impressed with the speed with which the technology has progressed since 1974 and can be confident that if we invest wisely, this rate will be maintained or even increased.

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Monoclonal Antibodies for Diagnosis of Infectious Diseases in Humans

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Within its unique ecological niche the human organism serves as a biological reservoir for a vast array of microorganisms, ranging from viruses and bacteria to fungi and multicellular parasites (*1*). Most of the host's interactions with these microorganisms are without consequence to health, because physical barriers in the body (such as the gut) or the immune system maintain the microorganisms at a tolerable level. However, in instances of immune nonresponsiveness, or in circumstances involving infection with highly pathogenic organisms, this normal equilibrium is upset and the health of the host is threatened. When this occurs, there is a pressing need to rapidly and specifically identify the overgrowing organisms with a view to designing therapies capable of either restoring the appropriate biological balance or entirely eliminating the pathogens from the body. This need for specific, rapid diagnosis and prompt, targeted therapy has become of paramount importance with (i) the dramatic increase of antibiotic-resistant bacteria in our society, (ii) the growing importance of progressive infections in the immunocompromised host, and (iii) the advent of newer forms of antiviral therapy.

Conventional Diagnosis of Infectious Diseases

Infectious diseases are generally diagnosed by four methods (*2*): (i) microscopic examination of tissue specimens and exudates, with the visual identification of either virus-infected cells (that may show inclusion bodies), bacteria, fungi, or parasites; (ii) culture methods, with the use of selective growth media that allow the amplification of small numbers of organisms that can be tested for susceptibility to potential therapeutic agents; (iii) immunological identification in tissues or body fluids of antigens associated with specific pathogens; or (iv) measurement of specific antibodies produced in the patient as a result of infection with an organism.

Given the complexity of infectious diseases, it is not surprising that no one diagnostic method has proved optimal for all situations. Instead, depending on the particular infection, laboratories commonly use a combination of two or more of the four different diagnostic methods. Thus, while microscopic identification may yield an unequivocal diagnosis of a multicellular parasite, this method is of limited value in identifying viruses. Culture methods, in contrast, provide an unambiguous determination of infectivity and are extremely sensitive; however, these methods also tend to be labor intensive and to involve lengthy incubation periods, and many common pathogens are difficult to grow in culture. Direct identification of pathogens by antibodies, while providing a rapid and specific method for diagnosis, is dependent on the specificity and strength of the antiserums used, which are known to vary considerably. In addition, since many microorganisms are antigenically related to each other, antibodies may demonstrate cross-reactions between pathogenic and nonpathogenic forms.

Monoclonal Antibody Technology

Recently, monoclonal antibody techniques have provided an opportunity to reevaluate the role of immunological methods for the diagnosis of infectious diseases. As a result of the pioneering studies of Kohler and Milstein (*3*), it is now possible to create immortal cloned

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cell lines that continuously and reproducibly produce unique antibody molecules. Since these cell lines can be obtained at high frequency (10^{-5} to 10^{-6}) it is relatively easy to scan an enormous repertoire of cells in order to select those that produce antibodies that critically distinguish antigens of different microorganisms.

Immortal cell lines are prepared by the chemically mediated fusion in vitro of lymphocytes from immunized mice and cells from a mouse tumor (myeloma) (*3*). The resultant hybrid cells acquire both antibody-producing potential (from the normal lymphocyte) and the ability to grow permanently in culture (from the myeloma). Cloned hybrid cells produce individual monoclonal antibodies in a continuous and virtually endless supply. Further, inoculation of the hybrid cells into the peritoneal cavity of compatible mice results in a tumor (referred to as a hybridoma) that secretes high concentrations (1 to 20 milligrams per milliliter) of monoclonal antibody into the tumor ascites fluid. By tapping the ascites fluid and purifying the monoclonal antibody, individual mice can sometimes provide sufficient antibody to perform 10,000 to 50,000 diagnostic assays. As would be expected from their clonal derivation, these antibodies (i) demonstrate extremely precise specificity, (ii) react with uniform avidity, and (iii) can be readily purified to homogeneity, providing reagent-grade materials for analysis.

Monoclonal antibody techniques are now widely practiced and antibodies of diagnostic potential have been prepared in research laboratories against a battery of viruses (*4*), bacteria (*5*), and parasites (*6*). In our laboratories at Genetic Systems Corporation and the University of Washington, we are routinely using monoclonal antibodies for the detection of human sexually transmitted infections. In a joint program with Syva Company (Palo Alto, California) these antibodies will be used to develop a line of rapid, easy-to-perform diagnostic tests. Since the results of our collective studies point to advantages and disadvantages of monoclonal antibody–based diagnostics, we use them in this article for further illustration and discussion.

Summary. Monoclonal antibody techniques are now widely practiced, and antibodies of diagnostic potential have been prepared in research laboratories against a battery of viruses, bacteria, fungi, and parasites. In this article the diagnostic utility of monoclonal antibodies for gonococcal, chlamydial, and herpesvirus infections is described. With immunofluorescence being used as an assay system, the antibodies demonstrated sensitivities of 94 to 99 percent for culture confirmation and 85 to 90 percent for direct diagnosis of specimens smeared on microscope slides. Since the direct diagnostic tests only required 15 to 20 minutes to perform, they represented a major advance in the diagnosis of infections which previously required 3 to 6 days to accomplish. It is anticipated that in the next decade an entirely new spectrum of antibody-based diagnostics will be developed, allowing the rapid, precise, and semiautomated diagnosis of many infectious diseases.

