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Effect of Acyclovir and Prednisolone on the Serological Response in Herpes Zoster

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The serological response of patients with acute herpes zoster was studied to determine whether a diagnosis could be made on a single serum sample, and whether this response was modified by treatment with antiviral and/or steroid therapy. The patients received one of four regimes of acyclovir and prednisolone, Varicella zoster virus (VZV) IgG, IgM, and IgA responses were measured by commercial and in-house enzyme immunoassays (EIA) using serum samples taken at days 0, 7, and 21 after entry into the study. Samples were also tested for IgM to Epstein-Barr virus (EBV) viral capsid antigen (VCA), and cytomegalovirus (CMV) IgM and for herpes simplex virus (HSV) antibodies by the complement fixation test (CFT). Analysis was carried out on data from 71 patients. VZV IgM was detected in 72%, VZV IgA in 78%, and either VZV IgM or IgA in 88% of patients tested, at some time during the 3-week study period. The optimal time to detect either class of antibody was approximately 1 week after the onset of the vesicular rash, when 85% of patients had one or both classes of acute phase antibody in their serum. There was no evidence of cross reaction with EBV, CMV, or HSV antibodies. Neither treatment with prednisolone nor the length of therapy with acyclovir affected significantly the VZV IgM or IgA responses. Therefore it is possible to make a serological diagnosis of herpes zoster on a single sample, optimally 1 week after the onset of the rash, in patients treated with acyclovir alone or with acyclovir and steroids.

KEY WORDS: VZV IgM, VZV IgA, enzyme immunoassay, diagnosis

INTRODUCTION

Herpes zoster is caused by the reactivation of latent varicella zoster virus (VZV), and the diagnosis is usually made clinically. Serological diagnosis, however, may be helpful in cases with an atypical rash or in those without a rash (zoster sine herpete). Serological diagnosis is based on a rise in VZV IgG titres, but this may take several weeks as acute and convalescent serum samples are required to be tested. Early diagnosis has become more important as antiviral therapy is now available, and to be effective this should be given as early as possible. Several studies have shown that VZV IgM and IgA can be detected in serum taken within a week of the onset of the vesicular rash [Tovi et al., 1985; Haikin and Sarov, 1982; Wittek et al., 1983; Ross and McDaid, 1972]. We studied the VZV IgG, IgM, and IgA responses in patients with acute herpes zoster who had received one of four regimes of acyclovir and prednisolone [Wood et al., 1994] to determine whether the antibody response was modified by treatment with antiviral and or steroid therapy and whether a diagnosis could be made on a single serum sample.

SUBJECTS AND METHODS

Patient Selection and Assessment

Patients with a clinical diagnosis of herpes zoster with a rash of less than 72 hours duration, and with zoster-related pain were eligible for enrollment in the study. Participants were 18 years of age or older with no known immunosuppressive illness. Patients were assessed clinically on entry into the study and were questioned on prodromal illness and duration of vesicular rash. The major dermatome involved, the number of lesions in that dermatome, and the presence of lesions in adjacent or distant dermatomes were also recorded. The patients were seen on days 0, 1, 2, and 3 and then reassessed twice weekly thereafter until day 21 and on day 28 for evidence of new vesicles and crusting of existing lesions. The patients were also assessed for zoster-related pain for up to a period of 6 months. A serum sample was taken at days 0, 7, and 21. The sera were stored at −20°C.

One hundred patients were enrolled in the study, 25 in each treatment group. The groups were similar with respect to age and sex. The analysis was confined to the 71 patients from whom all three serum samples were collected and tested for VZV IgG and VZV IgM. The sera from a representative sample of 15 patients from each treatment group were tested for VZV IgA.

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The demographic and clinical details have been reported elsewhere together with the effect of the study drugs on the clinical outcome [Wood et al., 1994].

Treatment Regimes

At the initial visit (day 0) the patients were allocated randomly to one of four treatment groups according to a prearranged protocol [Wood et al., 1994]. Groups I and II were treated with acyclovir for 7 days, plus either prednisolone or placebo for 21 days, respectively. Groups III and IV received 21 days of acyclovir plus either prednisolone or placebo for 21 days, respectively. The dose of acyclovir was 800 mg five times a day. The prednisolone was given as a tapering regimen: 40 mg/day for the first 6 days, 30 mg/day on days 7–10, 20 mg/day on days 11–14, 10 mg/day on days 15–18, and 5 mg/day on days 19–21.

Serological Methods

The stored sera were tested for the presence of IgG, IgM, and IgA antibody to VZV. VZV IgG and IgM were detected using commercially available sandwich enzyme immunoassays (HUMAN, Taunusstein, Germany). The tests were done according to the manufacturer's protocol.

