspension of protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden). After 10 min at room temperature, the mixture was centrifuged, and the supernatant fluid was used. IgG1, IgG2, and IgG4 are absorbed by protein A (5).

* Corresponding author.

IF. Indirect IF staining for detection of VZV IgG antibodies was performed as described by Grandien et al. (3).

ELISA. (i) Assay conditions. Coating of microplates (Nunc-Immunoplate I; Nunc, Aarhus, Denmark) was performed at room temperature overnight with 100- μ l volumes of antigen. The plates were then stored at 4°C. Before use they were washed three times with saline containing 0.05% Tween 20. Buffers were 0.05 M sodium carbonate (pH 9.5) for dilution of coating antigens or antibodies; phosphate-buffered saline without Ca^{2+} or Mg^{2+} (pH 7.4) with 0.05% Tween 20 and 0.5% bovine serum albumin for dilution of antisera, subclass-specific monoclonal antisera, or conjugates; and phosphate citrate buffer (pH 5.0) for dilution of the substrate *ortho*-phenylenediamine.

(ii) IgG viral antibody assays. IgG viral antibody assays were performed as described previously (16, 17).

(iii) IgG subclass-specific antibody assay. Microplates coated with VZV or HSV antigen were incubated with 100 μ l of patient serum diluted 10-fold (10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4}) for 2 h at 37°C. After being washed, the subclass-specific monoclonal antisera diluted as described above were added and incubated for 105 min at 37°C. The plates were washed, and 100 μ l of peroxidase-conjugated rabbit anti-mouse IgG diluted 1:800 (Dakopatts, Copenhagen, Denmark) was added. After 105 min of incubation, the plates were washed, and the substrate *ortho*-phenylenediamine was added. After 60 min at room temperature, the reaction was terminated with 2 M H_2SO_4 , and the absorbance at 450 nm (A_{450}) was measured in a Titertek Multiscan (Flow Laboratories, Irvine, Scotland).

RESULTS

Reactivity of HSV and VZV antibody-negative serum samples in subclass-specific ELISA. Ten sera, negative for HSV antibodies in CF and IgG ELISA, all had A_{450} values below 0.25 in a serum dilution of 1:10 when assayed in the subclass-specific ELISA. The background levels were calculated as described for IgG subclasses to CMV (7). The background values for antibodies to HSV were $A_{450} = 0.30$ (IgG1) and 0.25 (IgG2-4). The same background levels applied to 12 out of 15 VZV IF-negative sera. Among the 15 VZV IF-seroneg-

ative sera, 3 were positive in ELISA for VZV IgG. The subclass responses were shown to be IgG4 only. A_{450} values for VZV IgG4 of 1.105 to 1.904 were seen in serum dilutions of 1:10. These sera were also tested for subclasses to HSV antigen; one had no antibodies to HSV, one had measurable antibodies of subclasses IgG1, IgG3, and IgG4, and one had predominantly IgG4 HSV antibodies.

Subclass-specific antibodies against HSV and VZV in seropositive samples. In serum samples from healthy persons known to have IgG antibodies to HSV ($n = 10$) and VZV ($n = 10$), the distribution of different IgG subclasses was studied. All had IgG1 antibodies directed against HSV (Fig. 1), although the levels varied among individuals. None had detectable IgG2 to HSV. A simultaneous occurrence of IgG1, IgG3, and IgG4 was seen in five patients when they were assayed for HSV antibodies. In addition, one person (no. 1) had a high level of IgG4 antibodies against HSV but low or nondetectable antibodies against HSV of other subclasses.

Ten out of ten sera also contained IgG1 antibodies against VZV. The amounts of VZV-specific IgG2 and IgG3 were low. Two samples contained high amounts of IgG4 antibodies (Fig. 1).

In commercial gamma globulin and zoster immunoglobulin, HSV antibody activities were seen in IgG1 and IgG3; VZV antibody activity was mainly in IgG1.

Subclass-specific antiviral IgG in sera treated with protein A. Decreased total antiviral IgG titers were seen when sera were assayed in HSV ELISA IgG after absorption with protein A. Absorption of a selected convalescent-phase serum with protein A reduced the levels of IgG1 antibodies as measured in the HSV subclass-specific ELISA, whereas the level of virus-specific IgG3 remained unaltered.