Human Sexually Transmitted Diseases

As changes in sexual attitudes and activities have occurred in our society, sexually transmitted diseases (STD) have become more commonplace. In the United States alone, new infections with three of the most common STD pathogens: *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and herpes simplex virus (HSV) type 2 are believed to approach 10 million cases annually. In recent years the role of these infections in a wide spectrum of diseases has emerged. These three STD pathogens and their associated diseases are described below.

Neisseria gonorrhoeae. Approximately 1 million new cases of gonorrhoea are reported annually to the Centers for Disease Control in Atlanta (*7*), and it is estimated that the true incidence of gonorrhoea in the United States exceeds 2 million cases annually. *Neisseria gonorrhoeae* causes urethritis and epididymitis in men; cervicitis, urethritis, endometritis, and salpingitis in women (*8*); and proctitis and pharyngeal infection in both sexes. Approximately 1 to 3 percent of infected individuals develop disseminated gonococcal infection, with systemic complications including arthritis, dermatitis, endocarditis, and meningitis (*8*). The bacterium has also been implicated as a cause of morbidity during pregnancy, including chorioamnionitis, premature rupture of membranes, and premature delivery. Neonatal conjunctivitis as a result of infection at birth remains an important cause of blindness in some developing countries.

Detection of the gonococcus is accomplished either by Gram stain and microscopic examination of a patient's specimen, or by culture of the bacterium on a selective medium (*8*). Results of the culture are confirmed by oxidase reaction, morphology, and sugar utilization tests, or by immunological analysis. In males with urethritis, the Gram stain has a positive predictive value of > 95 percent and requires only 3 to 5 minutes for performance; the same technique in females is considerably less sensitive and at best detects only about 40 to 50 percent of the cervical infections diagnosed by culture. Consequently, cultures with confirmatory tests are the preferred methods for diagnosis in women. These methods, however, require 48 to 72 hours for completion.

Chlamydia trachomatis. Since chlamydial infections are not reported to federal agencies, their incidence can only be estimated. While the prevalence of *C. trachomatis* infection is slightly higher than that of *N. gonorrhoeae* infection in STD clinic populations, the prevalence of chlamydial infection is several times higher than that of gonococcal infection in obstetric, family planning, and student health clinic populations. Thus, the incidence of *C. trachomatis* infections probably is at least 5 to 10 million cases annually in the United States (*9*).

Like *N. gonorrhoeae*, *C. trachomatis* causes infections of the urethra, cervix, rectum, and conjunctivae, and commonly leads to endometriosis, salpingitis, and epididymitis (*10*). Thus, chlamydial infections mimic gonococcal infections. In addition, *C. trachomatis* is a common cause of pneumonia in young infants, may be a precipitating factor in Reiter's syndrome, and in developing countries is the cause of lymphogranuloma venereum (*10*).

The clinical similarities of these two infections are further complicated by the fact that *N. gonorrhoeae* and *C. trachomatis* often are co-transmitted. Approximately 20 percent of heterosexual males and 40 percent of women with gonorrhoea are also infected with chlamydia. Separately, or in combination, chlamydial and gonococcal infections are thought to be responsible for most of the estimated 850,000 annual cases of pelvic inflammatory disease in the United States, resulting in permanent infertility in an estimated 15 to 20 percent of these women (*10*). Approximately 5 to 10 percent of pregnant women previously studied in the United States have been infected with chlamydia, and transmission to the newborn results in ocular, nasopharyngeal, or respiratory infections in about two-thirds of the exposed infants (*10*).

Direct detection of chlamydia infections is not possible at present, except possibly in the diagnosis of neonatal chlamydial conjunctivitis. Since chlamydia are obligate intracellular pathogens, they can be isolated only in chick embryos or mammalian cells. The incubation period for isolation in mammalian cell cultures is 3 days to 1 week (*11*). These cultures are technically difficult and expensive to perform; as a consequence, the inability of most clinicians to conveniently diagnose chlamydia has markedly impaired control of these infections.

Herpesviridae. Herpes simplex virus (HSV) infections are among the most common infections of humans (*12*). Once acquired, these infections demonstrate a life-long pattern of episodic recurrence, such that each infected individual serves as a permanent carrier who is intermittently infectious. The virus can be classified into two subgroups according to genetic and antigenic composition, and by their patterns of infection: HSV type 1 is responsible for recurring orolabial lesions (cold sores), pharyngitis, ocular keratitis, and encephalitis; HSV type 2 is responsible for most genital herpes in adults, and for most neonatal infections. It is now apparent that the "anatomical" mode of classifying these viruses is not truly accurate, as a significant proportion (15 to 50 percent) of primary genital herpes is caused by HSV 1 (*13*). However, the probability of recurrent genital herpes is significantly lower after primary genital HSV 1 infection than after primary genital HSV 2 infection.

Infections with HSV 1 are widespread, particularly in populations of lower socioeconomic status. Infections with HSV 2, although less prevalent than HSV 1 infections, are becoming increasingly common. It has been estimated that approximately 300,000 to 600,000 new cases of genital herpes occur each year, and from 5 to 10 million new or recurrent episodes of genital herpes occur each year in the United States (*14*).

