

GENITAL HERPES

Virological Diagnosis and Antibody Response

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Abstract. Virological findings and the serological response of 44 patients with clinically diagnosed herpetic infection were studied. Virus identification and typing direct from exfoliated cells of lesions by indirect immunofluorescence was compared with conventional isolation and typing procedures. The sensitivity of immunofluorescence was somewhat less than that of isolation but its specificity permitted direct typing of the infectious herpes virus type. Antibody response was studied by kinetic neutralization and indirect immunofluorescent antibody assay. Patients without herpes virus type 2 antibodies in initial sera responded serologically to the infection, while patients with preexisting type 2 antibodies did not show a rise in antibody titre. The lack of correlation between the previous history and initial antibody status indicates the possibility of primary genital herpes infections with only minor symptoms.

The high frequency of Herpesvirus Hominis (HVH) type 2 antibodies observed in cervical cancer patients has aroused new interest in the epidemiology of this virus (6, 11). The prevalence of HVH type 2 antibodies in normal population varies considerably in different socioeconomic groups. Approximately one-tenth of normal adults have been found to possess these antibodies according to recent studies (8, 9). The venereal transmission of this infection in adults has been well documented (reviewed in (4)). In primary infections the clinical picture is often characterized by vesicular eruption and systemic signs. In recurrent forms and perhaps also in reinfections the symptoms may be milder. The lesion often consists of erosions which must be rapidly differentiated from syphilis and other venereal diseases. Serological tests and the demonstration of treponemes are used to prove syphilis. On the other hand, a rapid and reliable demonstration of herpesvirus helps in the differential diagnosis

and renders the presence of venereal disease unlikely.

Direct immunofluorescence has been successfully used in the identification and typing of herpesvirus from exfoliated cells (3). The indirect technique has been used in this laboratory for typing of herpesvirus isolates (to be published), and it was therefore of interest to evaluate the possibilities of this modification for the rapid diagnosis of genital herpetic infection. The sensitivity of different cell lines for conventional virus isolation was compared. Finally, the antibody response in patients with reference to initial antibody pattern and clinical data about previous episodes was studied.

MATERIAL AND METHODS

Clinical material

Patients attending the Venereal Diseases Clinic of the University Central Hospital of Helsinki and who had been clinically diagnosed as suffering from genital herpetic infection were included in this study. They represented different phases of the illness but most of them had had symptoms for few days only (Table I). The majority of these patients were males (40 males, 4 females) and their mean age was 29.2 years. Fifteen of them had had previous attacks.

Collection of samples

Samples for virus isolation were taken from lesions with a cotton swab and transported to the virus laboratory in balanced salt solution with 1% bovine serum albumin and antibiotics. Cells for immunofluorescence were scraped with another swab from lesions and dispersed on slides with ringed spots. After air-drying, the slides were fixed in acetone for 5 min. Serum samples were taken at the first visit and a second sample was obtained from most patients 2 weeks or more later.

Table 1. Duration of symptoms before examination in patients with genital herpes

	Duration of symptoms, days				
	1-3	4-5	6-7	≥ 8	it ^a
No. of patients	18	8	6	7	5

^a Unknown.*Virus isolation*

In the laboratory the swabs were removed from the transport medium after agitation. Into each tissue culture monolayer tube 0.2 ml of the fluid was inoculated. Four types of cells were used during the study: Human amnion (HA) (Bio-Cult and Flow Laboratories, Irvine, Scotland), primary human embryonic skin fibroblasts (HES), continuous monkey kidney cells (BSC-1) and primary monkey kidney cells (MK) (Flow Laboratories). At least two different cell lines were inoculated with each sample. When a cytopathic effect (CPE) suggesting herpetic infection was observed, cells were detached mechanically, washed twice with PBS and dispersed on slides. The cells were fixed with acetone for 3 min at +4° and the virus strain was typed with indirect immunofluorescence (P. L. in press).

Immunofluorescent staining of smear cells (FA)

The slides were washed for 10 min in phosphate-buffered saline, rinsed in distilled water and air-dried. In the staining procedure, type 1 rabbit antiserum was used in a dilution reacting with homologous virus only and type 2 antiserum in a dilution 4 times stronger than its heterologous, i.e. type 1 titre, which reacted strongly with both types. The conjugate was commercially available (Sevac, Prague) anti-rabbit gamma-globulin of swine origin with a Fluorescein/Protein ratio 1.7-3.0. Amido black was used as a counterstain. The working dilution contained 4 units as determined from plateau titre in checkerboard titrations. Cells giving a positive reaction with only type 2 antiserum were regarded as infected with type 2 virus and cells positive with both sera as infected with type 1 virus.