An in-house method was developed for the detection of VZV IgA based on the HUMAN VZV IgG assay with several modifications. The optimal dilutions of the serum and conjugate to be used were first determined by screening serial log dilutions (10^{-1}–10^{-4}) of the conjugate, against serial log dilutions (neat to 10^{-5}) of a positive (convalescent sample from a patient with chickenpox) and a negative (sample from a VZV IgG-negative patient) serum. The sera were diluted in the HUMAN VZV IgM assay diluent. This diluent absorbs IgG class antibody, so that rheumatoid factor in the serum does not interfere with the test. A conjugate dilution of 1 in 100 and a serum dilution of 1 in 10 were found to be the optimal dilutions which differentiated most clearly positive from negative sera. These were the dilutions used subsequently for the IgA assay. Thirteen sera from patients without recent history of chickenpox or zoster were then assayed in triplicate, on different days, to establish the cut-off for the assay. The cut-off values were calculated as the mean of the negative controls plus 0.1. A positive control (convalescent sample from a patient with chickenpox) and three negative controls were included in each VZV IgA assay.

To exclude concurrent infections with other herpes viruses, samples taken on day 7 from the 71 patients in the study were tested for Epstein-Barr virus (EBV) capsid antigen (VCA) IgM (Sigma, St. Louis, MO) and cytomegalovirus (CMV) IgM (CAPTIA™, Kentocor, Malvern), by enzyme immunoassay. Samples found to contain EBV VCA IgM were also tested for IgG to Epstein Barr Nuclear Antigen (EBNA) (Sigma, St. Louis, MO). Paired samples (day 0 and 21) were tested for herpes simplex virus (HSV) antibody by complement fixation test (CFT) [Grist et al., 1979].

Statistical Analysis

The chi-squared test was used for statistical analysis, and a P value of $<.05$ was considered statistically significant.

RESULTS

VZV IgG, IgM, and IgA Response

As expected all samples had detectable VZV IgG at days 0, 7, and 21, absorbance values ranging from 0.5 to >2.1.

Fifty-one (72%) patients developed VZV IgM at some stage during the study period (Table I). IgM antibody was detected first in the day 0, 7, and 21 samples from 19, 26, and six patients, respectively (Table II). Peak IgM level was present in the 7th day sample in 27 (53%) patients, and rising values from day 0 or 7 to day 21 with a peak on day 21 were detected in 20 (39%) patients. Four (8%) patients had peak VZV IgM values at day 0 with levels falling subsequently (Table II). Once detected, VZV IgM remained detectable at day 21 in all but five (10%) patients. Of these five patients, four had a peak IgM level in the day 7 sample with IgM becoming negative by day 21, and the fifth patient had an IgM peak at day 0 and had lost the IgM by the 7th day.

Forty-seven (78%) patients had detectable VZV IgA in at least one of the three samples (Table I). IgA antibody was detected first in the day 0, 7, and 21 samples from 22, 24, and one patient, respectively (Table II). Thirty-six (76%) patients had a peak IgA value on day 7, five (11%) patients on day 21, and six (13%) patients on day 0 (Table II). Sixteen (34%) patients lost their VZV IgA during the study period. Four of these had peak IgA level detected at day 0, and two each became negative at day 7 and day 21, respectively. Twelve patients had peak IgA level on day 7 and became negative by day 21.

Combined VZV IgM and IgA Response

Of the 60 patients tested for both VZV IgM and IgA, 53 (88%) had VZV IgM, IgA, or both classes of antibody detected at some time during the study period with 51 (85%) being positive in the day 7 sample (Table III). Seven patients had neither VZV IgM nor VZV IgA detected at any stage during the study period. In 34 patients, both classes of antibody were detected (Table IV). In 14 of these, both classes of antibody appeared initially in the same sample, in nine VZV IgM was detected first, and in 11 VZV IgA was detected first. In six and 13 patients, respectively, VZV IgM and VZV IgA alone was detected.

Serological Response by Treatment Group

The VZV IgG response was similar in all four treatment groups (results not shown). The number of patients from each treatment group who had detectable IgM and IgA at days 0, 7, and 21 and the serological trends observed for VZV IgM and VZV IGA are shown in Table V. Table V also shows, according to treatment group, whether IgM or IgA was detected first or whether both were detected at the same time.