Diagnosis of HSV infection is routinely performed by cell culture. In most cases, cultures yield definitive evidence of virus (that is, cytopathic effect) within 3 to 6 days. After growth of the HSV in culture, detailed typing for HSV 1 or HSV 2 can be accomplished by either immunological analysis (*15*) or by restriction endonuclease analysis of viral DNA (*16*). Classification of HSV's into either of the two subgroups serves several purposes, including (i) prognosis, since the recurrence rate of genital HSV 1 infection is considerably less than that of genital HSV 2 (*13*); (ii) treatment, since certain antiviral drugs demonstrate preferential activity for one of the HSV types [for example, (E)-5-(2-bromorinyl)-2′-deoxyuridine is much more active against HSV 1 than against HSV 2] (*17*); and (iii) epidemiological, for assessing the association of HSV infection with other disease processes (for example, cervical carcinoma) (*12*).

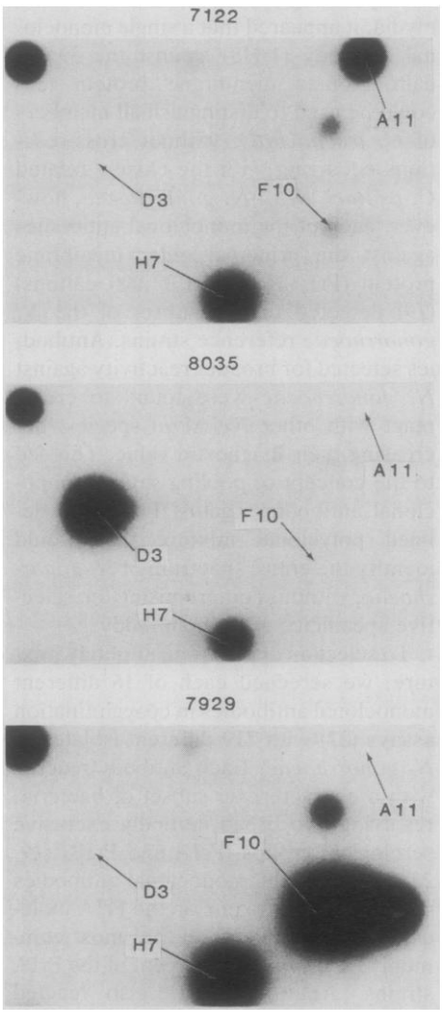

Fig. 1. Replicate-plating method for the detection of monoclonal antibodies against *N. gonorrhoeae*. Culture fluids from a 96-well microtest plate were replicate-plated into microtest plates onto which three different *N. gonorrhoeae* antigen extracts were adsorbed. Immune reactions were detected by the addition of ^{125}I-labeled protein A and subsequent autoradiography.

Preparation of Monoclonal Antibodies

Inherent in the hybridoma technology are difficulties associated with the fusion of somatic cells and the intermingling of two separate sets of genetic information. During early cell divisions after fusion hybrids undergo random loss of chromosomes. As a consequence, the ability to produce antibody may be lost from some of the hybrids. Since hybrids that do not produce antibody grow more rapidly than those that do, overgrowth of the former is certain to occur in mixed cell cultures. The antibody-producing hybrids must therefore be removed and cultured separately. Further, the segregation of chromosomes from the hybrid cells also results in considerable variability in growth properties, making it necessary for investigators to devote individual attention to each cell line. Since the hybridization procedure yields far too many cell lines for individual attention (perhaps 10^4 different hybrids in a good fusion), the initial screening for antibody production is of critical importance.

To obtain maximum information in the early screening, we have adapted replicate-plating techniques from bacterial genetics for the testing of antibody specificities (*18*). To accomplish this, we place hybrid cells in 96-well microtest plates. A small sample of culture fluid is then removed from each well and placed in replicate plates with care being taken to maintain the same physical orientation of the samples. Each of the replicate

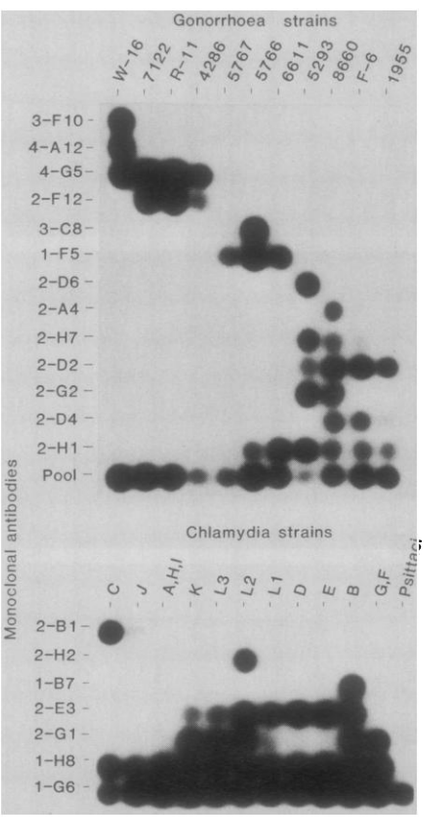

Fig. 2. Binding assays of monoclonal antibodies with different isolates of *N. gonorrhoeae* or *C. trachomatis*. Culture fluids were tested on common serotyping reference strains. Also included were tests with a pool of three culture fluids containing antibodies against *N. gonorrhoeae*. Immune reactions were detected by the addition of ^{125}I-labeled protein A and subsequent autoradiography.

plates contains a different antigen adsorbed onto the surfaces of the wells. As many as eight to ten antigen-adsorbed plates can be used, and immune reactions are detected by means of a radioimmunoassay in which ^{125}I-labeled protein A is added to each of the wells which are then examined by autoradiography.