Antibody titrations

A micromodification of the complement fixation technique (CF) was used with type 1 virus antigen.

Immunofluorescent assay (IFAT) for type-specific herpes virus antibodies has been described (1).

For neutralizing antibody titration (NT), serial two-fold dilutions of heat-inactivated patients' sera were incubated with about 400 plaque-forming units (PFU) of reference type 1 and type 2 virus strains (1) for 1 hour at room temperature. From each dilution the surviving virus was assayed in cultures of Vero cells. Virus incubated with normal rabbit serum was used as a control. A pN-value (neutralizing capacity) was calculated according to the formula $pN = \log A + \log \log V_0/V$ (12) where V is the amount of surviving virus incubated with pa-

tients serum. V_0 the amount of surviving virus incubated with normal rabbit serum and A the dilution of the serum. For each dilution a pN value was calculated and the mean value was the pN value of the serum.

The type of antibody was determined according to Nahmias et al. (5) by calculating a pN difference $pN1 - pN2$, where pN1 means the neutralizing capacity of the serum with type 1 virus and pN2 with type 2 virus. A value <0.05 indicates type 2 antibodies.

With IFAT, and index

$$\left(1 - \frac{\log \text{type 2 titre}}{\log \text{type 1 titre}} \cdot 100\right) > 85$$

indicated type 2 antibodies, while $I < 85$ indicated type 1 antibodies (10).

RESULTS

Clinical manifestations

The clinical manifestations of probable genital herpes in the present series are shown in Table II. In the majority of patients a group of erosions was present. In seven cases these were combined with regional lymphadenitis or fever. In two cases, vesicles were present 9 and 10 days after the onset of symptoms. In most cases, however, the vesicles were ruptured very early—in 15 patients erosions were the only manifestation of the disease even 1 to 3 days after the onset of symptoms.

Virus isolations

From the patients, 30 herpes virus strains were isolated. These 30 strains together with 26 other herpes virus strains from patients with various cutaneous infections were isolated with the same technique. This permitted the comparison of the sensitivity of the cell types used. HA, HES and BSC-1 cells were equally sensitive, while MK was definitely less sensitive (Table III).

Twenty-seven of the strains isolated from patients with genital herpes were of type 2. From one patient type 1 virus was isolated from a penile lesion. In addition, two type 1 strains were isolated from a patient with simultaneous outbreak

Table II. Distribution of clinical manifestations in patients with genital herpes

V, vesicles; E, erosions; L, lymphadenitis; F, fever; P, papules

	Manifestations						
	V	VF	VE	E	EL	EF	P
No. of patients	8	1	8	19	5	2	1

of herpetic erosions on forehead, mouth, and penis. The strains from forehead and mouth were type 1 while the penile strain was type 2.

Sensitivity and specificity of

FA technique

Adequate samples for both isolation and FA staining were obtained from 35 of the present 44 patients. From 14 patients with vesicles as a clinical sign of the disease FA and virus isolation were positive in 8 and 10 cases, respectively. In patients with only erosions as the local sign of the disease, FA was positive in 8 out of 19 cases and virus isolation was positive in 18 out of 22 cases. In two cases FA was positive but isolation negative, although serological evidence of herpesvirus infection was obvious in both cases. From non-venereal patients studied at the same time, 35 patients were positive with both techniques; FA was negative in 25, though virus could be isolated, and in 9 cases FA was positive and isolation negative. These results show that the sensitivity of FA is about one-third smaller than the conventional isolation technique (Table IV).

FA seems to be quite efficient in the differentiation between types 1 and 2; only in one case out

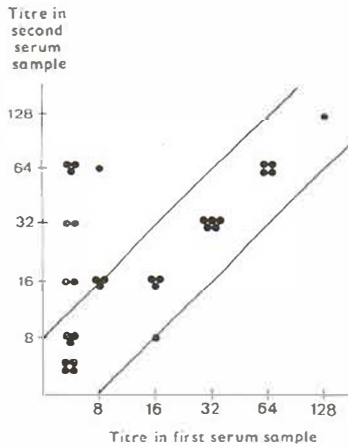


Fig. 1. Complement-fixing HHV antibodies in paired sera of patients with genital herpes.

of 15 did FA give a false type when compared with the subsequent typing of the isolate.

