JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1988, p. 1623–1625
0095-1137/88/091623-03$02.00/0
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Vol. 26, No. 9

Use of Murine Monoclonal Antibodies for Laboratory Diagnosis of Varicella-Zoster Virus Infection

CURT A. GLEAVES,* CARRIE F. LEE, CARLOS I. BUSTAMANTE,† AND JOEL D. MEYERS

Program in Infectious Diseases and Diagnostic Virology Laboratory, Fred Hutchinson Cancer Research Center, and University of Washington School of Medicine, Seattle, Washington 98104

Received 28 December 1987/Accepted 18 May 1988

The laboratory diagnosis of varicella-zoster virus (VZV) infection was reevaluated by direct immunofluorescent-antibody staining (DFA) and centrifugation culture with newly available murine monoclonal antibodies. Specimen smears were examined by DFA using monoclonal antibodies to VZV and to herpes simplex virus types 1 and 2. Specimens were also inoculated into shell vials for centrifugation culture and into standard tube cell culture. Of 68 specimens tested from 60 patients, 39 (57%) were positive for VZV by at least one method. DFA was positive in 36 of 39 (92%); centrifugation culture was positive at 24 h in 23 of 39 (59%) and at 48 h in 31 of 39 (79%); and standard culture was positive in 25 of 39 (64%). Twenty-three of the 39 positive specimens (59%) were positive by all three techniques. Forty-three of the 60 patients were considered to have VZV by clinical criteria, and 35 of these 43 (81%) had laboratory confirmation of the diagnosis. These data confirm that DFA is the method of choice for the rapid laboratory confirmation of VZV infection. The centrifugation culture assay can provide an alternative method to DFA for the laboratory diagnosis of VZV infection.

Varicella-zoster virus (VZV) can cause severe or fatal disease in the immunocompromised patient (13, 14). Providing a rapid specific diagnosis of VZV infection can facilitate the earlier administration of antiviral chemotherapy (2, 10). It is also important to differentiate VZV infection from the vesicular eruptions caused by bacterial agents, hypersensitivity reactions, or other viruses including herpes simplex virus (HSV) so that appropriate therapy can be initiated and unnecessary treatment can be avoided.

Viral isolation remains the definitive diagnostic procedure. However, growth of VZV in cell culture is usually too slow to be clinically useful, and in many cases VZV may not be isolated in routine cell culture due to the lability of this virus (3, 9). Previous studies have shown that detection of VZV by direct immunofluorescent-antibody staining (DFA) on specimens prepared from skin lesions with VZV-specific polyclonal antisera is more rapid and sensitive than and as specific for the laboratory diagnosis of VZV infection as standard cell culture (3, 8, 9).

With the recent development of murine monoclonal antibodies specific for VZV and the increasing use of the centrifugation culture assay, we reevaluated the laboratory diagnosis of VZV infection. In this study the sensitivity of DFA and centrifugation culture with new murine monoclonal antibodies specific for VZV were compared with isolation of VZV in standard cell culture for the laboratory confirmation of VZV infection.

MATERIALS AND METHODS

Specimens. A total of 68 specimens were obtained from skin lesions from 55 marrow transplant recipients and 5 normal persons with clinical signs suggestive of reactivated (56 patients) or primary (4 patients) VZV infection. By using a Dacroswab A (Spectrum Diagnostics, Glenwood, Ill.), cells were obtained from the base of the lesion(s) by vigorous scraping, and specimen smears were made on each well of a three-well slide (Carlson Scientific, Peotoene, Ill.). A companion swab taken from the same or an adjoining lesion was inserted into viral transport media (veal infusion broth containing 2% gelatin and antibiotics). The air-dried slides were then fixed in cold acetone for 10 min and stained by the DFA method within 2 to 24 h. The swabs contained in viral transport media were inoculated into centrifugation and standard cell culture the same day.

DFA. Specimen smears were examined by DFA by using fluorescein-labeled monoclonal antibodies specific for VZV (Ortho Diagnostics, Carpinteria, Calif.) (12), and HSV type 1 (HSV-1) and HSV-2 (Syva Co., Palo Alto, Calif.) (7). These reagents all contained Evans blue as a counterstain. Samples of 50 μl of reagent were applied to the appropriate wells, and the slides were incubated at 36°C for 30 min in a moist chamber. After incubation the slides were rinsed in distilled water and air dried, after which they were mounted in a glycerol-based mounting medium (pH 9.0; Syva) and examined with an epifluorescence Zeiss microscope at a magnification of ×250.

