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Rapid Assay for Mycobacterial Growth and Antibiotic Susceptibility Using Gel Microdrop Encapsulation

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Effective control of tuberculosis transmission in vulnerable population groups is dependent on rapid identification of the infectious agent and its drug susceptibility. However, the slow growth rate of mycobacteria has undermined the ability to quickly identify antimicrobial resistance. These studies describe a mycobacterial growth assay based on microencapsulation technology used in conjunction with flow cytometric analysis. Mycobacteria were encapsulated in agarose gel microdrops approximately 25 μm in diameter, and colony growth was monitored by using flow cytometry to evaluate the intensity of auramine staining after culture for various times at 37°C. By this method, colony growth of *Mycobacterium bovis* and *M. smegmatis* could be quantified within 1 to 3 days after encapsulation. Inhibition of growth by rifampin and isoniazid was also evaluated in this time period, and the presence of an isoniazid-resistant subpopulation representing 3% of the total microorganisms could be detected. This use of encapsulation and flow cytometry has the potential to facilitate rapid and automated evaluation of inhibition of growth by antimicrobial agents and shorten the time frame for analysis of clinical specimens.

In the past decade, antimicrobial agent-resistant infections have become rampant worldwide, increasing the morbidity, mortality, and cost associated with disease (5, 9). For tuberculosis (TB), tests for antimicrobial susceptibility take weeks and delayed therapy can compromise the patient as well as lead to an increase in disease incidence. Among infectious diseases, TB remains the leading cause of death in the world. From 1985 to 1993, the number of reported TB cases in the United States increased 18%, after having decreased every year since 1882 (20). This resurgence is mainly attributed to human immunodeficiency virus-TB coinfection and increased transmission in vulnerable populations (2). Efforts to control TB are severely hampered by the time required for growth, identification, and susceptibility testing by current methods (2, 9). Other than staining for acid-fast bacilli in sputum, the commonly used diagnostic methods for TB require 2 to 8 weeks, primarily because of the slow growth of pathogenic mycobacteria, which have characteristic doubling times of 15 to 24 h (23). Systems such as the BACTEC 460 (Becton Dickinson, Sparks, Md.) which use radiometric detection for measurement of bacterial growth and metabolism have reduced the time required for growth and antibiotic susceptibility testing to 2 to 4 weeks (17). Definitive identification of the bacteria, however, may still require conventional culture and biochemical testing. Recent developments have been directed towards integrating automated methods with nucleic acid probe technology (14, 16, 30). However, current probe technology is used only for culture identification and must be combined with growth or metabolic assays for antibiotic susceptibility testing.

Weaver et al. first demonstrated the feasibility of using encapsulation technology to rapidly detect and enumerate individual microorganisms in patient samples (28). This method involves the encapsulation of cells in gel microdrops (GMDs), which function as microscopic testing regions for assay of growth and metabolism. Successful encapsulation and growth of several species of microorganisms have been demonstrated with GMD technology (1, 28). In addition, encapsulation of mammalian cells did not significantly affect viability, growth, or secretion and viable hybridoma cells have been recovered after flow sorting for subsequent culture (13, 27). The ability to make measurements of cell function within the microdrop offers several potential advantages for microbiological testing, including increased speed and sensitivity. The production of GMDs can be controlled to generate microspheres of microscopic proportions (≤100 μm in diameter), which results in rapid diffusion through the agarose hydrogel (27–29) and allows measurement of large numbers of GMD-encapsulated cells by flow cytometry (1, 12, 18).

Analysis of mycobacteria by flow cytometry is difficult, primarily because of their small size and propensity to aggregate. Recently, successful flow cytometric analysis of recombinant *Mycobacterium bovis* BCG for use as a potential drug delivery vaccine has been reported (24). However, in contrast to the situation with larger bacteria such as *Escherichia coli*, which are routinely analyzed by flow cytometry (3, 4, 26), reports of clinical analysis of mycobacteria by this method are rare. GMD encapsulation allows "bacteria to masquerade as larger particles" (11) and provides a discrete microenvironment for colony growth. Thus, GMD encapsulation can reduce problems associated with detecting small particles and eliminate cell aggregation in the flow line.