Subclass-specific antiviral IgG in primary and reactivated disease. In convalescent-phase sera from patients with primary HSV infections, IgG3 was the predominant subclass containing virus-specific antibody activity (Fig. 2). In addition, all had IgG1, one had barely measurable IgG2, but none had IgG4 virus-specific antibodies. Sera collected during an extended period from five additional patients with primary HSV infections were studied. Four of the patients showed the IgG1 and IgG3 subclass distribution described above for

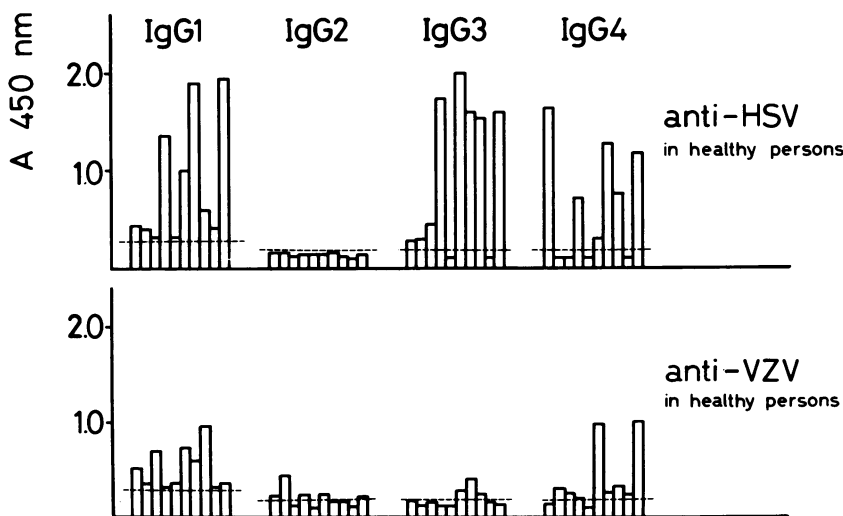


FIG. 1. Distribution of HSV- and VZV-specific IgG subclasses in sera of 10 healthy seropositive donors. The A_{450} values for serum dilutions of 1:10 are shown. The background value for each clone is shown (—).

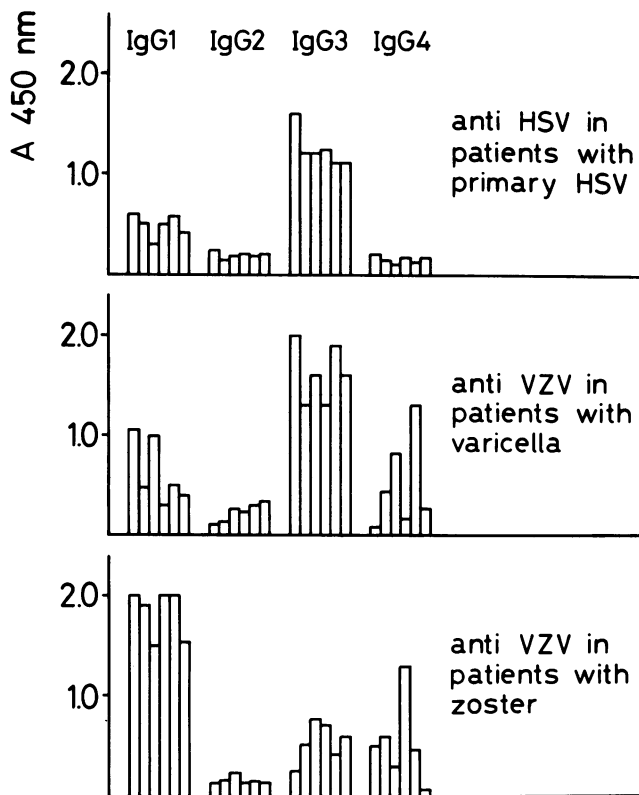


FIG. 2. IgG subclass patterns to VZV and HSV in convalescent sera from six patients with HSV, varicella, or zoster infections. Sera were taken 2 to 3 weeks after onset of illness, and the A_{450} values for serum dilutions of 1:10 are shown.

primary HSV infections. This commonly seen subclass pattern is illustrated by patient Wi (Fig. 3). The IgG1 peak was found in a serum taken 13 weeks after onset of illness; IgG3 was elevated from 3 weeks up to 41 weeks. After recurrences of cold sores at 19 and 23 weeks, no significant titer rise was seen by CF or total IgG ELISA. The levels of IgG4 were, however, increased considerably after these recurrences.

