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Behaviour of IgG Antibody Avidity for the Antigen and of IgA Antibody in Active Cytomegalovirus, Epstein–Barr Virus, Herpes Simplex Virus and Human Herpes Virus 6 Infections. Adaptation of a Commercial Test

J. Gutiérrez*, M. Rodríguez, M. C. Maroto, G. Piédrola and J. Peirón

Department of Microbiology, Universitary Hospital San Cecilio, University of Granada, Spain

The clinical value of specific IgA and IgG antibody avidity to herpes simplex virus, cytomegalovirus, Epstein–Barr virus and human herpes virus 6 for the detection of active disease and primary infection, respectively, was evaluated. The IgG avidity test, with a break point of 55%, for the detection of primary infection, and of the IgA test for the detection of disease, were associated with a sensitivity of 97% and 64%, respectively; specificity of 100% and 82%; a positive predictive value of 100% and 76%; and a negative predictive value of 96% and 72%, respectively.

Introduction

During infection by herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein–Barr virus (EBV) and human herpes virus-6 (HHV-6), IgM antibody (Ab) are not sufficiently consistent for use in the diagnosis of primary infection, reinfection or reactivation, whether symptomatic or not. These Abs can give false-negative results, because they are produced in small quantities which are difficult to detect or not produced.[1] False-positive results can be due to their long persistence and their presence in processes unrelated to the actual infection;[2] positive findings may derive from reactivation or from reinfection,[3–6] as also observed in patients infected with the human immunodeficiency virus (HIV). Common epitopes in viruses, e.g. HSV and varicella zoster virus (VZV) may lead to simultaneous laboratory detection of various IgMs. Finally there are heterotypical reactions between circulating Abs and white blood cells, the main target cells for CMV, EBV and HHV-6.[7,8]

The diagnosis of active herpes virus infection by the detection of a four-fold increase in the IgG titre is difficult. The titre rise may be failed, as IgGs usually appear very soon after the onset of symptoms.[9]

In this context, the study of Abs of the IgA and IgG classes with low avidity for the antigen could constitute a good marker for recent primary infection. IgA Abs are present in primary infection, they appear later than IgM Abs, and they are found in reactivation. Quantification of specific IgG Abs avidity by simple techniques is a new sensitive and specific method for the serological diagnosis and follow-up of certain infections.[3,4] This method could be used to determine the approximate point of the primary infection and to distinguish between reactivation, reinfection and primary infection. Generally, the affinity of IgG for the antigen is low after the first antigen contact and rises during the following weeks and months due to maturation of the B lymphocytes and the decrease in antigen stimulus.

For this reason we set out to determine the clinical value of the study of IgA and specific IgG avidity for the detection of active disease and primary infection, respectively, caused by the herpes viruses CMV, EBV, HSV and HHV-6.

Materials and Methods

Serum samples from patients and healthy subjects with past infection were analysed in order to study specific IgG avidity and to detect IgA to CMV, EBV, HSV and HHV-6. IgA were not studied in samples from healthy subjects with a past HHV-6 infection. The samples studied and their clustering into panels are shown in Table I. The samples from patients were analysed retrospectively, after being taken 15–25 days following the onset of clinical symptoms, at intervals of 20–25 days where various samples were examined for follow-up (Fig. 1).

Healthy subjects with past infection

It was made up of individuals with a mean age of 25 ± 5 years, without symptoms and underlying disease known

* Address correspondence to: Dr J. Gutiérrez. C/ Camino Bajo de Huetor 84, 1-A, E-18008 Granada, Spain.
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Table I. Serum sample panels studied and their relationship to clinical.

