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Monoclonal Antibodies Reactive with All Strains of *Haemophilus ducreyi*

ERIC J. HANSEN* AND THERESA A. LOFTUS

Department of Microbiology, Graduate School of Biomedical Sciences, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Received 17 October 1983/Accepted 4 January 1984

We have isolated plasma cell hybridomas which secrete monoclonal antibodies directed against *Haemophilus ducreyi*. Two of these monoclonal antibodies recognize all strains of *H. ducreyi* tested to date and are capable of detecting the presence of *H. ducreyi* in skin lesions produced by this pathogen in experimental animals. These monoclonal antibodies which react with apparently all strains of *H. ducreyi* have the potential to be developed into a rapid immunodiagnostic test for chancroid.

Chancroid is one of the classic sexually transmitted diseases and has traditionally been regarded as occurring primarily in tropical countries (1, 2). However, an increasing number of reports over the past few years indicate that this disease, which is characterized by painful genital ulcers with or without lymphadenopathy, also occurs in most of the industrialized nations of Europe and North America (1, 7, 9, 14). Unfortunately, there is a paucity of information concerning the incidence and epidemiology of chancroid, primarily owing to the difficulties inherent in making an accurate diagnosis of chancroid. Early studies of chancroid were hampered by the fact that *Haemophilus ducreyi*, the causative agent of chancroid, is a fastidious microorganism, and only recently have improved methods for the isolation and identification of *H. ducreyi* been developed (8, 9, 12, 16, 21). Consequently, diagnosis of chancroid has often been based on a combination of the clinical picture and the exclusion of the other etiological agents, such as *Treponema pallidum* and herpes simplex virus type 2, of genital ulcer disease (4).

The most rapid methods for diagnosis of infectious disease usually involve immunological techniques which employ antibodies as specific probes for a pathogen or its antigen(s) in clinical specimens. There has been only one report of an experimental serological test for the diagnosis of chancroid, and this procedure involved the use of animal sera which had to be extensively adsorbed with other bacteria to eliminate cross-reacting antibodies (2). However, the development of monoclonal antibody technology has permitted the ready isolation and essentially unlimited production of antibodies specific for microbial antigens with diagnostic importance. Monoclonal antibodies specific for *Chlamydia trachomatis* (22), *T. pallidum* (19), and herpes simplex virus type 2 (17) have recently been produced and will assuredly be useful in the development or improvement of rapid diagnostic tests for their associated sexually transmitted diseases. To prepare highly specific biological reagents with potential for development into a rapid immunodiagnostic test for chancroid, we isolated a number of plasma cell hybridomas which produce monoclonal antibodies directed against *H. ducreyi*.

H. ducreyi strains were grown on the medium described by Hammond et al. (9). Hybridoma production involved the use of 8-week-old BALB/c mice immunized by intraperitoneal injection with 10^9 CFU of *H. ducreyi* strain 35000 suspended in phosphate-buffered saline (pH 7.2) or in Freund complete adjuvant (Difco Laboratories, Detroit, Mich.). A month later, these mice were given a second injection of *H. ducreyi* identical to the first, and 3 days later, spleens were removed from these animals and used in a standard hybridoma fusion procedure described previously (18). Culture supernatant fluids of the resultant hybrids were screened for the presence of monoclonal antibodies directed against *H. ducreyi* in an enzyme-linked immunosorbent assay system which employed cell envelopes from sonically disrupted *H. ducreyi* cells as antigen (10 μg of protein per well) (18). Hybridomas producing *H. ducreyi*-specific monoclonal antibodies were cloned by the use of a limiting dilution technique (18).

A total of 56 hybrids producing monoclonal antibodies directed against *H. ducreyi* were identified in this manner. All of these monoclonal antibodies react with the immunizing strain of *H. ducreyi* (strain 35000) which was an isolate obtained from the 1975 chancroid outbreak in Winnipeg, Canada (9). Radioimmunoprecipitation analysis demonstrated that several of these monoclonal antibodies are directed against proteinaceous cell envelope antigens of *H. ducreyi* (Fig. 1). *H. ducreyi* cells were radioiodinated by a lactoperoxidase-catalyzed procedure and then solubilized in a detergent solution exactly as described previously (5, 6). A portion of the solubilized *H. ducreyi* cells was then incubated with each individual monoclonal antibody, and radioimmunoprecipitation analysis was performed exactly as described previously, except that 50 μl of protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) was used in place of protein A-bearing *Staphylococcus aureus* cells to precipitate soluble immune complexes involving IgG antibodies and *H. ducreyi* antigens. The protein A-Sepharose–antibody–antigen complexes were washed extensively and dissociated by being heated at 100°C in digestion buffer (6); the protein A-Sepharose was then removed from suspension by centrifugation. Radioiodinated *H. ducreyi* proteins present in the immune precipitates were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and identified by autoradiography (5, 6).