Patient W-B, a pregnant woman with a genital infection, had a predominance of IgG4, both initially and during the follow-up period (Fig. 3). This patient had CF titers to HSV

in only one serum, obtained 4 weeks after onset of illness (4). IgG4 was the predominant subclass to VZV also.

The subclass pattern was compared in convalescent-phase sera from patients with varicella or zoster (Fig. 2). The patients with varicella had higher levels of IgG3, whereas those with zoster had higher levels of IgG1. Both patients with current varicella or zoster had higher IgG3 levels to VZV than did healthy persons (Fig. 1). Figure 4 shows the VZV IgG subclasses in acute- and convalescent-phase sera from one patient with varicella and another patient with zoster. Dilution curves illustrate the rapid increase of virus-specific IgG1 and IgG3 in primary disease and of IgG1 in reactivated disease.

In both HSV and VZV infections, IgG2 levels were low. IgG4 levels seemed to occur occasionally in individuals and were not correlated with whether the infection was primary or reactivated.

DISCUSSION

The distribution of the virus-specific antibodies among the four IgG subclasses was studied in patients with primary and recurrent infections of HSV and VZV. With the monoclonal antibody to IgG1 available when this assay was first developed for CMV, the sensitivity for IgG1 detection was not as high as that for the other subclasses (7). We, therefore, used the assay to study the relative occurrence of IgG subclasses to HSV and VZV and the changes during and after infection. Most patients had antibodies to HSV and VZV of subclasses IgG1 and IgG3. In serial follow-up studies, IgG3 was seen to appear first, followed by IgG1. This was similar for primary HSV and varicella infections. In zoster, IgG1 was the predominant subclass, in keeping with the finding in zoster immunoglobulin. HSV- and VZV-specific IgG4 occurred occasionally and sometimes was the only detectable subclass. IgG2 had low reactivity with HSV or VZV. We cannot, however, rule out the possibility that some viral antigens (9) with which IgG2 may react were lacking in our preparations.

Beck (1) found anti-HSV activity in the IgG3 fraction as determined in a neutralization test. These findings differ from those obtained by Ratner et al. (13), who showed a decreased neutralizing activity to HSV in sera after protein A treatment. In our assay, virus-specific IgG1, IgG3, and IgG4 antibodies could be detected in sera from healthy persons known to have IgG anti-HSV. In convalescent sera obtained 2 to 4 weeks after onset of a primary HSV infection, we demonstrated both IgG3 and IgG1 anti-HSV.

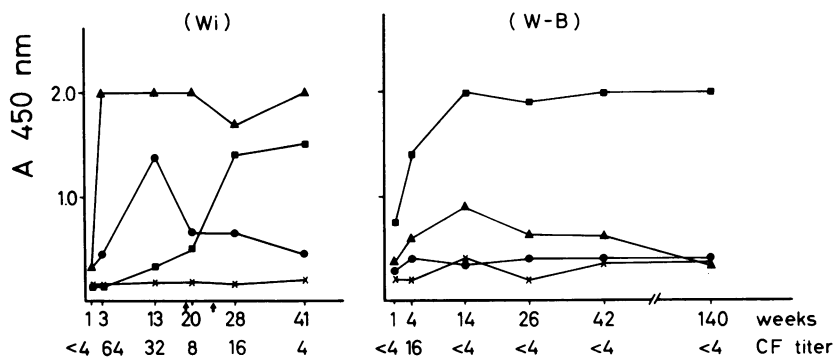


FIG. 3. Appearance of the HSV-specific IgG subclasses IgG1 (●), IgG2 (X), IgG3 (▲), and IgG4 (■) in two patients with primary HSV infections. One patient (Wi) had two recurrences of HSV at weeks 19 and 23 (arrows) which increased levels of IgG4 anti-HSV. One patient (W-B) had a predominance of IgG4 anti-HSV during the follow-up period.