TABLE I. VZV IgM and IgA Response in the Study Group

	No. of patients (%)	
	IgM	IgA
Total tested	74	60
Antibody detected in at least one sample	51 (72)	47 (78)
Antibody not detected in any sample	20 (28)	13 (22)

TABLE II. Sequential IgM and IgA Responses in the Possible Group of Patients (51 of IgM, 47 of IgA)

	IgM	IgA
Antibody initially detected a		
Day 0	19 (37)	22 (47)
Day 7	26 (51)	24 (51)
Day 21	6 (12)	1 (2)
Trend in absorbance values		
Peak at day 21[a]	20 (39)	5 (11)
Peak at day 7	27 (53)	36 (76)
Peak at day 0[b]	4 (8)	6 (13)
Antibody falling to undetectable levels during the study period	5 (10)	16 (34)

[a]Rising throughout the study period, i.e., peak at day 21.
[b]Falling throughout the study period, i.e., peak at day 0.

TABLE III. Number of Patients Positive for VZV IgM and VZV IgA at Days 0, 7, and 21 Post Herpes Zoster

		No. of positive patients (%)			
Antibody tested	No. tested	Day 0	Day 7	Day 21	Total
IgM	71	19 (27)	44 (62)	46 (65)	51 (72)
IgA	60	22 (37)	44 (73)	31 (51)	47 (78)
IgM and IgA[a]	60	31 (53)	51 (85)	47 (78)	53 (88)

[a]The definition of positive patients in this group in those with either VZV IgM and/or VZV IgA detected.

TABLE IV. Appearance of VZV IgM and VZV IgA Antibody According to Treatment Group

	No. of patients					
Treatment regimes	Group I	Group II	Group III	Group IV	Total	*P*
Only IgM detected	3	1	0	2	6	*
Only IgA detected	2	2	4	5	13	*
Both IgM and IgA detected	9	10	8	7	34	**
IgM detected first	3	3	1	2	9	**
IgA detected first	3	4	3	1	11	**
IgM and IgA detected at same time	3	3	4	4	14	**
Neither IgM nor IgA detected	1	2	3	1	7	**

*$.5 > P > .1$.
**$P > .5$.

The five patients in whom the VZV IgM antibody became undetectable had all received steroids (groups I and III). Sixteen patients had lost the VZV IgA by day 21 of study period; nine of these had received steroids (groups I and III) and seven had not received steroids (groups II and IV).

The 34 patients in whom both IgM and IgA were detected were distributed similarly among the four treatment groups (Table IV).

EBV, CMV, and HSV Serology

Four (6%) out of 71 patients were positive for EBV VCA IgM and EBNA IgG on day 7 samples. The four EBV VCA IgM-positive patients were amongst the 53 patients positive for VZV IgM and/or IgA at some time during the study period but not necessarily on the day 7 sample. A fourfold rise in HSV CFT antibody was detected in three (4%) patients. These three patients were also amongst the 53 patients who had both VZV IgM and/or IgA detected but were different from those positive for EBV VCA IgM. CMV IgM was not detected in any of the samples (Table VI).

DISCUSSION

Overall, VZV IgM was detected in 72%, VZV IgA in 78%, and either VZV IgM or IgA in 88% of patients, at

TABLE V. Details of VZV IgM and IgA Response According to Treatment Group

Treatment regimes	Group I		Group II		Group III		Group IV		P	
Mean age (yr)	56		64		62		64			
Sex (M/F)	4/13		6/12		4/14		5/13			
	No. of patients									
	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA	IgM	IgA
No. of patients analysed	17	15	18	15	18	15	18	15		
Antibody detected in at least one sample	14	11	14	12	11	12	12	12	*	**
Antibody not detected in any sample	3	4	4	3	7	3	6	3		
Antibody initially detected at										
Day 0	5	6	6	6	3	6	5	4	**	**
Day 7	11	10	13	12	10	10	10	12	**	**
Day 21	12	9	14	9	8	5	12	8	*	*
Trend in absorbance values										
Peak at day 21[a]	4	2	6	1	5	1	5	1	**	**
Peak at day 7	8	7	8	9	5	9	6	11	**	**
Peak at day 0[b]	2	2	0	2	1	2	1	0	**	**
Not detected in any sample	3	4	4	3	7	3	6	3	*	**
Antibody falling to undetectable levels during the study period	2	2	0	3	3	7	0	4	*	*
Antibody only detected at day 21	2	1	1	0	1	0	2	0	**	*

[a]Rising throughout the study period, i.e., peak at day 21.
[b]Falling throughout the study period, i.e., peak at day 0.
*$.5 > P > .1$
**$P > .5$.