Figure 1 shows an example of a radioimmunoassay of culture fluids containing antibodies against *N. gonorrhoeae*. In this test the culture fluids from a single 96-well plate were replicate-plated onto membrane extracts of three different gonorrhoeal strains (NRL 7122, 8035, and 7929). Four unique antibodies were identified in this test; three of the antibodies (wells A-11, F-10, and D-3) reacted predominantly with only a single bacterial antigen, whereas one of the antibodies (well H-7) reacted with all three of the antigens. In this manner it was possible to rapidly compare individual antibody activities and to select those antibodies desired for continued development.

Selection of Antibodies with Diagnostic Potential

Replicate-plating methods have been used to prepare three independent panels of monoclonal antibodies that distinguish *N. gonorrhoeae*, *C. trachomatis*, and HSV (HSV 1 and HSV 2) from each other and from other common microorganisms (*19–21*). Each antibody in these panels reacts with a single specificity, identifying a particular antigenic determinant of one of these microorganisms.

Examination of more than 1000 monoclonal antibodies against these microorganisms revealed a remarkable diversity in the repertoire of antigens recognized by the mouse (*19–21*). In some instances the monoclonal antibodies identified antigens that were broadly distributed on a variety of bacteria or viruses, while in others, the antibodies identified antigens that were contained within only an extremely small subset of organisms. For the purposes of diagnosis, we attempted to select antibodies that would react with all members of a particular phylogenetic group (for example, *Neisseria gonorrhoeae*), but not with members of other phylogenetically related groups (for example, other *Neisseria* species). To detect such antibodies we used a two-tiered selection system: in the first tier, we identified antibodies that reacted exclusively with organisms within a single phylogenetic group; in the second tier, we selected a subset of these antibodies for their ability to individually identify as many members of the desired phylogenetic grouping as possible.

Figure 2 shows representative results of radioimmunoassays with two panels of monoclonal antibodies prepared against the membrane proteins of *N. gonorrhoeae* and *C. trachomatis*. Antibodies of different specificity could be readily distinguished. In the case of chlamydia, it appeared that a single monoclonal antibody (1-H8) against the 39,000 dalton outer membrane protein (*20*) could be used to distinguish all members of *C. trachomatis*, without cross-reactions occurring with the closely related *C. psittaci*. With *N. gonorrhoeae*, however, each of the monoclonal antibodies against the principal outer membrane protein (PrI; 34,000 to 37,000 daltons) (*19*) detected only a subset of the *N. gonorrhoeae* reference strains. Antibodies selected for broader reactivity against *N. gonorrhoeae* were found to cross-react with other *Neisseria* species, decreasing their diagnostic value. This led to the concept of pooling several monoclonal antibodies against PrI into a defined polyclonal mixture that would identify the entire spectrum of *N. gonorrhoeae*, without compromising the selective specificity of each antibody.

To select an appropriate antibody mixture, we screened each of 16 different monoclonal antibodies in coagglutination assays (*22*) with 719 different isolates of *N. gonorrhoeae*. Each antibody reacted with a characteristic subset of bacteria, resolving two broad mutually exclusive serological groups (PrIA and PrIB) (*19, 22*). Three of the monoclonal antibodies detected determinants on the PrIA molecule. Antibody 4-G5 reacted most commonly, identifying 99 percent of the PrIA strains. Antibody 2-F12 also reacted with a broad spectrum of PrIA strains, identifying 94 percent of strains, whereas antibody 4-A12 demonstrated a more restricted range of reaction, detecting only 44 percent of the PrIA strains. Six other monoclonal antibodies detected determinants on the PrIB molecule. Antibody 2-H1 reacted most commonly, identifying