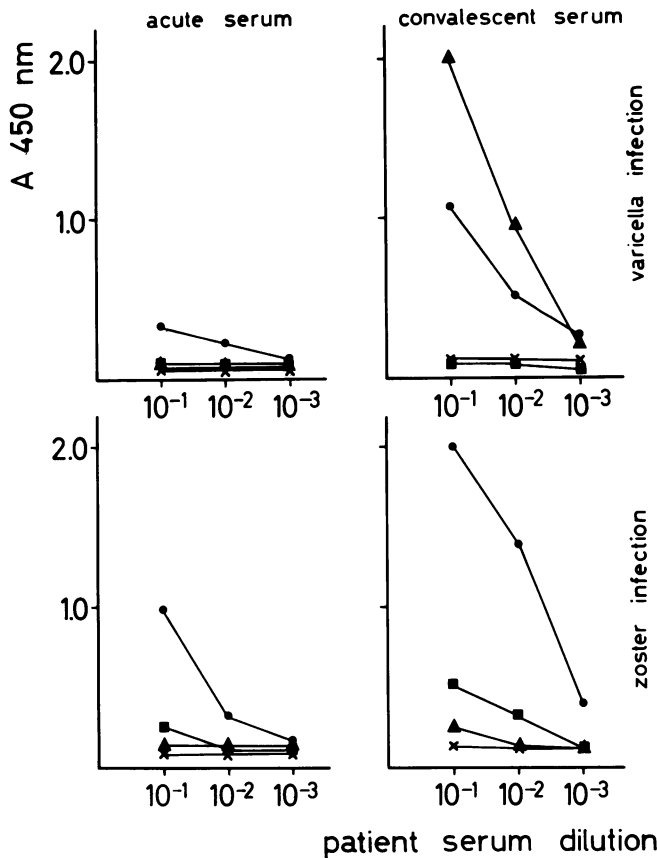


FIG. 4. Antibodies to VZV of subclasses IgG1 (●), IgG2 (X), IgG3 (▲), and IgG4 (■) in acute- and convalescent-phase sera from a patient with varicella and a patient with herpes zoster. Acute-phase sera were obtained within 1 week after onset of illness, and convalescent-phase sera were obtained 2 weeks after onset of illness.

The common occurrence of both IgG1 and IgG3 with anti-herpes reactivity could explain why Beck found antiviral IgG3 (1), whereas Ratner et al. (13) found antiherpes activity in sera without IgG3. The differences in neutralizing capacity may be due to differences in methodology or to the choice of sera used in their tests.

IgG3 constitutes about 5% of the total IgG (11). It is noteworthy that this subclass appeared to be the first to react with HSV and VZV antigens in primary infections. A similar pattern, with detection of IgG3 to CMV in sera taken early during convalescence, was noted regularly (7). A subclass restriction of the IgG response, with IgG1 and IgG3, has been noted recently for hepatitis B surface antigen (10). The different functional properties of IgG subclasses may confer important differences in the ability of the patient to suppress the viral infection.

In viral serology, certain precautions must be taken due to the restricted antiviral response. The use of protein A labeled with ¹²⁵I or enzymes as an antiimmunoglobulin for detection of antibodies to HSV (2) or VZV may exclude detection of an important part of the antibody response to these viruses. Similarly, animal sera directed against human IgG, which are often used as second antibodies in immune reactions for viral antibodies, must be confirmed to have reactivity with all IgG subclasses.

In sera of normal individuals, a much wider range of total

IgG4 values was found than for the other subclasses (11). The same authors also presented evidence that the capacity of a given individual to respond to an antigen by producing antibodies of predominately one or another IgG subclass may be under genetic control. One patient with a long-standing IgG4 anti-HSV antibody response had CF titers to HSV for a very short period. The CF activity was possibly due to an IgM response, since IgG4 does not fix complement. In 3 of 15 patient sera which were negative in IF studies for antibodies to VZV, IgG4 anti-VZV was detected by ELISA. Presumably the IgG4 antibodies were not detected by the second antibody used in IF. Thus, an HSV or VZV infection with an IgG4 response may go undetected by CF or IF serology. Leonard et al. (6) described two subclasses of IgG produced by varicella and zoster infections. These subclasses differed in their electrophoretic mobilities and abilities to neutralize VZV. In the slow fraction, anti-VZV was found by IF and neutralization in both varicella and zoster infections. In the fast IgG fraction, neutralizing activity was demonstrable only in zoster infections. IgG4 is known to have a faster electrophoretic mobility than the other subclasses, and the fast IgG fraction described by Leonard et al., therefore, probably was IgG4.

ACKNOWLEDGMENTS

We thank Erling Norrby, Karolinska Institute, for valuable advice and Eva Ohlsson for excellent technical assistance.