Antibody titrations

In the initial serum samples, 46% of the patients were seronegative with CF. With IFAT the number of seronegatives was smaller; only 11% did not react with type 1 virus infected cells at a dilution of 1/10, while 22% were negative with type 2 infected cells. However, some of the first serum samples were taken more than 5 days after the onset of symptoms. If these are omitted, the percentage for seronegative patients with CF was 56%, with IFAT type 1 24%, and type 2 43%.

Less than one-fourth of patients showed a seroconversion with the CF technique. 17 patients had constant titre and 4 remained seronegative. With IFAT the number of seroconversions was the same as with CF but in addition 5 patients showed a significant increase in titre. All patients had herpes virus antibodies in their convalescent sera, thus demonstrating the greater sensitivity of this technique compared with CF (Figs. 1 and 2). Four of the 5 patients with no initial HHV antibodies had a type 2 antibody response (Table V). From 1 patient both type 1 and type 2 viruses were isolated. He had a history of previous attacks in spite of the absence of herpes virus antibodies in his initial serum sample. When measured with IFAT, he developed a type 1 antibody pattern, while with neutralization his antibodies could be considered as type 2.

Nine patients had initial type 2 antibodies

Table III. Comparative sensitivity of cell lines used for primary isolation

Cell lines inoculated 1 / 11	Positives 1 / 11
HA / HES ^a	7 / 8
HA / BSC-1	22 / 23
BSC-1 / HES	15 / 16
MK / Other ^b	5 / 9

^a For abbreviations, see text.

^b Either HA, HES, or BSC-1.

Table IV. Immunofluorescent demonstration of herpesvirus from exfoliated cells (FA) compared with virus isolation in patients with genital and other herpes infections

Patients	n	FA-positive (%)	Isolation-positive (%)
Genital herpes	35	48	77
Others	69	64	87

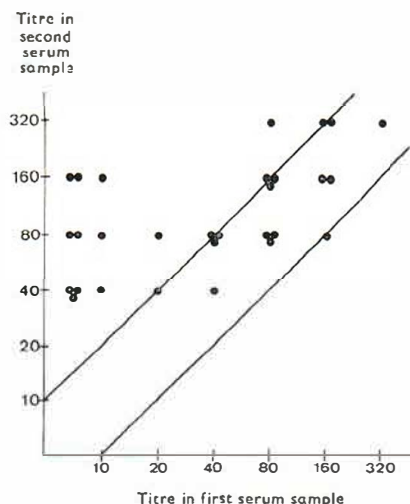


Fig. 2. Immunofluorescent HVH type 2 antibodies in paired sera of patients with genital herpes.

(Table VI). In only one of them was a significant increase in titre observed.

In the 9 patients with type 1 initial antibodies, 7 showed a significant increase in IFAT type 2 antibody titre. Two had no significant change in titre and from these, evidence of type 1 virus infection was obtained (Table VII).

Correlation between previous history and clinical and serological data

Fifteen patients had had previous attacks and 2 of them lacked initial HVH type 2 antibodies. Both these patients had a seroconversion during

Table V. Antibody titre in acute and convalescent sera of 5 patients with a seroconversion

IFAT 1 = Immunofluorescent antibody titre with type 1;
IFAT 2 = Immunofluorescent antibody titre with type 2;
pN1-pN2 = neutralization capacity difference between type 1 and type 2 virus

Patient no.	IFAT 1	IFAT 2	pN1-pN2
4	<10	<10	
	10	40	-0.3
18	<10	<10	
	320	160	-0.1
31	<10	<10	
	20	160	-0.9
44	<10	<10	
	20	80	-0.6
22	<10	<10	
	160	40	-0.3 ^a

^a From this patient both type 1 and type 2 viruses were isolated. See text.

Table VI. Antibody titre in acute and convalescent sera of patients with initial type 2 antibodies

pN1-pN2 <0.05 and/or log IFAT type 2/log IFAT type 1 $\times 100 > 85$

Patient no.	IFAT 1 ^a	IFAT 2	pN1-pN2
2	160	80	-0.2
	80	160	-0.2
6	40	40	-0.0
	80	80	-0.1
15	40	80	-0.1
	40	160	-0.1
19	160	80	-0.2
	80	80	-0.2
21	20	80	n.d. ^b
	80	320	
26	80	160	n.d.
	80	160	
39	40	160	n.d.
	80	160	
41	160	320	n.d.
	160	320	
45	80	160	n.d.
	40	80	

^a For explanation, see Table V.