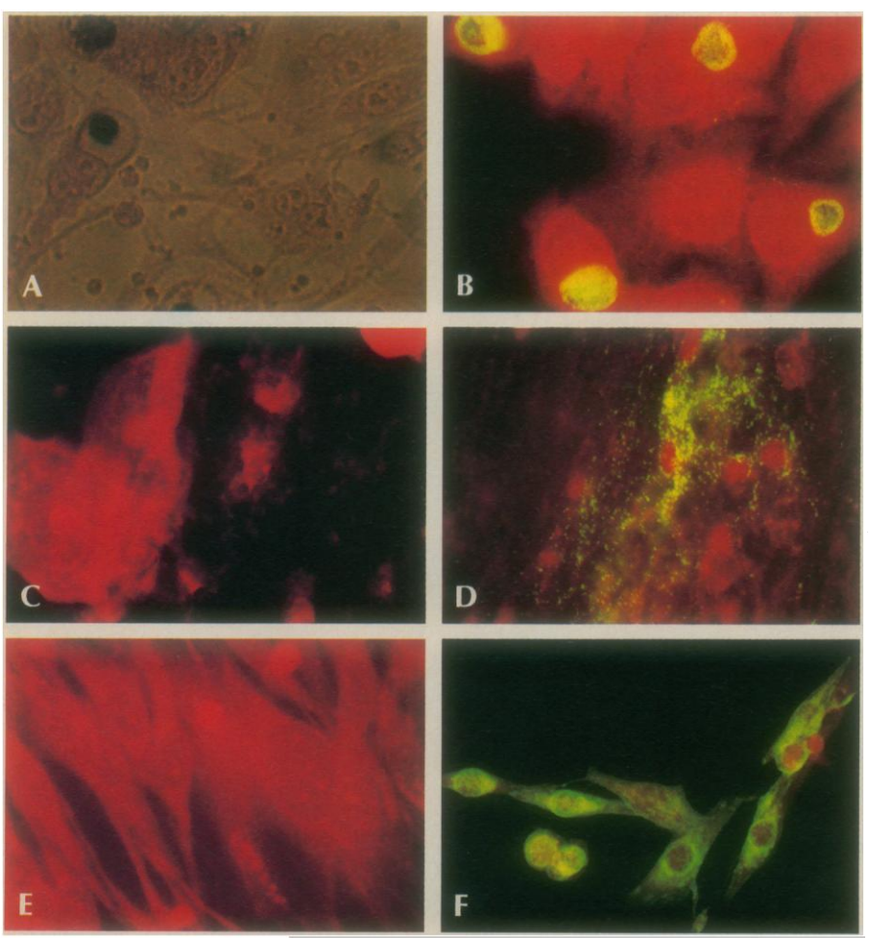

Fig. 3. Diagnosis of chlamydia and herpesvirus infections with monoclonal antibodies. (A and B) Detection of chlamydia inclusion bodies in cells infected in culture by (A) iodine stain or (B) IF test with a monoclonal antibody. (C and D) Immunofluorescence tests for chlamydia elementary bodies on (C) a cervical smear from a patient that tested chlamydia-negative by culture, and (D) a cervical smear from a patient that tested chlamydia-positive by culture. (E and F) Immunofluorescence tests for a herpesvirus antigen (HSV 1; gC glycoprotein) on (E) cells infected with HSV 2, and (F) cells infected with HSV 1.

93 percent of the PrIB strains. In order of their reactivities, antibodies 3-C8, 2-D6, 1-F5, 2-G2, and 2-D4 reacted with 78, 52, 52, 18, and 17 percent of the PrIB strains, respectively.

On the basis of their patterns of reaction, we pooled three of the monoclonal antibodies (4-G5, 2-H1, and 3-C8) and tested this mixture with the 719 isolates. As could be predicted from the results of tests performed with the individual antibodies, the antibody mixture identified 716 (99.6 percent) of the isolates tested. This same antibody mixture, when tested on 18 different *Neisseria* species, reacted exclusively with *N. gonorrhoeae* (*19*). Thus, in the case of gonorrhoea, the construction of an antibody mixture proved to be a satisfactory method to overcome the limited specificity observed with individual antibodies.

Diagnosis of *Chlamydia trachomatis* with Monoclonal Antibodies

Chlamydia have a life cycle that is reminiscent of viruses (*10, 11*). The infectious form of the organism (referred to as the elementary body) is an extracellular element (300 nanometers in diameter) that is transmissible from one cell to another. Upon entering a cell the organism is contained within an endocytic vacuole. During a 48- to 72-hour cycle the organism replicates to form a large inclusion body that contains several hundred new elementary bodies. Lysis of the cell leads to release of the elementary bodies into the extracellular space and continued rounds of infection in neighboring cells. Because of an accumulation of glycogen in the inclusion body of the infected cell, it is possible to detect intracellular chlamydia 48 to 72 hours after infection by staining with iodine (*11*).

Diagnosis of chlamydia in culture is commonly performed with mammalian cell lines (*11*). To improve sensitivity, duplicate cultures are usually prepared; one is stained with iodine 72 hours after infection, while the other is used for secondary passage onto yet another culture. This secondary culture is stained after another 72 hours after infection, resulting in a total testing period of 6 days. Of all infections detected by culture, approximately 65 to 80 percent are detected in the first passage while 20 to 35 percent are detected in the second passage.

In preliminary studies, the efficacy of a monoclonal antibody against the major membrane protein of *C. trachomatis* was compared to that of iodine for the detection of intracellular chlamydia inclusion bodies (Fig. 3). At intervals of 18, 24, 48, and 72 hours after infection, the cells were fixed in ethanol, stained with either fluorescein-conjugated antibody or iodine, and then examined by immunofluorescence (IF) or light microscopy. Staining of infected cells with monoclonal antibody revealed characteristic inclusions at 18 hours which could be accurately counted at 24 hours (Fig. 4). The total number of inclusions remained constant throughout the 48- and 72-hour time periods, although at later time periods the inclusions increased in size and the specimen was easier to read. In contrast, staining of a parallel set of infected cells with iodine failed to yield visual evidence of infection until 48 hours, with 72 hours being required for routine quantitation. Throughout a thousandfold range of infectivity, inclusion counts by both the IF and iodine methods were linear, indicative of single-hit kinetics and infection caused by a single chlamydia elementary body. At each point in the titration the IF method detected 8 to 11 times more inclusions than the iodine method. Similar enhanced sensitivity (approximately fourfold) has been demonstrated by comparing the IF monoclonal antibody method to Giemsa staining of chlamydia-infected cells (*23*).

Table 1. Diagnosis of *C. trachomatis* with monoclonal antibodies. Specimens from 2785 patients were inoculated in duplicate into cells cultured in 96-well microtest plates, processed through two culture passages, and then fixed and stained with either iodine or fluorescein-conjugated monoclonal antibody in order to detect intracellular inclusion bodies. Since 417 of the specimens were positive in both tests and 2288 were negative in both tests, the results were concordant in 97 percent of the specimens.