These studies were designed to evaluate the feasibility of using GMD encapsulation technology for rapid assay of mycobacterial growth and susceptibility to antimicrobial agents. *M. bovis* BCG and *M. smegmatis* were used as a model system to investigate the efficacy of encapsulation and detection of clonal growth by flow cytometry. The results indicate that GMD cell encapsulation technology can be used in conjunction with flow cytometry as a method to rapidly evaluate mycobacterial colony growth and antibiotic susceptibility.

MATERIALS AND METHODS

Mycobacterial culture. Mycobacteria obtained from the American Type Culture Collection (ATCC) (Rockville, Md.) were used as a model system. This research has primarily focused on the vaccine strain of bacillus Calmette-Guérin,

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M. bovis BCG, including both isoniazid (INH)-susceptible (ATCC 35734) and INH-resistant (ATCC 35747) strains, as well as *M. smegmatis* (ATCC 19420), a mycobacterium classified as a rapid grower. *M. kansasii* (ATCC 12479), *M. intracellulare* (ATCC 13209), and *M. xenopi* (ATCC 19250) were also used in some experiments.

Mycobacteria received from the ATCC were resuspended in Middlebrook 7H9 medium (Difco, Detroit, Mich.), and aliquots were inoculated onto Lowenstein-Jensen slants (Becton Dickinson Microbiology Systems, Cockeysville, Md.) as well as into tissue culture flasks containing Middlebrook 7H9 medium supplemented with 0.05% Tween 80 (Sigma Chemical Co., St. Louis, Mo.) and albumin, glucose, and catalase (ADC) (Becton Dickinson Microbiology Systems). These cultures were incubated under standard conditions at 37°C in humidified atmosphere of 5% CO_2. Cultures in suspension for longer than 3 to 4 weeks were discarded in favor of fresh inoculations from the Lowenstein-Jensen slants. Suspension cultures were passaged according to density and used for encapsulation shortly thereafter to ensure that they were in a growth phase rather than stationary phase. The cells were harvested for encapsulation by centrifugation at 1200 × *g* for 7 min, and the pellet was resuspended in 1 ml of Middlebrook 7H9 medium. To promote disaggregation, 100 μl of Tween 80 was added and the suspension was vortexed vigorously. The cell suspension was then passed through a 27.5-gauge needle and filtered through a 5-μm-pore-size filter to remove any remaining cell clumps. The resulting preparation was counted and adjusted to obtain approximately 10^7 cells per ml.

Microencapsulation. The CellSys 100 microdrop maker (One Cell Systems, Cambridge, Mass.) was used for GMD formation. Instrument features critical to formation of microdrops with desired specifications include the blade and shaft design and variable shaft rotation speed. The mycobacterial encapsulation protocol was designed to obtain a GMD preparation with a mean diameter of 25 μm. All procedures were performed in a laminar flow hood and were designed to minimize aerosol formation. The high viscosity of the oil and the configuration of the microdrop maker prevent aerosol production during the emulsification process.

Agarose (SeaPrep; FMC, Rockland, Maine) was added to 1 ml of cold phosphate-buffered saline (PBS) at a concentration of 4%, and the mixture was heated to 90°C for 10 min and cooled to 37°C. The cell suspension was equilibrated to 37°C, and 500 μl was added to an equal volume of agarose. A 700-μl aliquot of the agarose-cell mixture was added to 15 ml of CelMix 200 emulsion matrix (One Cell Systems) at 37°C, and the sample was emulsified in a CellSys 100 microdrop maker. The GMDs were then removed from the oil by centrifugation and washed in PBS. A sample of the GMD pellet was fixed immediately and examined by phase microscopy to determine the GMD size and the number of organisms per GMD (occupancy rate). An aliquot of the day zero fixed sample was also stored at 4°C for analysis.

Analysis of encapsulated-cell growth. Encapsulated mycobacteria were cultured in Middlebrook 7H9 medium as described above in the absence or presence of INH and rifampin (Sigma). At various times, aliquots were taken and fixed by incubating the cell sample with an equal volume of 80% MeOH for 60 min at 37°C. To ensure that the fixation protocol was effective and that no viable mycobacteria remained, samples were inoculated on Middlebrook 7H10 agar to assay for colony growth. In addition, the *Bac*light Live/Dead stain (Molecular Probes, Eugene, Oreg.) was used to assess viability. Incubation in MeOH for 60 min at 37°C resulted in fixation, and no viable mycobacteria were observed with either of these methods. After incubation with MeOH, the GMDs were centrifuged (500 × *g*, 6 min) and the pellet was resuspended in PBS and stored at 4°C until analysis.