Infection	Clinical phase	Panel	Total patients	Patients followed up
HSV	Primary infection	1a	31	3
	Reactivation	1b	6	0
	Past infection	1c	50	0
CMV	Primary infection	2a	75	21
	Reactivation	2b	3	0
	Past infection	2c	50	0
EBV	Primary infection	3a	100	30
	Past infection	3b	50	0
HHV-6	Primary infection	4a	5	2
	Past infection	4b	50	0

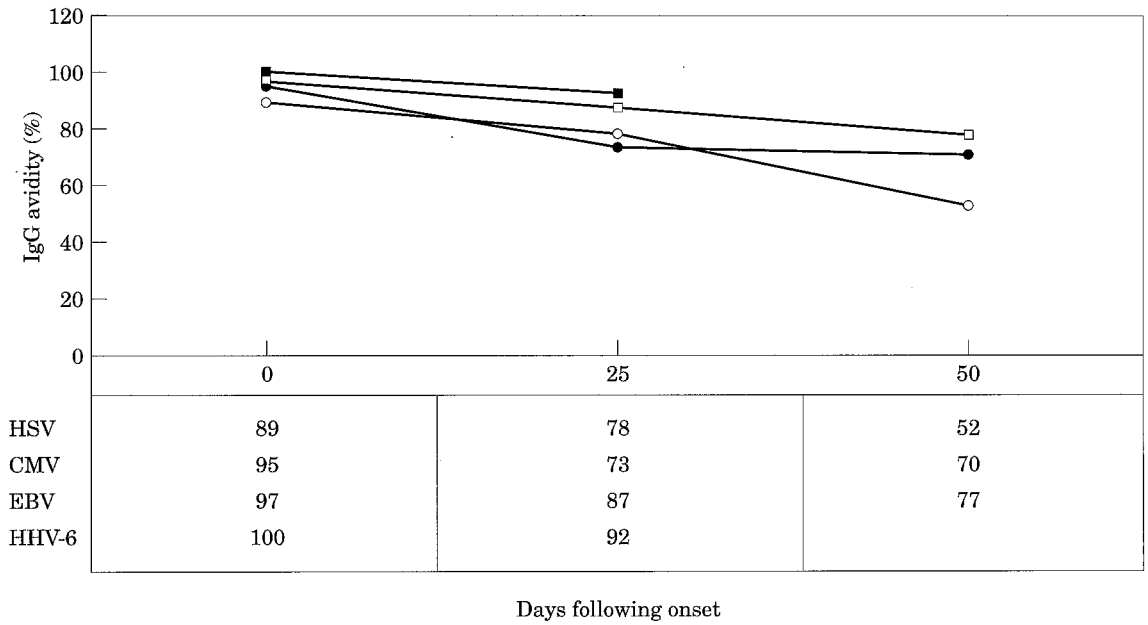

Figure 1. Evolution of IgG without avidity by antigen in the infection by HSV, CMV, EBV and HHV-6. (○) HSV; (●) CMV; (□) EBV; (■) HHV-6.

to be associated with immunological or neoplastic diseases, with specific IgG and without IgM, which sera were obtained more than or equal at 6 months after the initiation of previous symptoms of infection.

Patients

The subjects who had underlying disease known to be associated with immunological or neoplastic diseases, other HIV infection, were excluded from the study. Primary infection (mean age of 5 ± 2 years) and reactivation (mean age of 25 ± 2 years) from patients were defined in these cases by:

(1) Primary infection characterized by previous absence of specific IgG and IgM and symptoms of illness since the birth at the time of the study.

(2) Reactivation characterized by previous presence of specific IgG and some previous episode at the time of the study with specific IgM and symptoms of illness.

Illness by CMV

It was defined by the presence of antigen p65 of CMV from blood in a number >40 leucocytes infected by 2×10^5 white cells detected by immunoperoxidase test (Insctar); of specific IgM (with anti-IgG) and IgG detected by indirect ELISA (Behringwerke) at 15–25 days following the onset of clinical symptoms and the absence of IgM to the other human herpes viruses (except human herpes virus 7, which was not tested). The patients had fever, infectious mononucleosis or retinitis.