Number of specimens showing positive reaction			
In both tests	With monoclonal antibody only	With iodine only	With neither test
417	57	23	2288

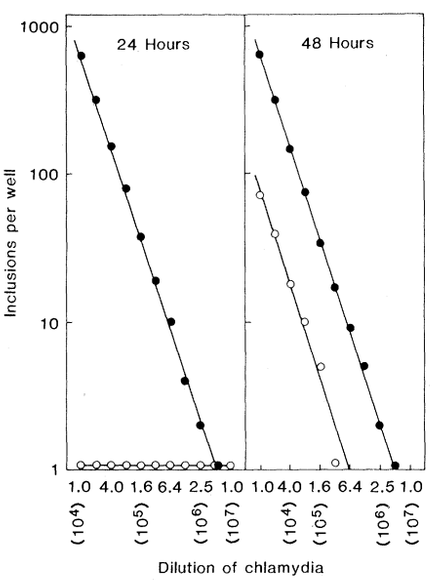

Fig. 4. Comparison of iodine staining and IF tests for the detection of chlamydia inclusion bodies in culture. Cells were infected in duplicate with a serial twofold titration of *C. trachomatis* (serovar I). Twenty-four and 48 hours later the plates were fixed, stained with iodine or fluorescein-conjugated monoclonal antibody, and examined by light or IF microscopy.

The IF and iodine staining methods have also been compared for the detection of chlamydia in cultures derived directly from patient specimens (Table 1) (*24*). For this purpose, each patient's specimen was inoculated in parallel into four microtiter wells containing cell monolayers. After 72 hours of incubation, one of the wells was tested by the IF method and another by staining with iodine (first passage). The remaining two monolayers were disrupted and passed onto fresh monolayers for another 72-hour incubation period (second passage) and were then stained in the same manner as the first passage specimens. Concordant results with the two assays were observed in 97 percent of the 2785 specimens tested. Of the 440 specimens that were positive by iodine stain, 417 were also positive in the IF test, indicating a sensitivity of 95 percent. The IF test detected 25 percent more positive specimens on first passage; by second passage, however, the tests showed closer equivalence with the IF test detecting only 5 percent more positive specimens than the iodine stain. With almost all specimens the IF method detected an average of eight times more inclusions per monolayer than the iodine method, confirming results of the serial titration study.

In an effort to further decrease the time required to diagnose chlamydia infections, IF tests with monoclonal antibodies have also been performed on specimens obtained directly from patients by means of swabs. One swab from the urethra or cervix of each patient was streaked onto a microscope slide for IF testing, while a sample from a duplicate swap was suspended in transport medium for inoculation into cell cultures.

Examination of urethral and cervical smears by IF demonstrated a pattern of staining that was characteristic of chlamydial infection. In approximately 90

Table 2. Typing of herpes simplex viruses. One hundred and twenty-two different isolates of HSV were typed as HSV 1 or HSV 2 by three different methods: (i) immunoperoxidase stain with rabbit antiserum against HSV 1 or HSV 2. Each antibody was tested on virus-infected cells by twofold serial titration; specimens were scored as HSV 1 or HSV 2 if one of the antiserums demonstrated a fourfold or greater difference in reactivity. (ii) Immunofluorescence tests with monoclonal antibodies against HSV-1 or HSV 2. Antibodies were tested at a single dilution (1/50) of ascites fluid. (iii) DNA restriction endonuclease digestion of ^{32}P-labeled HSV infected cells. Total cellular DNA was subjected to endonuclease digestion with Bam HI and analysis by polyacrylamide gel electrophoresis. Five of the 122 specimens were shown to be mixed HSV 1 and HSV 2 infections by the IF and restriction endonuclease methods; this was confirmed by subsequent plaque purification of HSV 1 and HSV 2 from the mixtures.

Test method	Number of virus isolates scored as			
	HSV 1 alone	HSV 2 alone	Indeterminate	Mixed HSV 1/HSV 2
(i) Rabbit antiserum	11	70	41	Not applicable
(ii) Monoclonal antibodies	34	83	0	5
(iii) Restriction endonuclease	34	83	0	5

percent of the specimens that were positive when stained with iodine in culture, this pattern consisted of large numbers (> 50 per smear) of extracellular elementary bodies which appeared under the microscope as individual small pinpricks of light. In heavily infected specimens the fluorescent elementary bodies produced a delicate "starry sky" pattern that could be observed throughout the sample. Representative examples of these IF patterns are presented in Fig. 3, A and B. It was of interest to note that although intracellular inclusions of chlamydia were readily observed in cells infected in vitro, direct clinical specimens rarely contained cells with obvious inclusions.

Since the direct test can be performed in less than 30 minutes, it represents a significant advantage over the culture method. Efforts are now under way to further assess the sensitivity of the IF technique with a larger number of patient specimens.

Identification of HSV with Monoclonal Antibodies

For typing HSV in culture, we have developed a panel of four monoclonal antibodies that unambiguously distinguish HSV 1 from HSV 2 (*21*). Monoclonal antibody 3-G11 reacts with the HSV 1–specific 80,000 to 120,000 dalton glycoprotein (gC) complex, antibody 6-A6 reacts with an HSV 2–specific protein of 140,000 daltons, antibody 6-E12 reacts with an HSV 2–specific protein of 55,000 daltons, and antibody 6-H11 reacts with an HSV 2–specific protein of 38,000 daltons. With this panel, we and our collaborators have now typed over 500 different isolates of HSV (*21, 25, 26*).