Fluorescent staining and flow cytometry. Staining with propidium iodide (PI) (Sigma) at a final concentration of 0.25 mg/ml or auramine O (Sigma) at a final concentration of 0.5 mg/ml was used to monitor cell growth by flow cytometry and fluorescence microscopy. Fixed samples were stained shortly before examination with an Olympus BH2 fluorescence microscope or analysis by flow cytometry. Flow cytometry was done with a FACScan instrument (Becton Dickinson, San Jose, Calif.) at the Flow Cytometry Center at the Dana Farber Cancer Institute (Boston, Mass.). Empty GMDs carried through the staining protocol were used as a control. Analysis of forward scatter and fluorescence intensity was done by using Lysis II software. Auramine and PI fluorescence were measured on the FL1 and FL2 channels, respectively, by using a standard 488-nm excitation laser.

Mycobacterial cell identification. Cultures were examined every few weeks by using the BBL Quick Stain kit (Becton Dickinson) to confirm that only acid-fast bacteria were present. Preliminary experiments were also done with nucleic acid hybridization probes (GenProbe, San Diego, Calif.). Accuprobe Culture Identification kits specific for *M. tuberculosis* complex and *M. kansasii* were used, and hybridization was assayed with the Optocomp II luminometer (MGM Instruments, Hamden, Conn.).

RESULTS

Encapsulation of mycobacteria. Encapsulation protocols developed for other applications, such as measurement of antibody secretion by hybridoma cells, result in approximately 10% of the GMDs containing a single cell, with the remaining 90% of the GMDs in the preparation left unoccupied. In these studies, it was important to avoid multiple occupancy, since measurement of secretion from individual cells was a selection criterion for fluorescence-activated cell sorting.

The characteristic tendency of mycobacteria to aggregate made it particularly challenging to obtain a suspension containing predominantly single cells. Methods investigated for dispersing the aggregated cells included addition of various concentrations of Tween 80, sonication, cell harvesting at different culture densities, use of shear forces resulting from aspiration through a small-gauge needle, and filtration. The most reproducible protocol involved harvesting recently passaged cells and mixing them with 10% Tween 80 before aspirating them through a 27.5-gauge needle. The dispersed cells were then put through a 5-μm-pore-size filter to remove any remaining large clumps. This relatively uncomplicated procedure efficiently removed large cell aggregates without seriously undermining yield. This protocol results in a preparation of GMDs with an average occupancy of four cells (ranging from one to six) per microdrop. The data from initial experiments indicated that with six or fewer organisms per GMD, growth of both *M. bovis* BCG and the more rapidly growing *M. smegmatis* could be measured (25). Although the optimal sensitivity would involve encapsulation of only a single organism per GMD, these initial studies suggest that single occupancy may not be necessary, and the additional processing required to generate only single cells significantly decreases the yield.

Effect of encapsulation on cell growth. The impact of GMD encapsulation on mycobacterial growth was determined for *M. bovis* BCG, *M. smegmatis*, *M. kansasii*, *M. xenopi*, and *M. intracellulare*. Microscopic examination of the GMDs by using PI, a fluorescent nucleic acid stain, indicated colony growth for each of these strains after encapsulation. This result was confirmed by inoculating agar plates with GMD-encapsulated cells and observing the presence of CFU. In addition, after 3 to 7 days in suspension culture (depending on the strain and the number of cells encapsulated), outgrowth of mycobacteria from the GMDs into the nutrient medium was seen. These data indicated that GMD encapsulation did not inhibit mycobacterial growth.

As discussed in Materials and Methods, the encapsulation procedure is carried out in a biosafety cabinet to maintain sterility and minimize exposure to mycobacteria. The protocol also minimizes aerosol formation since the encapsulation is done in a closed system and encapsulated mycobacteria are cultured in closed flasks until analysis. The analysis, microscopic or flow cytometric, is done with samples which have been MeOH fixed and do not contain any viable mycobacteria. Consequently, this method does not involve analysis of potentially infectious samples by flow cytometry.