Illness by HSV

It was defined by the presence of HSV antigen determined by capture ELISA (Murex) from vesicle liquids in the onset of clinical symptoms; of specific IgM (with anti-IgG) and IgG detected by indirect ELISA (Behringwerke) at 15–25 days following the onset of clinical symptoms and the absence of IgM to the other human herpes viruses (except human herpes virus 7, which was not tested). The patients had fever and erythematous vesiculopapules in the mucose, skin or urethra.

Illness by EBV

It was defined by the presence of anti-VCA IgM (with anti-IgG) and IgG detected by IFA (Gull) at 15–25 days following the onset of clinical symptoms and the absence of IgM to the other human herpes viruses (except human herpes virus 7, which was not tested). The patients had fever or infectious mononucleosis.

Illness by HHV-6

It was defined by the presence of specific IgM (with anti-IgG) and IgG detected by IFA (Stellar) at 15–25 days following the onset of clinical symptoms and the absence of IgM to the other human herpes viruses (except human herpes virus 7, which was not tested). The patients had fever or infectious mononucleosis.

Study of IgA

An indirect ELISA, with a commercial solid phase of the virus in question, an anti-IgA conjugate and prior treatment of the samples with anti-IgG (Behringwerke) was used for HSV, CMV and ABV. To determine the break point we followed the instructions of the manufacturer (for cut-off of 0.2). In the case of HHV-6 a commercial IFA (Stellar) was used, with a 1/40 sample dilution and prior treatment with anti-IgG. The result was expressed as positive or negative.

Study of IgG avidity

(1) For HSV, CMV and EBV the testing was carried out with an automated and modified system (Behring ELISA Processor III) with a commercial test (indirect ELISA, Behringwerke). Testing was performed once without denaturing agent and repeated with 8 M urea, added for 10 min after incubation of the samples. For exact evaluation of the IgG the ALPHA-METHOD calculation system (Behringwerke) was used.[10] The results are expressed in IU/ml for EBV and titres for CMV and HSV. To determine the incidence of low-avidity IgG, we calculated the difference between the values obtained from the two test runs (with and without urea) and the result was expressed as a percentage of the total IgG. The test was repeated and the mean value was used, provided the deviation did not exceed 10%, to insure the accuracy of the result. If it exceeded 10% the test was repeated. We analysed the reliability of levels of IgG without avidity by antigen above 55% and those between 50 and 55% for diagnosis of recent acute infection, values associated with other recent infections in previous studies.[3,4]

(2) For HHV-6 the method utilized was IFA with serial dilutions of serum, carried out once without denaturing agent and repeated with 8 M urea, added for 5 min after incubation of the samples and before the PBS rinses. We analysed the reliability of decrease in two dilutions of the quantity of IgG detected from serum sample using urea.[3,4]

The sensitivity, specificity and positive and negative predictive value were studied for the avidity test and IgA when compared to the groups of primary infection, reactivation or past infection.

Results

The percentages of low-avidity IgG and IgA-positive for panels 1, 2 and 3, in the first serum sample, are shown in Table II. For panel 4a (HHV-6) all samples displayed low-avidity IgG and IgA; except that IgA was not found in one of them. In panel 4b no serum sample had low-avidity IgG. The change in avidity over time for the samples from panels 1 to 4 is shown in Fig. 1. The reliability of the IgG avidity test, with break points of 50 and 55%, for the detection of primary infection, and of the test for IgA in the detection of active disease, are shown in Table III.

Discussion

Because the study of IgG and IgM does not satisfy the needs for diagnosing herpes virus infections, new serological markers are being evaluated, such as IgG avidity and specific IgA.

Several workers have shown that the avidity of IgG changes with time since infection, whether viral[3,11–15] or bacterial.[16–20] When the percentage of IgG with a low avidity for the antigen is $\geq 50\%$ a recent primary infection may be assumed.[4] We found that, using break points

Table II. IgG Avidity for the antigen and IgA in the first samples from the panels studied by ELISA.