Table 2 shows a comparative analysis in which 122 HSV isolates from 107 patients were typed by three independent methods (immunoperoxidase-labeling with type-specific rabbit antiserums, IF with monoclonal antibodies, and restriction endonuclease analysis of viral DNA) (*25*). The results obtained with the monoclonal antibody demonstrated 100 percent concordance with restriction endonuclease analysis of viral DNA. In 117 isolates, the HSV was unambiguously typed as either HSV 1 or HSV 2. In five different isolates from three patients, the monoclonal antibodies typed a mixed infection of HSV 1 and HSV 2. The presence of mixed infections in each of these specimens was confirmed by plaque purification of viruses from the mixture and by restriction endonuclease analysis of both the virus mixture and the plaque-purified viruses. In contrast, antiserums prepared in rabbits were capable of typing only 66 percent of the 122 isolates; the remaining 34 percent yielded indeterminate antigen patterns from which a definitive identification could not be made.

In addition to their utility in culture systems, the monoclonal antibodies provided sufficient specificity to enable diagnosis and typing of HSV directly on primary clinical specimens (*21*). For this purpose, cells were obtained from herpes lesions by scraping with swabs and were smeared onto microscope slides for IF tests with monoclonal antibodies against HSV 1 or HSV 2. In each test a duplicate sample of the specimen was inoculated into cell cultures.

Immunofluorescence tests were performed in this way on specimens (of oral, genital, mucocutaneous, and ocular sites) obtained from 59 patients with clinically suspected HSV and 43 control patients with unsuspected HSV infection.

Herpesvirus was isolated in tissue culture from 54 of the specimens obtained from suspected herpes lesions, and in 48 (88 percent) of these, HSV antigens were detected in IF tests. The monoclonal antibodies detected HSV antigens in six clinically suspect specimens from which the culture method did not reveal infectious virus. These latter findings confirmed the results of other investigators showing that IF methods for HSV can demonstrate viral antigens in clinical specimens in the absence of infectious virus (*27*). The monoclonal antibodies did not detect HSV antigens in any of the 43 specimens obtained from the control population.

The efficacy of the monoclonal antibodies in typing HSV in clinical specimens was most clearly illustrated when the test predicted types of virus in clinical materials that would be unexpected according to the anatomical site from which they were derived. Thus, we have identified by IF tests with direct specimens (and confirmed by restriction endonuclease analysis and secondary IF tests on culture isolates) examples of genital HSV 1 infection, oral HSV 2 infections, and HSV 2 infections of diverse mucocutaneous sites such as on the tips of fingers, the elbow, and in the axilla.

Advantages, Disadvantages, and the Future of Immunodiagnostics

Until the advent of monoclonal antibodies, immunodiagnostic tests relied exclusively on antibodies obtained from the serum of animals. Although immunization could be used to increase the titer of certain antibodies in antiserums, antibodies per se were always obtained as mixtures of immunoglobulins. Since the precise composition of antiserums could not be controlled, the activity of any particular antiserum (that is, its specificity and affinity) was the sum of the reactions of its multiple constituents. For the most part, "specificity" of a particular antiserum was determined by those antibodies that were in highest concentration (dominant antibodies); the activities of antibodies in lower concentration (minor antibodies) were generally masked by the dominant antibodies.

The ability to control the composition of antibody reactants is of critical importance in the diagnosis of infectious diseases. Since most microorganisms have numerous phylogenetic relatives with common antigens, antiserums prepared in animals cross-react with a spectrum of organisms, including pathogenic and nonpathogenic varieties. In addition, antigen preparations used for immuniza-

tion are commonly contaminated with unrelated biological materials, resulting in immunization with secondary antigens and the formation of antibodies that cross-react with normal host constituents.

Individual monoclonal antibodies react with only a single antigenic determinant and thus provide a degree of specificity far greater than that of conventionally prepared antibodies. Further, and perhaps most important, since the hybridoma method provides the ability to scan an enormous repertoire of antibodies, it is possible to select individual monoclonal antibodies with highly defined characteristics, for example, specificity, avidity, and isotype. Both minority and dominant antibodies can be studied and used with equal facility. This provides significant opportunity, for, in many instances, minority antibodies are those with preferred specificity for diagnostic tests. For example, antiserums prepared in mice against HSV 1 or HSV 2 demonstrate extensive cross-reaction between the types. In contrast, hybrid cells from these mice can be selected for the production of monoclonal antibodies that react with only one of the types.

Hybrid cell lines also provide a remarkably constant and economic source of antibody. Antibodies of identical chemical structure can be obtained for years and preparations of such antibodies can be easily standardized. Most monoclonal antibodies can be produced on a large scale by passage of hybridomas in mice. As much as 1 to 20 milligrams of homogeneous antibody can be purified from each milliliter of tumor ascites fluid. This high level of antibody production, as well as the permanent growth properties of the hybrid cells, allows a virtually unlimited supply of a standardized reagent.

Within the context of these advantages, though, it should be emphasized that the clonal origin of these antibodies also poses certain problems. Each monoclonal antibody has highly defined properties, and it is not uncommon to observe order-of-magnitude differences between individual monoclonal antibodies for properties such as (i) retention of activity after labeling with radioisotopes or fluorochromes, (ii) solubility, as influenced by the ionic strength of *p*H of buffer, and (iii) stability over long-term storage. Such differences have tended to restrict the routine preparation of monoclonal antibodies to sophisticated laboratories. Other problems include: (i) their high specificity, considered to be too narrow by some critics; (ii) their avidity, which is sometimes lower than antibodies in hyperimmune antiserums; and (iii) the rates at which they are produced by hybridomas, which may vary, and the possibility that their production may cease. Although numerous examples of these problems can be cited, it should also be realized that hybridoma technology is yet in its infancy. Only several years ago it was considered a notable achievement to prepare a single monoclonal antibody against an antigen of choice.