TABLE VI. EBV, CMV, and HSV Serology in the Study Group†

	No. tested	EBV VCA IgM		Rise in HSV CFT titre	
		+*	−	+*	−
VZV IgM or IgA +	53	4	49	3	50
VZV IgM or IgA −	18	0	18	0	18

†+ = positive; − = negative.
*$.5 > P > .1$.

some time during the 3-week study period. The VZV IgM and IgA was detected only for a short duration in 10% and 34% of the patients, respectively, becoming negative by day 21. The optimal time to detect either class of antibody was approximately 1 week after the onset of the vesicular rash. At this time, VZV IgM, IgA, or both classes of antibody were detected in 85% of the patients studied.

Previous studies have shown that VZV IgM and VZV IgA are specific for acute VZV infection [Tovi et al., 1985; Haikin and Sarov, 1982; Wittek et al., 1983; Ross and McDaid, 1972]. These studies have involved small numbers of patients and various techniques, e.g., immunofluorescence [Tovi et al., 1985], radioimmune [Haikin and Sarov, 1982], and immunoperoxidase [Wittek et al., 1983] assays. Enzyme immunoassays (EIA) are widely used in diagnostic virology laboratories. This study shows that this technique can be used to detect acute phase antibody in a majority of patients with acute herpes zoster.

Cross reactions between EBV, CMV, HSV, and VZV antibodies have been noted previously [Karner and Bauer, 1994]. We screened all the patients in the study group for evidence of a current or recent infection with EBV, CMV, and HSV to determine that the VZV IgM and IgA positivity was not due to cross reaction with antibodies to other herpes viruses. None of the 71 patients in the study group were positive for CMV IgM. Four patients had positive EBV serology, which was indicative of either EBV reactivation or late convalescence from EBV infection, as both EBV VCA IgM and EBNA IgG were positive. Three patients had a rise in HSV CFT antibody titre. The four and three patients with positive EBV and HSV serology, respectively, were amongst the 53 patients with positive VZV IgM or IgA; none of the 18 patients with negative VZV IgM and IgA antibodies had either EBV IgM, or CMV IgM detected or a rise in HSV antibodies. However, the association between positive EBV IgM and VZV IgM/IgA positivity and rise in herpes titre and VZV IgM/IgA positivity did not reach statistical significance ($P > .5$ for both). The four EBV IgM-positive patients were also different from these who had a rise in HSV CFT antibody titre. We did not, therefore, find any evidence of genuine cross reaction between VZV, EBV, CMV, and HSV antibodies in our study group. The positive EBV and HSV serology in the four and three patients, respectively, may indicate a genuine re-

cent infection or reactivation with EBV and HSV which was co-incidental with their herpes zoster.

The patients in the four treatment groups were generally well matched on entry into the study as regards demographic and clinical parameters [Wood et al., 1994]. There was no significant difference between the treatment groups as to the number of patients who had VZV IgM or IgA detected, the day peak level of VZV IgM or IgA was detected, or trend in absorbance values of the VZV IgM or IgA during the study period (Table V). The VZV IgM and IgA response was not affected by any of the parameters used to assess the clinical disease before treatment or disease progression during the 3-week study period (results not shown); however, patients with a longer prodromal illness were more likely to have a falling rather than a rising VZV IgM response over the study period. There were five patients in whom VZV IgM fell to undetectable levels during the study, and all had a relatively long and mild clinical disease. However, each of these five patients also received steroids, but the contribution of each of these factors to the early loss of VZV IgM cannot be determined as the numbers were too small for statistical evaluation. There were 16 patients in whom the VZV IgA became undetectable by day 21. Nine of these had received acyclovir plus steroids and seven had received acyclovir alone; this difference was not statistically significant (Table V). There were 15 patients from each treatment group who were tested for VZV IgA in addition to VZV IgM. There was again no significant differences between the treatment groups whether VZV IgM or IgA were detected alone or together or in the order in which each class of antibody appeared (Table IV).

Earlier studies in patients with chickenpox treated with either acyclovir or placebo showed that the cell-mediated and humoral responses were not affected by treatment with a 5-say course of acyclovir [Robart et al., 1993; Dunkle et al., 1991]. Our study shows that longer courses of acyclovir and treatment with steroids also have little effect on either the VZV IgM or VZV IgA responses.

Classically, the diagnosis of herpes zoster is based on the typical clinical presentation of dermatomal rash with sensory changes. Atypical rashes, especially in immunocompromised patients, and herpete sine zoster, may make the clinical diagnosis difficult. We found that steroid treatment did not affect the VZV IgM or VZV IgA antibody response; therefore similar antibody responses may occur in other similarly immunocompromised patients. We therefore conclude that it is usually possible to make a serological diagnosis of herpes zoster on a single serum sample taken within 3 weeks of the onset of the illness, optimally around day 7 of the rash.

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