Quantitation of mycobacterial growth. PI staining was used initially for evaluating growth, but the intensity of the fluorescent signal was variable. In contrast, staining with auramine, which binds to mycolic acid in the mycobacterial cell wall, generated much more consistent results. Auramine is routinely used in clinical settings for mycobacterial staining (6, 19) but has not been widely used as a fluorescent dye for flow cytometry. Analysis of encapsulated *M. smegmatis* stained with auramine by flow cytometry is shown in Fig. 1. In this experiment, colony growth in the GMDs was assayed after 1 and 2 days in culture. With the more rapidly growing *M. smegmatis*, cells grow out of the GMDs into the surrounding medium by day 3.

In Fig. 1, the profile obtained from a sample of unoccupied microdrops is included to illustrate that the nonspecific fluorescence associated with the GMDs is low. In addition, the

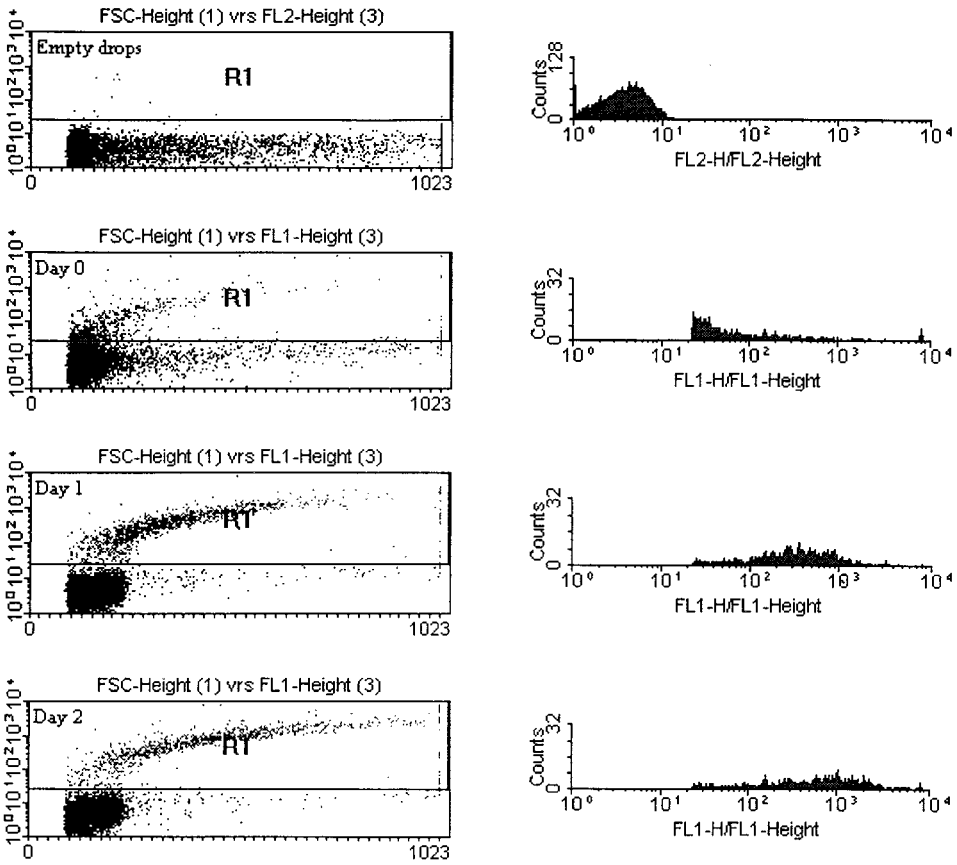

FIG. 1. Analysis of growth of encapsulated *M. smegmatis*. *M. smegmatis* cells were encapsulated as described in the text, and samples were taken after the indicated times in culture at 37°C, fixed in MeOH, and stained with auramine. (Left panels) Analysis of forward scatter and fluorescence intensity (FL channel 1) by using a FACScan cell sorter. The increase in fluorescence associated with colony growth is delineated by the R1 region. (Right panels) A corresponding histogram of the fluorescence signal intensity associated with occupied GMDs. The background fluorescence from the staining of unoccupied GMDs shown in the top panels (empty drops) is not included. The signal corresponding to encapsulated mycobacteria demonstrates a time-dependent increase from a mean fluorescence of 122 units at day zero to 406 and 726 units after 1 and 2 days in culture, respectively.

large size of the GMD in relation to mycobacteria is useful in flow analysis in that it facilitates defining the region of interest. The encapsulation protocol described here results in approximately 20 to 30% of the GMDs containing mycobacteria, the remainder being unoccupied. Consequently, the largest number of events is detected in the region associated with unoccupied GMDs. The growth of encapsulated *M. smegmatis* can clearly be seen by comparing the numbers of events detected in the R1 region in the left-hand panels of Fig. 1 immediately after encapsulation and after culture. Histograms of these data are presented in the right-hand panels and show the increase in GMD-associated fluorescence, which represents colony growth. Although PI staining is still used in some applications, the data presented in this report are based on auramine staining, which is an established clinical method for mycobacterial identification.