Centrifugation culture. Clinical specimens obtained in viral transport media were vortexed and inoculated at a volume of 0.2 ml per vial into each of four or six 1-dram (ca. 3.7-ml) shell vials containing cover slip cultures of MRC-5 cells; 50 specimens were set up in six shell vials, whereas 18 specimens were set up in four shell vials only (see below). Centrifugation was performed at 700 × *g* for 1 h at 34°C, after which 1.0 ml of Eagle minimal essential medium containing 2% fetal bovine serum and antibiotics was added to each vial. Cultures were then incubated at 36°C for 24 to 48 h. After incubation the medium was removed, and the cover slips were washed twice with phosphate-buffered saline–Tween 20 (pH 7.2) and then fixed with cold acetone for 20 min inside the vial (4, 5).

All 68 specimens were stained in the vial by indirect immunofluorescence with murine monoclonal antibodies B5

* Corresponding author.
† Present address: University of Maryland Cancer Center, Baltimore, MD 21201.

TABLE 1. Detection of VZV from 68 clinical specimens by three laboratory methods

Test	Antibody[a]	Time (h)	No. of positive specimens
DFA[b]			36
Centrifugation culture	B5	24	23
	B5	48	31
	H6	24	16[c]
	H6	48	28[c]
Standard cell culture			25
Any method			39

[a] Monoclonal antibody used in indirect immunofluorescence (see text).
[b] Ortho VZV monoclonal antibody used.
[c] All were positive with the B5 antibody.

and H6 (Syva). The B5 monoclonal antibody is reactive with the major VZV glycoprotein (gpI) (gp90/gp58) and primarily stains cytoplasmic antigens. The H6 monoclonal antibody reacts with the 170,000-molecular-weight nonglycosolated phosphoprotein and stains predominantly nuclear antigen. One cover slip of each specimen was stained at 24 and 48 h with each of the separate monoclonal reagents B5 and H6. Briefly, 150 μl of the appropriate reagent was added to each vial and incubated at 36°C for 30 min. The cover slips were washed twice for 5 min each in phosphate-buffered saline. To each vial 150 μl of goat anti-mouse fluorescein isothiocyanate-labeled conjugate (Tago, Burlingame, Calif.) was then added and incubated as described above. The cover slips were washed as described above, counterstained with Evans blue, and mounted cell side down on a glass slide in gycerol-buffered medium (pH 9.0).

Additionally, 50 of the 68 specimens were stained by direct immunofluorescence with the directly conjugated monoclonal antibody (Ortho Diagnostics). This antibody also detects gpI (gp90/gp58) of VZV (12). As described above, one cover slip of each specimen was stained at 24 and 48 h with 150 μl of the reagent. After incubation at 36°C for 30 min, the cover slips were rinsed briefly with distilled water, blotted, mounted, and examined as above.

Standard cell culture. Specimens were inoculated at a volume of 0.2 ml per tube into two 16- by 125-mm culture tubes containing human foreskin fibroblast monolayers. Cultures were maintained for 5 weeks before being considered negative. Cultures showing typical cytopathic effect for VZV were confirmed by subculture and subsequent staining by direct immunofluorescence with the direct monoclonal reagent (Ortho).

RESULTS

VZV was identified in 39 of 68 specimens (57%) from 35 of 60 patients by at least one of the laboratory methods. Thirty-two patients had reactivation disease, and three had primary VZV infection. Of these 39 positive specimens, 36 specimens (92%) were positive by DFA. Centrifugation culture detected 23 of 39 (59%) at 24 h and 31 of 39 (79%) at 48 h. VZV was isolated in standard cell culture from 25 specimens (64%) at a mean of 12.5 days (Table 1). The DFA assay appeared better then centrifugation and standard cell culture for VZV detection. With McNemar's paired analysis, there was a significant difference in the DFA test compared with standard culture ($P < 0.01$) and with the 24-h centrifugation culture assay ($P < 0.01$). Comparison of DFA with the 48-h centrifugation culture showed no significant difference.

Of the monoclonal antibodies used in the centrifugation culture assay, B5 detected VZV in all 23 specimens positive by this technique at 24 h and all 31 specimens positive at 48 h. The H6 antibody detected VZV in 16 of these 23 specimens at 24 h and 28 of these 31 specimens after 48 h. Staining with the B5 reagent was also much easier to interpret than staining with the H6 reagent. The directly conjugated monoclonal antibody reagent was used in staining of only 50 of the 68 specimens. Of these 50, 15 specimens were positive at 24 h and 20 were positive at 48 h. The staining characteristics were similar to that of the B5 reagent. All specimens positive with the direct reagent were also positive in corresponding cover slips with the B5 reagent, and the direct reagent did not fail to detect any specimens which were positive with the B5 reagent.

Of the 39 positive specimens, 23 (62%) were detected by all three laboratory methods. Of the 16 specimens with discrepant results, 8 were positive by DFA only, 5 were positive by DFA and centrifugation culture, 2 were positive by centrifugation culture and standard culture, and 1 was positive by centrifugation culture only. The three specimens negative by DFA all had smears containing no or relatively few cells.