	Subjects (percentages)		
Panel*	50–55% low-avidity IgG	>55% low-avidity IgG	IgA-positive
1a	9 (29)	22 (70.96)	26 (83.87)
1b	1 (16.66)	0	3 (50)
1c	5 (10)	0	9 (18)
2a	7 (9.33)	64 (85.33)	59 (78.66)
2b	1 (33)	0	1 (33)
2c	5 (10)	0	1 (2)
3a	1 (1)	99 (99)	85 (85)
3b	3 (6)	0	4 (8)

* HSV: Primary infection (1a), reactivation (1b), past infection (1c); CMV: primary infection (2a), reactivation (2b), past infection (2c); EBV: primary infection (3a), past infection (3b).

Table III. Reliability of IgG avidity at 50% & 55% break points and of IgA.

	Percentages										
	Herpes simplex virus Low-avidity IgG		IgA	Cytomegalovirus IgG low-avidity		IgA	Epstein–Barr virus IgG low-avidity		IgA	Human herpes virus 6 IgG low-avidity	IgA
	50%	55%		50%	55%		50%	55%		(+)	
Sensitivity	96.7	100	78.3	96	97.6	64.1	100	99	79.7	100	80
Specificity	98	100	82	98	100	98	98	100	92	100	
Positive predictive value	96.7	100	76.3	97.9	100	97.1	98.6	100	93.6	100	
Negative predictive value	98	100	82	96	96.8	72	100	99	75.4	100	

higher than 50%, study of IgG avidity can help to distinguish between short-term and long-term acute infection. The reliability of this assay is close to 100% for all micro-organisms. Exceptions may be due to various causes: persistence of immature (and perhaps not very protective) Abs in patients with a past infection or reactivation; rapid maturation of Ab avidity in patients with a primary infection and problems in the commercial antigen used. This would mean that the antigen in question is not suited for Ab detection against a particular virus subtype with sufficient sensitivity. Alternatively, different denaturing agents may be needed to detect low-avidity Abs with greater sensitivity. In this setting 8 M urea may be less sensitive than diethylamine, because urea may not be able to detect low-adivity Abs more than 3 months after infection, as reported in the case of rubella.[8] However, in each individual case the use of one or other denaturing agent has to be adapted to the type of organism inducing the Ab response and the strength of the denaturing effect caused.[21]

In the literature, revised break points suggested for low-avidity Abs vary between 70 and 75% in some paper[22–25] and are set at 30% in others.[26–27] Estimates depend on the denaturing agent employed, the type of organism in question and the requirements of the individual author. Authors, who argue in favour of higher break points, use 6 M urea or diethylamine as a denaturing agent.[22–25] We used a 50% break point initially and later 55%, discarding samples with values between 50 and 55%. We found that the yield improved slightly – except for HHV-6 (panel 4), where the break point was not set in the same way. A diagnostic yield of near 100% was thus obtained with all viruses. If we take into account that the IgM are not detected in all cases of acute infection, IgG avidity testing and IgM study might resolve conflicting cases. The various settings are IgM-negative primary infection with low-avidity IgG and IgM-positive reactivation with normal avidity IgG, which would indicate a non-recent infection.[21]

Other authors[28] have obtained similar results with EBV using specific ELISA for viral capsid antigen or early antigen. Nevertheless, our system used a pool of antigens

(EBNA, VCA and EA) and it is more simple and automated.

In patients for whom we were able to carry out a serological follow-up, and under the conditions of our study, we found a normalization of avidity 50 days after symptoms of primary infection. These results confirm, with high significance, the value of our study for the differentiation of a short-term or long-term infection.

Taking into account the avidity of IgG in recent primary infections, the study of this parameter may be useful for ascertaining the aetiology of an infection when there are various IgM with cross-reactivity in antigen populations (between HSV and VZV) or because of heterotypical reactions between CMV, EBV and HHV-6.