For a monoclonal antibody to be useful in a diagnostic test for chancroid, it must be capable of reacting with most, if not all, strains of *H. ducreyi*. A colony blot-radioimmunoassay system (6) modified from that described by Henning et al. (11) was employed to screen these monoclonal antibodies against a panel of 12 *H. ducreyi* strains collected from

* Corresponding author.

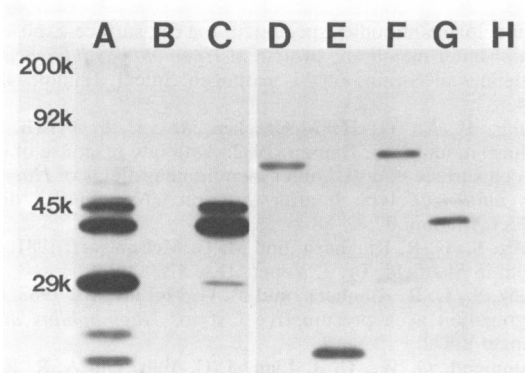

FIG. 1. Radioimmunoprecipitation of ^{125}I-labeled *H. ducreyi* proteins by monoclonal antibodies. ^{125}I-labeled proteins present in solubilized *H. ducreyi* cells employed as antigen (lane A). ^{125}I-labeled proteins precipitated by 500 μl of the following control reagents or hybridoma culture supernatant fluids containing monoclonal antibodies: phosphate-buffered saline (lane B); monoclonal antibody 9D12 (lane C); monoclonal antibody 5B9 (lane D); monoclonal antibody 8H4 (lane E); monoclonal antibody 11H3 (lane F); monoclonal antibody 11F7 (lane G); phosphate-buffered saline (lane H). Molecular weight position markers are provided on the left side of the autoradiograph (k, kilodaltons). Some variable and nonspecific binding to the protein A-Sepharose of a protein with an apparent molecular weight of 29,000 is visible in certain lanes. Autoradiography was performed as described previously (6).

several different countries around the world (Fig. 2). A small quantity of bacterial colony material from each strain was spotted onto a strip of filter paper (Whatman no. 40), which was then dried at 37°C for 1 h, and soaked for 1 h in phosphate-buffered saline (pH 7.2) containing 1% normal rabbit serum (NRS buffer) to block non-specific protein binding sites on the paper or colony material. Each filter strip was then incubated for 2 h in NRS buffer containing a different hybridoma culture supernatant fluid (at 10% final concentration) as the source of monoclonal antibody. Each filter strip was then washed four times (30 min each) with NRS buffer to remove unattached antibody, and then each strip was incubated in NRS buffer containing affinity-purified and radioiodinated rabbit anti-mouse immunoglobulin (10^6 cpm) as a probe for mouse monoclonal antibodies attached to *H. ducreyi*. Unattached radioactive probe was then washed away and the strips were exposed to X-ray film for autoradiography. A dark spot developed on the film if the monoclonal antibody reacted with the individual *H. ducreyi* strain.

The vast majority of these monoclonal antibodies, as exemplified by monoclonal antibody 8A7 (subclass IgG1), recognize both the immunizing strain of *H. ducreyi* and several other strains in the test panel, but do not react with all 12 strains (Fig. 2). In contrast, several different monoclonal antibodies, as exemplified by monoclonal antibodies 8H4 (subclass IgG2a) and 9D12 (subclass IgG2a), react with all the strains in the test panel. These latter two monoclonal antibodies are directed against different *H. ducreyi* antigens, as determined by radioimmunoprecipitation analysis (Fig. 1).

