Confirmation of herpes simplex virus type 2 infections in herpes-like genital lesions by a simple complement-fixation test

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SUMMARY   The presence of complement-fixing antibody to an early herpes simplex virus type 2 (HSV-2) antigen (the AG-4 antigen) was correlated with HSV-2 infection in the sera of patients with genital herpes. Eighty-eight per cent of sera taken two weeks after clinical diagnosis of a primary or recurrent herpes infection in patients, confirmed to have HSV-2 by virus isolation and typing, contained the anti-AG-4 complement-fixing antibody. None of the patients with genital HSV-1 had the antibody, and only 9% of controls or patients with facial HSV-1 infection had positive results for the antibody. This correlation was used to identify genital HSV-2 infections when either no virus sample had been taken or when virus isolations had been unsuccessful. Thus, a simple complement-fixation test can confirm an HSV-2 virus infection without isolation of the virus from the herpetic lesion.

Introduction

Two types of herpes simplex virus have been isolated from genital lesions. While herpes simplex type 2 (HSV-2) is the type most commonly found in ulcerative genital lesions (ranging from 60% to 90% depending on the population under study)[1 2] herpes simplex type 1 (HSV-1) has also been identified in genital lesions.[1 2] Isolation of the herpes simplex virus from the lesion is usually followed by typing by a variety of different methods, such as virus neutralisation,[3] immunoperoxidase methods,[4] thymidine kinase induction,[2] plaque morphology,[5] DNA restriction endonuclease analysis,[6] and others.[7] The unequivocal identification of HSV-2 type antibody in patients' sera has been difficult because of the cross-reaction between HSV-1 and HSV-2 antibody and the high incidence of antibody to type 1 virus in the community.[8] This problem may be overcome to some extent if a type-specific HSV-2 glycoprotein is used as the test antigen or if extensive absorption of sera with HSV-1-infected cells is performed.[9]

We report here that a simple complement-fixation test with a crude four-hour HSV-2-infected HEP-2 cell extract (containing the immediate early AG-4 antigen) can be used to distinguish rapidly between HSV-1 and HSV-2 infections. We have also used this simple test to confirm clinically diagnosed genital herpes as an HSV-2 infection.

Materials and methods

CELLS AND VIRUSES
Human epidermoid carcinoma (HEP-2) cells and HSV-2 strain G used to make the AG-4 antigen came from stocks held at the microbiology department, La Trobe University, Melbourne. (Both are also available from the American type culture collection, Rockville, Maryland, USA.) HSV-2 was propagated and titrated by plaque assay on HEP-2 cells maintained in Eagle's minimal essential medium (EMEM) supplemented with 10% newborn-calf serum (NBC) and 100 μg/ml neomycin.

BLOOD SAMPLES
Blood samples were obtained from 64 patients (40 women and 24 men, aged 18-30 years) with genital herpes-like lesions (from either Dr D Bradford of the Communicable Diseases Centre, Melbourne, or the physicians at La Trobe University Health Centre, Melbourne). Samples were also obtained from a control group of 23 patients, matched for age and sex distribution, without past or present HSV-2 infection. Blood was collected as soon as possible from these sources (usually within an hour) and sera harvested after storage of the blood overnight at 4°C.

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PREPARATION OF AG-4 ANTIGEN

HEP-2 cells were grown in Roux bottles in a humidified 5% CO_2 atmosphere at 37°C, and infected with HSV-2 strain G at a multiplicity of infection of 0·7 plaque-forming units (pfu)/cell for AG-4 preparation. The virus was adsorbed for one hour at 37°C and the cells then overlaid with EMEM supplemented with 1% newborn-calf serum and 100 μg/ml neomycin and incubated for a further four hours at 37°C. The medium was then removed, and the cells scraped off in sterile barbitone-buffered saline (BBS) and washed three times. The final cell pellet was resuspended in 0·7 ml of BBS per 1 × 10^7 cells, frozen and thawed four times, centrifuged at 1600 × g for 15 minutes to remove cell debris, and stored at −70°C until used. Control antigen (AG-H) was prepared in the same way with mock-infected HEP-2 cells. The antigen concentration was adjusted to 2·0 mg/ml of protein.[10]