Lesions of 25 of the 60 patients were not confirmed as VZV by any of these laboratory methods. Thirteen of these 25 patients had other diagnoses ultimately made. These included six with specimens positive for HSV by DFA and standard cell culture (three HSV-1 and three HSV-2), two diagnosed as roseola infantum, one as porokeratosis, and four as graft-versus-host disease. Of the six specimens positive for HSV, the VZV antibodies (the Ortho antibody used in DFA, B5 used in centrifugation culture) did not cross-react with these HSV samples. Conversely, by DFA the positive VZV specimens did not cross-react with the HSV monoclonal antibodies. Four patients had no final laboratory or clinical diagnosis. Eight specimens came from eight patients considered on clinical grounds to have true VZV infection. Of these eight, two had inadequate DFA smears and one patient had received 5 days of acyclovir treatment at the time of testing. If these 8 are included in the calculation of the sensitivity of diagnosis, the combined sensitivity of these techniques appeared to be 83% (39 of 47) based on specimens examined or 81% (35 of 43) based on patients examined.

DISCUSSION

DFA with a murine monoclonal antibody reactive with VZV was more sensitive than either centrifugation culture or standard cell culture for the rapid laboratory diagnosis of VZV infection, detecting 36 of 39 (92%) positive specimens; eight of 39 specimens (21%) were positive only by DFA. The finding that DFA was more sensitive than either cell culture method was not unexpected. Using polyclonal reagents, both Schmidt et al. and Drew and Mintz showed that direct detection of VZV in cellular smears of vesicular lesions was more sensitive than isolation of the virus in standard cell culture (3, 9). Among the proposed reasons for this difference in sensitivity is that VZV is a very labile agent and thus specimens must be processed rapidly for ideal viral isolation (13), and that VZV antigen may be detected by DFA in specimens taken late after the onset of infection and after the time at which infectious virus is present (9).

Centrifugation culture was also more sensitive than standard cell culture, although we did not include blind passage of standard cultures, which might have increased the yield

(1, 4–6). Recently, Smith reported that 3 of 11 VZV isolates detected by centrifugation culture (8 at 48 h and 3 at 120 h postinoculation) were not recovered in standard cell culture (11). Examination of centrifugation cultures beyond 48 h was not part of our study design, and the possibility exists that some of the eight specimens positive only by DFA might have been positive in centrifugation culture at later times. Monoclonal antibody B5 and the directly conjugated monoclonal antibody were equally sensitive in detecting VZV in those specimens examined with both reagents in centrifugation culture. Both detected more specimens than the H6 monoclonal antibody. Both produced similar staining of cytoplasmic antigens, which was easily recognized by immunofluorescence, whereas the staining pattern of the H6 antibody was weak in intensity and difficult to interpret. In all likelihood the B5 antibody and the Ortho VZV antibody are detecting the same glycoprotein antigen, namely, gpI, which has been reported by Weigle and Grose to be the predominant VZV glycoprotein (12). Either the B5 or the directly conjugated antibody would be acceptable for use in centrifugation culture. Only the direct monoclonal antibody is presently available commercially.

Eight patients (all marrow transplant patients) were considered to have true VZV infection by clinical criteria but were not confirmed as such by any of the three laboratory methods used in this study. As described above, specimens from two of these patients were inadequate for DFA and another patient had received 5 days of antiviral treatment before the specimen was obtained. The remaining five specimens were all considered adequate for DFA examination, and no technical problems were observed with either of the two cell culture techniques. There are several possible explanations for these apparent false-negatives. Specimen smears were taken by a variety of medical personnel, and it is possible that variability between DFA and culture techniques was thereby introduced. Lability of VZV in culture is mentioned above. It is possible that even these newer methods for the laboratory detection of VZV or the reagents used in these assays are indeed only about 85% sensitive when used to examine clinically obtained specimens. This agrees with the findings of Drew and Mintz; 86% of the patients in their study who had clinical features of typical herpes zoster were confirmed as having VZV infection by DFA when polyclonal reagents were used (3).

The data from this study confirm that DFA is most sensitive and therefore the method of choice for the laboratory diagnosis of VZV infection. Thirty-two of 35 patients in this study had reactivation VZV disease, but we presume that results would be similar among patients with primary VZV infection. The availability of a directly conjugated murine monoclonal antibodies increases the ease with which these tests can be performed in the clinical laboratory, although the sensitivity was not greater than that achieved with polyclonal antibodies in other studies (3, 9). The centrifugation culture assay can provide an alternative rapid method for the laboratory diagnosis of VZV infection, especially if smears for DFA are not available or are not optimal for examination.

ACKNOWLEDGMENTS

This investigation by Public Health Service grants CA 18029 and CA 15704 from the National Cancer Institute and grant HL 36444 from the National Heart, Lung, and Blood Institute.

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