With the IgA test we obtained lower sensitivity and specificity for the detection of active disease (primary infection or reactivation) as the presence of IgA in healthy subjects reduces the value of this test, as has already been reported.[29-30]

In conclusion, in recent primary infection the IgG have low avidity for the antigen. In past infection or reactivation most IgG have high avidity for the antigen. In recent primary infection there are levels higher than 55% of IgG with low avidity for the antigen. With levels below 55% the sequential study of the normalization of avidity is diagnostic for the suspected infection. Studying the avidity for IgG is relevant for the diagnosis of a primary infection with negative or low positive IgM and for infections, and a diagnosis of past infection with persistent IgM may be differentiated. The avidity test is simple and automated in the laboratory, in contrast to other commercial tests. Techniques quantifying the avidity of specific IgG to HSV, CMV, EBV and HHV-6, together with traditional serological methods (tests for IgG and IgM), are very useful for ascertaining, from a single serum sample, the time since infection. Conversely, the yield of the test for IgA is lower for the detection of active disease.

References

1 de Ory F. Viral infections: interpretation of specific IgM detection assays. *Span J Clin Microbiol* 1991; **25**: 110–112.

2 Grazia M, Percivalle E, Zannini M, Rossi V, Gerna G. Development and evaluation of a capture ELISA for IgM antibody to the human cytomegalovirus major DNA binding protein. *J Virol Meth* 1991; **35**: 315–329.

3 de Ory F, Antonaya J, Fernández V, Echevarría JM. Application of low-avidity immunoglobulin G studies to diagnosis of Epstein-Barr virus infectious mononucleosis. *J Clin Microbiol* 1993; **31**: 1669–1671.

4 de Ory F, Casa I, Domingo CJ, Echevarría JM. Application of fluoroimmunoassay for the identification of low avidity specific IgG against pathogenic human viruses and *Toxoplasma gondii*. *Clin Diagn Virol* 1995; **3**: 323–335.

5 Ward KN, Gray JJ, Fotheringham MW, Sheldon MJ. IgG antibodies to human herpesvirus-6 in young children: changes in avidity of antibody correlate with time after infection. *J Med Virol* 1993; **39**: 131–138.

6 Ward KN, Gray JJ, Joslin ME, Sheldon MJ. Avidity of IgG antibodies to human Herpesvirus-6 distinguishes primary from recurrent infection in organ transplant recipients and excludes cross-reactivity with other Herpesvirus. *J Med Virol* 1993; **39**: 44–49.

7 Fikar CR, Mckee MD. False positivity of IgM antibody to Epstein–Barr viral capsid antigen during acute hepatitis A infection. *Ped Infect Dis J* 1994; **13**: 413–414.

8 Hedman K, Lappalainen M, Söderlund M, Hedman L. Avidity of IgG in serodiagnosis of infectious diseases. *Rev Med Microbiol* 1993; **4**: 123–129.

9 Gray JJ, Caldwell J, Sillis M. The rapid serological diagnosis of infectious mononucleosis. *J Infect* 1992; **25**: 39–46.

10 Gutiérrez J, Maroto C, Piédrola G. Evaluation of a new reagent for anti-Cytomegalovirus and anti-Epstein-Barr virus IgG. *J Clin Microbiol* 1994; **32**: 2603–2605.

11 Blackburn NK, Besselaar TG, Schoub BD, O'Connell KF. Differentiation of primary Cytomegalovirus infection from reactivation using the urea denaturation test for measuring antibody avidity. *J Med Virol* 1991; **33**: 6–9.

12 Hedman K, Vaheri A, Brummer-Korvenkontio M. Rapid diagnosis of Hantavirus disease with an IgG-avidity assay. *Lancet* 1991; **338**: 1353–1356.

13 Meurman O, Waris M, Hedman K. Immunoglobulin G antibody avidity in patients with respiratory syncytial virus infection. *J Clin Microbiol* 1992; **30**: 1479–1484.