Since monoclonal antibodies represent the individual building blocks of antiserums, there is no a priori reason to consider that the specificity and avidity of the composite antiserum cannot be matched by the individual antibodies themselves. In some instances a single monoclonal antibody may provide the appropriate reactivity to constitute a diagnostic reagent, whereas in others, it may be necessary to mix and match different antibodies to obtain the desired specificity. This latter approach is illustrated by our studies with gonorrhoea, where a mixture of three monoclonal antibodies identified 99.6 percent of isolates tested without cross-reacting with 17 other closely related species of *Neisseria*.

In addressing the issue of stability, two factors need be considered—the stability of the antibody source (that is, the hybrid cell line) and the continued integrity of the antibody molecules produced by a particular cell line. As already mentioned, the stability of antibody production varies from one cell line to another. In our experience approximately 60 percent of hybridomas are sufficiently stable to warrant continued use. Although some lines continue to demonstrate variable antibody production, these can generally be maintained through careful attention, aggressive recloning, and the freezing of adequate samples of cells for future regeneration of stocks. With regard to the stability of the antibody products themselves, there have been recent reports of cellular mutations in hybridomas that affect the antibodies produced by the cells (*28*). In all instances, however, the mutant cells appeared at extremely low frequency (10^{-4} to 10^{-5}). These mutant cells did not show a selective growth advantage over nonmutant cells and they persisted only as a minority population. Thus, with nonselective culture conditions, as are commonly used, it is unlikely that significant drift in the quality of monoclonal antibodies would occur.

These considerations lead us to expect that future areas of development in this field will emphasize (i) the preparation of antibodies with specificities capable of performing novel diagnostic functions, (ii) improved methods of manufacturing antibodies, and (iii) the incorporation of antibody-based reagents into instrumentation.

Antibodies. Not only will the library of monoclonal antibodies necessary for the phylogenetic classification of microorganisms be completed, but new antibodies will be developed to specifically identify pathogenic organisms or the molecular factors responsible for their pathogenesis. The development of monoclonal antibodies against plasmid-encoded proteins responsible for drug resistance will facilitate rapid decisions concerning preferred modalities of treatment.

Manufacturing. Variable chromosome loss, with the concomitant overgrowth of hybrid cells that do not produce antibody, is the most problematic feature of the hybridoma method. As new knowledge is acquired concerning the chromosomal locations of mouse genes, it should be possible to induce mutations in the genes involved with cell metabolism that are closely linked to genes encoding immunoglobulin heavy and light chains. With defined growth media, it should then be possible to select against cells that lose chromosomes encoding either of the immunoglobulin chains.

Instrumentation. The highly defined and reproducible properties of monoclonal antibodies invite their use in instrumentation. For example, they will probably be incorporated into a new generation of instruments for automated blood and tissue typing, detection of specific antibodies in blood, and quantitative determination of microbial antigens in a variety of body fluids and tissues.

Conclusions

Monoclonal antibodies are, in effect, homogeneous immunological reagents of defined specificity, avidity, high specific activity, and selected isotype. Individual antibodies can be selected for their unique reactions with particular microorganisms. In certain instances these antibodies can identify antigenic relations between organisms that are not apparent in tests with conventional antiserums.

We have described the use of monoclonal antibodies for the diagnosis of gonorrhoea, chlamydia, and herpesvirus infections in humans. In each case the monoclonal antibodies showed patterns of specificity and reproducibility that far exceeded those available with conventionally prepared antibodies. Furthermore, direct tests for these organisms

required only 15 to 20 minutes to perform, representing a major advancement in the diagnosis of infections that previously required 3 to 6 days of culture to accomplish. In view of these advantages the continued development of techniques for the production and utilization of monoclonal antibodies should lead to great improvements in the quality of microbiological diagnosis.

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Immunotoxins: A New Approach to Cancer Therapy

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Approximately 75 years ago, Paul Ehrlich discussed the potential use of antibodies as carriers of pharmacologic agents (*1*). During the last decade, there has been considerable progress in the application of this concept to the elimination of cells that are reactive with antibodies coupled to toxic agents. In this article, we discuss work by ourselves and others concerning the elimination of normal and neoplastic target cells by conjugates containing antibody and toxin. We also present evidence that conjugates of toxin and antigen can induce specific immunologic unresponsiveness.

The term "immunotoxin" is used here to refer to a cell-binding antibody or antigen covalently bound to a plant or bacterial toxin. The toxin may be the whole molecule or a polypeptide portion carrying the toxic activity. Although much of our understanding of the mechanisms by which these toxins kill cells rests on studies of diphtheria toxin (*2*), the prevalence of diphtheria antitoxin in human populations renders this toxin unsuitable for clinical use. Therefore, most recent investigators have used ricin, a plant toxin. Like most toxic proteins produced by bacteria and plants, ricin has a toxic polypeptide (A chain) attached to a cell-binding polypeptide (B chain) (*3, 4*). The B chain is a lectin that binds to galactose-containing glycoproteins or glycolipids on the cell surface. By mechanisms that are not well understood, ricin A chain gains access to the cell cytoplasm. It is presumed, but has not been proved, that the route of entry is by receptor-mediated endocytosis (*5*) and that the A chain, which has a hydrophobic portion (*6*), penetrates the membrane of an endocytic vesicle or phagolysosome to enter the cytoplasm (*3*). By analogy with other toxins (*7*), it is possible that the B chain has a second function, namely, facilitating the translocation of the A chain through the membrane of the endocytic vesicle (*7–10*) by

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