The photomicrographs in Fig. 2 illustrate GMD-associated fluorescence from auramine staining of encapsulated *M. bovis* BCG 734 immediately after encapsulation (day zero) and after 4 days of culture at 37°C. The growth of mycobacterial colonies within the GMDs during this period is associated with an increase in the amount of auramine staining. In contrast to growth of encapsulated *M. smegmatis*, growth of encapsulated *M. bovis* BCG can be monitored for 3 or 4 days of culture since outgrowth is not seen until day 5.

Determination of antibiotic susceptibility. Since GMD en-

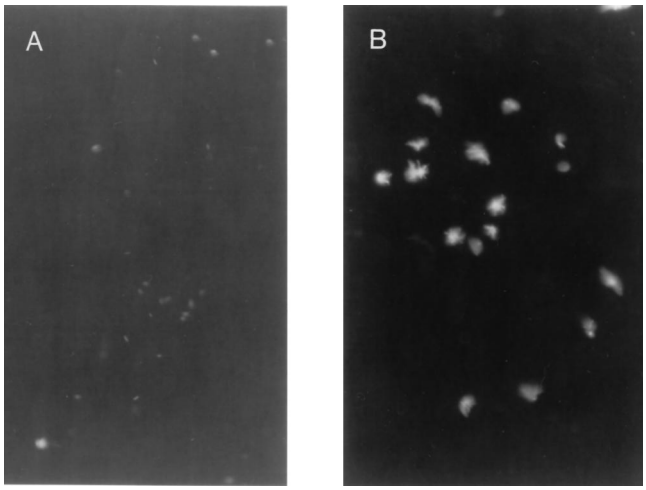

FIG. 2. Photomicrographs of GMD-encapsulated *M. bovis* stained with auramine. *M. bovis* BCG 734 cells were encapsulated as described in the text, fixed, stained with auramine, and examined with an Olympus BH2 fluorescence microscope (40× objective). (A) Encapsulated mycobacteria fixed immediately after encapsulation. (B) Sample from the preparation shown in panel A fixed after 4 days in culture.