COMPLEMENT FIXATION

The technique was performed essentially as described.[10] The test was carried out in polystyrene tubes (Disposable Products Pty Ltd, Salisbury, S Australia). Guinea-pig complement, haemolysin, and sheep red blood cells (SRBC) were obtained from the Commonwealth Serum Laboratories, Melbourne, Australia. The test sera were diluted 1/4 in BBS and inactivated by heat at 56°C for 30 minutes. For the test, 0·1 ml of diluted serum, 0·1 ml of antigen or BBS, and 0·2 ml (2 units) of complement were incubated at 4°C for 17 hours. The tubes were then warmed at 37°C for 10 minutes, and 0·1 ml of BBS, 0·1 of haemolysin (2 units), and 0·1 ml of 2% SRBC (5 × 10^7cells/ml) added. After 60 minutes' incubation at 37°C the results were read. In all tests one standard positive and one standard negative control serum were included. The reaction was considered positive for AG-4 if 10% or more complement was fixed with AG-4 than with the control AG-H preparation. Also included was a set of standards representing the range 0-100% complement fixation. In every case in this study, no complement was ever fixed with the control AG-H preparation, although in previous studies some AG-H reactivity was observed.[10]

Each serum sample was tested for activity against AG-4 and AG-H together with a control serum to establish any anticomplementary activity. All test samples were run in duplicate in at least three separate experiments. For routine purposes a 1/4 dilution of sera was used to measure the presence of AG-4 antibody but some sera can be diluted further.

VIRUS ISOLATION AND TYPING

Virus samples were usually collected from herpes-like lesions with a sterile cotton applicator and placed in transport media by the physicians at the time the patients attended with lesions. The samples were forwarded to the Fairfield Infectious Disease Hospital, Melbourne, for isolation and typing by a standard virus neutralisation method.[3] Essentially, the herpes virus isolate was initially grown and isolated in human embryo lung (HEL) cells. The virus (100 TCD_{50}) was then added to microtitre plate wells (Lindbro tissue culture, Flow Laboratories, Australia) together with diluted antisera to either HSV-1 (MacIntyre) or HSV-2 (MS).[10] The cytopathic effect of residual herpes virus infectivity was determined on HEL cells. The antisera used in these virus neutralisation tests were prepared in rabbits by repeated injection of HSV-1 or HSV-2 over a four-year period. At the dilutions used (usually 1/25) the anti-HSV-1 sera neutralised both HSV-1 and HSV-2 viruses while the anti-HSV-2 sera neutralised only HSV-2 viruses. The results of the herpes simplex virus typing reached the physicians about four weeks after the sample was sent. Only about 4% of genital herpes isolates were typed as HSV-1 by Fairfield Infectious Disease Hospital (K Hayes, personal communication). The results of these typing tests were available to us only after the anti-AG-4 antibody titres had been determined.

Results

TIME OF ANTIBODY DETECTION

The time-course appearance of complement-fixing antibody to AG-4 in recurrent HSV-2 and in primary HSV-1 and HSV-2 infections in patients with genital herpes is shown in the figure. Over the five-week period no anti-AG-4 complement-fixing antibody was detected in the patients with genital HSV-1 infections. The antibody was usually detected two weeks after a primary infection of HSV-2 and was still present five weeks later. On the other hand, in
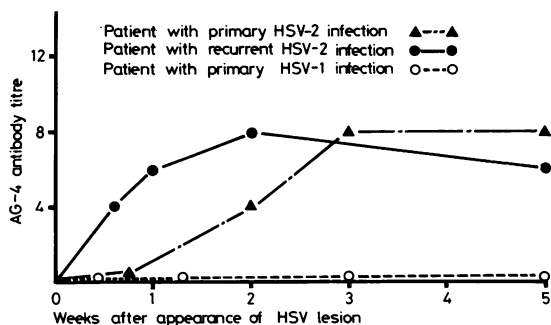

FIGURE *Appearance of anti-AG-4 complement-fixing antibody in the sera of three patients with herpes infection.*

50% of patients with recurrent HSV-2 infections (figure) the anti-AG-4 complement-fixing antibody could be detected at four days and increased to a maximum titre at two weeks after the lesions appeared. After this time, in unusual recurrent cases, the complement-fixing antibody titre may rise to as high as 1/64 or may fall rapidly.[11] Thus, the best time to take sera for the AG-4 complement-fixation test, for both recurrent and primary HSV-2 infections, is at two weeks after the appearance of lesions.