This same basic radioimmunoassay system was employed to evaluate these monoclonal antibodies for their diagnostic potential. Viable cells of the immunizing strain of *H. ducreyi* were deposited by intradermal injection into six sites on the back and sides of rabbits in the standard procedure employed to test the virulence of *H. ducreyi* isolates, as described by Hammond et al. (10). At 72 h postinoculation, a small sample of tissue was scraped from the surface of each of the resultant lesions. This tissue was homogenized in 1,000 μl of phosphate-buffered saline, and 1.0 μl of the resultant suspension was spotted onto a filter paper strip and processed in the radioimmunoassay system described above using monoclonal antibodies 8H4 and 9D12 as specific probes for *H. ducreyi*. Both of these monoclonal antibodies are able to readily detect the presence of *H. ducreyi* in the lesion material (Fig. 3). In contrast, these monoclonal antibodies do not react with either purified *T. pallidum* organisms or testicular tissue from rabbits with *T. pallidum*-induced orchitis (Fig. 3). In addition, in preliminary tests monoclonal antibody 8H4 has been shown not to react with either herpes simplex virus type 2 or *Neisseria gonorrhoeae*. Furthermore, monoclonal antibody 8H4 does not recognize either *Haemophilus influenzae* or *Haemophilus parainfluenzae* (data not shown), both of which can be part of the normal genital flora in men (13).

It must be emphasized that the technique used for the detection of *H. ducreyi* in skin lesions that was employed in this study does not represent the preferred embodiment for the use of these monoclonal antibodies in diagnostic tests. It should be possible to use these monoclonal antibodies to develop either direct or indirect immunofluorescent techniques or, preferably, more simple but equally accurate methods like coagglutination. Although the use of a single monoclonal antibody specific for a given antigenic determinant might prevent a coagglutination test from functioning properly, owing to little or no cross-linking, the existence of monoclonal antibodies which both recognize apparently all strains of *H. ducreyi* and react with different antigens of this pathogen indicates that a mixture of two or more monoclonal antibodies with different antigenic specificities might be employed successfully for this purpose. Finally, with the recent introduction of new and improved methods for coupling enzymatic reagents to antibody probes that also simul-

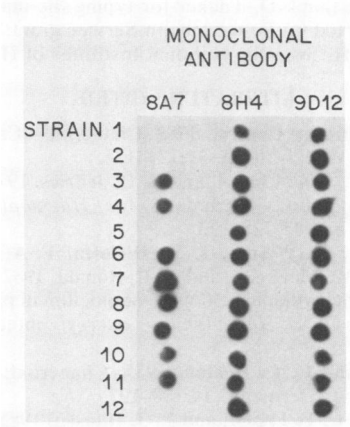

FIG. 2. Autoradiograph obtained from a colony blot radioimmunoassay illustrating the specificities of monoclonal antibodies 8A7, 8H4, and 9D12 for twelve different strains of *H. ducreyi*. Each dark spot indicates a positive reaction between a monoclonal antibody and a *H. ducreyi* strain. Strains 1 and 2 were isolated in Georgia in 1979 (21); strain 3 was isolated in Winnipeg, Canada in 1975 (9); strain 4 is a Pasteur Institute type strain; strain 5 was isolated in California in 1981; strain 6 was isolated in Baltimore in 1935; strain 7 was isolated in Kenya in 1979; strains 8, 9, and 10 were isolated from U.S. servicemen returning from the Far East (16); strains 11 and 12 were isolated in Singapore (20).

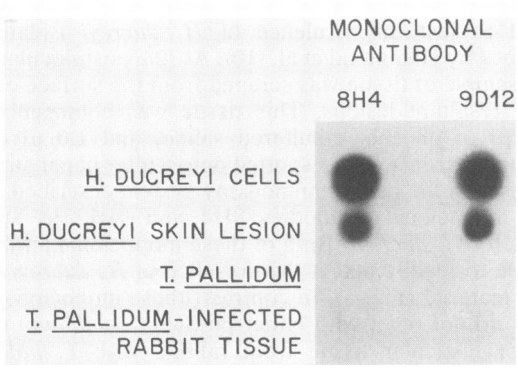

FIG. 3. Autoradiograph obtained from a colony blot-radioimmunoassay illustrating the specificity of monoclonal antibodies 8H4 and 9D12 for *H. ducreyi* cells from a colony on an agar plate, *H. ducreyi* cells or antigen in tissue scraped from a *H. ducreyi*-induced skin lesion in a rabbit, *T. pallidum* cells purified from rabbit testicular tissue, and *T. pallidum*-infected rabbit testicular tissue. A 1.0-μl amount of bacterial cells or tissue homogenized in phosphate-buffered saline was spotted onto the filter paper for use in the colony blot radioimmunoassay.

taneously increase the sensitivity of the detection system (23), it should be possible to utilize these *H. ducreyi*-specific monoclonal antibodies in the design of a simple and rapid diagnostic test for chancroid.

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