^b n.d. = not done.

the illness. Clinically, one of them had a vesicular eruption combined with fever, but in the other, only erosions without any additional signs were observed.

Table VII. Antibody titre in acute and convalescent sera of patients with initial type 1 antibodies

pN1-pN2 >0.5 and/or log IFAT type 2/log IFAT type 1 $\times 100 < 85$

Patient no.	IFAT 1	IFAT 2	pN1-pN2
5	40	20	+0.5
	40	80	+0.2
10	160	20	+1.3
	160	80	+0.8
11	20	10	n.d.
	40	160	
14	10	<10	n.d.
	40	80	
16	160	80	+0.7 ^a
	160	80	+0.7
17	40	10	+0.8
	40	40	+0.6
25	80	40	+0.6 ^b
	160	20	+0.6
32	20	<10	n.d.
	40	40	
38	10	<10	n.d.
	40	80	

^a From this patient type 1 virus was isolated.

^b From this patient type 1 virus was demonstrated with FA from lesion.

Nine of the patients with no history of previous attacks had type 2 antibody pattern in their initial serum. Vesicular eruption was observed in the genitals of 4 of them. Only 2 of the 5 patients with erosive lesions had regional lymphadenitis.

DISCUSSION

Indirect immunofluorescence offers two definite advantages in the diagnosis of HVH infections. It can be used in the identification and typing of the isolated strains, and for the physician, the rapidity of the diagnosis direct from exfoliated cells is of great value. The efficiency of this method is very much dependent on the amount and quality of the cells obtained (3). In our study the samples were taken by the physician. FA technique sensitivity was about equal to the conventional virus isolation technique in patients with vesicular lesions but in patients with erosive manifestations of the disease, virus isolation was definitely more sensitive than the FA technique. Inadequate number of cells in smears probably explains the inferior sensitivity. This technique is so simple, however, that with repeated samples the loss of sensitivity could possibly be compensated.

Positive identification of syphilis can, in the earliest stages of the disease, be achieved only by the demonstration of treponemes. The demonstration of herpes virus with the FA technique makes a rapid diagnosis possible in herpetic infections and helps to exclude syphilis.

A specificity of high degree can be achieved with FA techniques. In series of experimentally infected mice and clinical patients, Nahmias et al. (5) obtained a correct typing in 91% with direct FA. In the current study a similar specificity rate was observed with an indirect technique. Although the identification of herpes virus antigen is sufficient for the differential diagnosis of herpes genitalis from venereal diseases, the identification of type 2 antigen is important from the epidemiological point of view. It also has important implications in neonatal herpetic infections, where the identification of HVH type 2 in encephalitic newborns warrants the use of systemic IDU treatment (7).

The role of pre-existing HVH antibodies in the protection from heterotypic infections and rein-

fections is difficult to evaluate. Herpes can be inoculated into patients with preexisting antibodies (2). On the other hand, patients with a latent herpes infection can have undetectable antibody levels, as evidenced by the patient with a simultaneous outbreak of multiple herpetic lesions in this series. In a material of 122 cases with genital herpes, Nahmias et al. (5) found one-third of patients without initial herpes antibodies. Rawls et al. (9) found the same proportion of seronegatives by neutralization technique. In our material the proportion of patients without initial herpesvirus CF antibodies did not differ from the normal population. Also, with the more sensitive IFAT technique the number of seronegative patients was comparable to the normal population (P. Leinikki, O. Pettay, O. Widholm: unpublished observations). These results suggest that patients contracting clinically diagnosed genital herpes have an antibody pattern similar to that of a normal population of the same age. This speaks in favour of the view that initial HVH antibodies do not afford protection from genital herpetic infection.

The serological response in patients varied depending on the antibody status in their initial serum sample. All patients with a seroconversion presented a type 2 antibody response by neutralization technique. On the other hand, most patients with initial type 2 antibodies had a constant titre. The patients who initially had type 1 antibody pattern had a demonstrable increase in antibody titre during a HVH type 2 infection. This could be demonstrated with IFAT, where the type 1 titre mostly remained constant in spite of a four-fold or greater increase in type 2 titre.

Only partial agreement was obtained between the anamnestic information of previous history of genital herpetic attacks and the serological response. One-third of patients with no history had a type 2 antibody pattern in their initial sera. Furthermore, the mild clinical forms observed in patients with seroconversion seem to indicate that a primary herpetic infection of the genitals may, in the adult, be clinically very mild or possibly even remain unnoticed.

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