FREQUENCY OF ANTIBODY DETECTION

The frequency of appearance of anti-AG-4 complement-fixing antibody in a variety of patients with herpes-like lesions is shown in the table. The sera tested were taken 14 days after lesions appeared (although from most patients we had more than one serum sample at different time periods). In 29 of 33 (88%) patients with HSV-2 infections confirmed by virus isolation and neutralisation the antibody was present in their sera (at titres ranging from 1/4-1/64, mean titre 1/8; only three patients had titres >1/32). Sera with complement fixation at <1/4 were considered negative; those in controls that were positive had titres of 1/4-1/8.

In the genital HSV-1 infections no antibody was detected and in both the patients with facial HSV-1 and the controls only 9% of sera contained the antibody. Occasionally swabs from the herpes-like lesions yielded a virus that was not typed as herpes simplex and these patients did not have the antibody. When sera were taken from patients with genital herpes-like lesions, and no isolation of virus attempted, 82% contained anti-AG-4 complement-fixing antibody. The anti-AG-4 antibody titres were compared with the virus typing data only after the former were known.

If a similar extract of HEP-2 cells infected with HSV-1 for four hours (similar to the AG-4 pre-paration—that is, multiplicity of infection at 0·7 pfu/cell) was used in the test no complement fixation was detected with sera from patients with either HSV-2 or HSV-1 infections. If the HSV-1

infection rate was greater than 3 pfu/cell or the infection was allowed to proceed for 18 hours rather than four hours, however, most of the 14 days' HSV-2 sera (approximately 90%) would react with the HSV-1 extract in the complement-fixation test. This was probably because of the cross-reaction of HSV-1 antibody (HSV-neutralising antibody increases by twofold or threefold after infection[11]) with the late HSV-1 antigens.

Discussion

We have found that the antigen that patients with HSV-2 infections react to in HSV-2 four-hour-infected HEP-2 cells is an immediate early protein of 160 000 molecular weight.[11] This antigen has previously been used to detect anti-AG-4 complement-fixing antibodies in patients with cancer of the cervix.[10 12] We have found that it would also detect AG-4 antibodies in patients infected with HSV-2[10 11] but not in those infected with HSV-1.[10] Detection of the anti-AG-4 complement-fixing antibody in HSV-2 patients is, however, dependent on the time at which the sera are taken after recurrence or primary herpetic infection (figure). When sera are taken 14 days after the appearance of the herpes-like lesion, or sometimes earlier in recurrent episodes, this simple complement-fixation test can detect whether the lesion is due to either HSV-2 or HSV-1. Sera taken at the time of presentation with HSV-2 lesions were positive for the antibody only in some recurrent cases, but once the antibody appeared (in most cases) it was found to persist for six months.[11] Decline in the antibody titres has been found as early as four weeks[11] and in other recurrent cases it has persisted for at least 18 months. Thus it is possible in most cases to repeat or perform the complement-fixation test more than two weeks after the appearance of lesions.

Our results suggest that the sera of 88% of the patients with HSV-2 infections (diagnosed by virus isolation and typing by a neutralisation method)

TABLE *Frequency of anti-AG-4 complement-fixing antibodies in the various patient groups*

Patient group	No tested	Positive results for AG-4 antibody	
		No	%
Confirmed genital HSV-2	33	29	88
Confirmed genital HSV-1	4	0	0
Non-genital facial HSV-1	12	1	8·3
Herpes-like lesions but no virus isolation attempted (or virus sample not infectous)	11	9	82
Herpes-like lesions (virus other than herpes isolated*)	4	0	0
Controls (no HSV infection)	23	2	8·7

* Usually not identified other than as non-herpes virus. (Adenoviruses are occasionally isolated from the genital tract.[13])

contained the anti-AG-4 complement-fixing anti-body. It is unlikely that any herpes typing technique is completely unambiguous,[3] although restriction technology has claimed to be so.[6] This complement-fixation test is simple and the results are obtained quickly and do not require virus isolation. Moreover, the test was not interfered with by the presence of HSV-1 antibody in the sera, a problem occurring with most serological tests,[1] and could be used well after the initial appearance of the herpes lesions. The anti-AG-4 complement-fixation test was found to be most useful when either no virus isolation was attempted at the time of examination or it was un-successful in confirming that the genital lesion was caused by HSV-2 and not HSV-1.

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