LITERATURE CITED

1. Beck, O. E. 1981. Distribution of virus antibody activity among human IgG subclasses. *Clin. Exp. Immunol.* **43**:626-632.
2. Cleveland, P. H., D. D. Richman, M. N. Oxman, M. G. Wickham, P. S. Binder, and D. M. Worthen. 1979. Immobilization of viral antigens on filter paper for a (¹²⁵I) staphylococcal protein A immunoassay: a rapid and sensitive technique for detection of herpes simplex virus antigens and antiviral antibodies. *J. Immunol. Methods* **29**:369-386.
3. Grandien, M., P. Appelgren, Å. Espmark, and K. Hanngren. 1976. Determination of varicella immunity by the indirect immunofluorescence test in urgent clinical situations. *Scand. J. Infect. Dis.* **8**:65-69.
4. Gronowitz, J. S., and C. F. R. Källander. 1981. Occurrence of antibodies against herpes simplex virus thymidine kinase in human sera. *J. Med. Virol.* **8**:177-186.
5. Kronvall, G., and R. C. Williams, Jr. 1969. Differences in anti-protein A activity among IgG subgroups. *J. Immunol.* **103**:828-833.
6. Leonard, L. L., N. J. Schmidt, and E. H. Lennette. 1970. Demonstration of viral antibody activity in two immunoglobulin G subclasses in patients with varicella-zoster virus infections. *J. Immunol.* **104**:23-27.
7. Linde, G. A., L. Hammarström, M. A. A. Persson, C. I. E. Smith, V.-A. Sundqvist, and B. Wahren. 1983. Virus-specific antibody activity of different subclasses of immunoglobulins G and A in cytomegalovirus infections. *Infect. Immun.* **42**:237-244.
8. Lowe, J., P. Bird, D. Hardie, R. Jefferies, and N. R. Ling. 1982. Monoclonal antibodies (McAbs) to determinants on human gamma chains: properties of antibodies showing subclass restriction or subclass specificity. *Immunology* **47**:329-336.
9. Martin, M. L., E. L. Palmer, and R. E. Kissling. 1972. Complement-fixing antigens of herpes simplex virus types 1 and 2: reactivity of capsid, envelope, and soluble antigens. *Infect. Immun.* **5**:248-254.
10. Morell, A., B. Roth-Wicky, and F. Skvaril. 1983. Immunoglobulin G subclass restriction of antibodies against hepatitis B surface antigen. *Infect. Immun.* **39**:565-568.
11. Morell, A., F. Skvaril, A. G. Steinberg, E. van Loghem, and W. D. Terry. 1972. Correlations between the concentrations of the four subclasses of IgG and Gm allotypes in normal human

- sera. *J. Immunol.* **108**:195-206.
12. **Mortimer, G. E., and J. P. Widdowson.** 1979. Predominance of immunoglobulin G sub-class 3 among the complement-fixing antibodies to streptococcal M-associated protein. *Clin. Exp. Immunol.* **37**:247-258.
 13. **Ratner, J. J., B. A. Sanford, and K. O. Smith.** 1979. Use of protein A-treated sera in unmasking herpes simplex virus type 1 (HSV-1) immunoglobulin A and identifying HSV-1 immunoglobulin G as the predominant neutralizing antibody. *J. Clin. Microbiol.* **10**:415-418.
 14. **Riese, W. F., F. Skvaril, and D. G. Braun.** 1976. Natural infection of man with group A Streptococci. Levels, restriction in class, subclass and type, and clonal appearance of polysaccharide-group-specific antibodies. *Scand. J. Immunol.* **5**:383-390.
 15. **Sieber, G. R., P. H. Schur, A. L. Aisenberg, S. A. Wetzman, and G. Schiffman.** 1980. Correlation between serum IgG-2 concentrations and the antibody response to bacterial polysaccharide antigens. *N. Engl. J. Med.* **303**:178-182.
 16. **Sundqvist, V.-A.** 1983. Frequency and specificity of varicella-zoster virus IgM response. *J. Virol. Methods* **5**:219-227.
 17. **Sundqvist, V.-A., and B. Wahren.** 1981. An interchangeable ELISA for cytomegalovirus antigen and antibody. *J. Virol. Methods* **2**:301-312.
 18. **Winkelhake, J. L.** 1978. Immunoglobulin structure and effector functions. *Immunochemistry* **15**:695-714.