14 Schoub BD, Blackburn NK, Johnson S, McAnerney JM, Miller B. Low antibody avidity in elderly chickenpox patients. *J Med Virol* 1992; **37**: 113–115.

15 Thomas HIJ, Morgan-Capner P, Enders G, O'Shea S, Calicott D, Best JM. Persistence of specific IgM and low avidity specific IgG1 following rubella. *J Virol Meth* 1992; **39**: 149–155.

16 Chen HA, Johnson BD, Sims TJ *et al.* Humoral immune responses to *Porphyromonas gingivalis* before and following therapy in rapidly progressive periodontitis patients. *J Periodontol* 1991; **62**: 781–791.

17 Guigno D, Coupland B, Smith EG, Farrell ID, Desselberger U, Caul EO. Primary humoral antibody response to *Coxiella burnetii*, the causative agent of Q fever. *J Clin Microbiol* 1992; **30**: 1958–1967.

18 Lahesmaa-Rantala R, Lehtonen O, Granfors K, Toivaven A. Avidity of anti-*Yersinia* antibodies in yersiniosis patients with and without *Yersinia*-triggered reactive arthritis. *Arth Rheum* 1987; **30**: 1176–1180.

19 Lopatin DE, LaBelle D, Lee SW. Measurement of relative avidity of antibodies reactive with *Porphyromonas (Bacteroides) gingivalis* in the sera of subjects having adult periodontitis. *J Periodontol Res* 1991; **26**: 167–175.

20 Maes RF. Evaluation of avidity of IgG anti-mycobacterial antibodies in tuberculous patients serum by an A-60 immunoassay. *Eur J Epidemiol* 1991; **7**: 188–190.

21 Gutiérrez J, Rodríguez M, Moroto C, Piédrola G. Reliability of four methods for the diagnosis of acute infection by Epstein–Barr virus. *J Clin Lab Anal* 1997; **11**: 78–81.

22 Thomas HIJ, Morgan-Capner P, Connor NS. Adaptation of a commercial rubella-specific IgG to assess specific IgG avidity. *Serodiagn Immunother Infect Dis* 1993; **1**: 13–16.

23 Thomas HIJ, Morgan-Capner P, Cradeck-Watson JE, Enders G, Best JM, O'Shea S. Slow maturation of IgG1 avidity and persistence of specific IgM in congenital rubella: implications for diagnosis and immunopathology. *J Med Virol* 1993; **41**: 196–200.

24 Lappalainen M, Koskela P, Koskiniemi M *et al.* Toxoplasmosis acquired during pregnancy: improved serodiagnosis based on avidity of IgG. *J Infect Dis* 1993; **167**: 691–697.

25 Joynson DHM, Payne RA, Rawal BK. Potential role of IgG avidity for diagnosing toxoplasmosis. *J Clin Pathol* 1990; **43**: 1032–1033.

26 Kangro HO, Manzoor S, Harper DR. Antibody avidity following varicella-zoster virus infections. *J Med Virol* 1991; **33**: 100–105.

27 Enders G, Knotek F. Rubella IgG total antibody avidity and IgG

subclass-specific antibody avidity assay and their role in the differentiation between primary rubella and rubella reinfection. *Infection* 1989; **17**: 218–226.

28 Anderson A, Vetter V, Kreutzer L, Bauer G. Avidities of IgG directed against viral capsid antigen or early antigen: useful markers for significant Epstein–Barr virus serology. *J Med Virol* 1994; **43**: 238–244.

29 Rabie H, Valentine-Thon E, Steinman J, Nehrkorn A. Serological responses to herpes simplex virus type 1 (HSV-1) analysed with enzyme-linked immunosorbent assay (ELISA) and Western-blot (WB). *Acta Virol* 1991; **35**: 113–126.

30 Doerr HW, Rentschler M, Scheifler G. Serologic detection of active infections with human herpes viruses (CMV, EBV, HSV, VZV): diagnostic potential of IgA class and IgG subclass-specific antibodies. *Infection* 1987; **15**: 93–98.