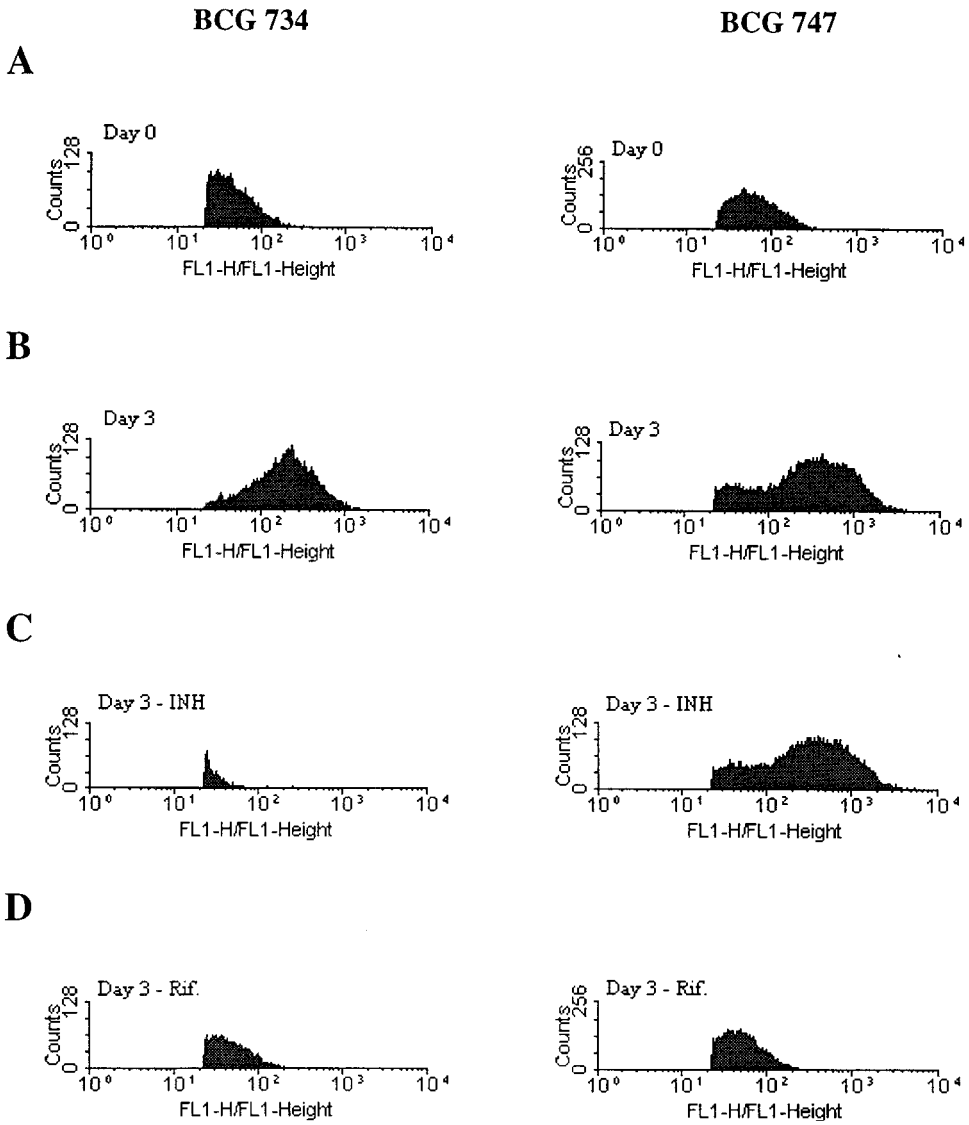

FIG. 3. Analysis of growth of encapsulated *M. bovis* BCG. *M. bovis* BCG 734 (left) and BCG 747 (right) were encapsulated as described in the text, stained with auramine, and analyzed with a FACScan cell sorter. Histograms of the GMD-associated fluorescence are presented. The gated region was defined to exclude the background obtained with empty drops. Samples were taken immediately after encapsulation (A) or after incubation at 37°C for 3 days (B). The mean intensity in this region increased 364% (70 to 255 units) for strain BCG 734 and 510% (51 to 260 units) for strain BCG 747. In the presence of 5 μg of INH per ml (C), the fluorescence intensity of the strain BCG 734 samples reflected complete inhibition of growth. In contrast, the INH-resistant strain, BCG 747, was unaffected by the antibiotic (mean fluorescence of 476 and 493 units in the absence and presence of INH, respectively). In the presence of 1 μg of rifampin (Rif.) per ml (D), mean fluorescence was indistinguishable from the value for the day zero samples for both strains.

capsulation technology allowed analysis of mycobacterial growth by flow cytometry, INH and rifampin were added to the cultures to determine if this approach could be used for rapid analysis of antibiotic susceptibility. In Fig. 3, flow cytometric analysis of auramine staining is illustrated for encapsulated *M. bovis* BCG 734 and BCG 747 cultured in both the absence and the presence of these antibiotics.

These data show the increase in GMD-associated fluorescence after 3 days of culture in the absence of antibiotics for both the BCG 734 and BCG 747 strains of *M. bovis*. The mean fluorescence increased 364% (70 to 255 units) for strain BCG 734 and 510% (51 to 260 units) for strain BCG 747. In the presence of 1 μg of rifampin per ml, the mean fluorescence was indistinguishable from that of the day zero samples for both cell lines, indicating complete growth inhibition. The presence of 5 μg of INH per ml also completely inhibited growth of the *M. bovis* BCG 734 samples. In contrast, growth of the INH-resistant strain, *M. bovis* BCG 747, was unaffected by the antibiotic. In other experiments, inhibition of *M. bovis* BCG 734 growth by INH was shown to be concentration dependent; complete inhibition was seen with 5 μg of INH per ml, whereas a 1-μg/ml concentration resulted in a 59% decrease in fluorescence intensity in comparison with the result for the control at day 3 (data not shown).

Detection of an antibiotic-resistant subpopulation. To determine if this method could be used to detect small subpopulations of drug-resistant mycobacteria, various amounts of INH-resistant *M. bovis* BCG 747 were mixed with *M. bovis* BCG 734, encapsulated, and cultured in the presence or absence of antibiotics. In the experiments shown in Fig. 4, the

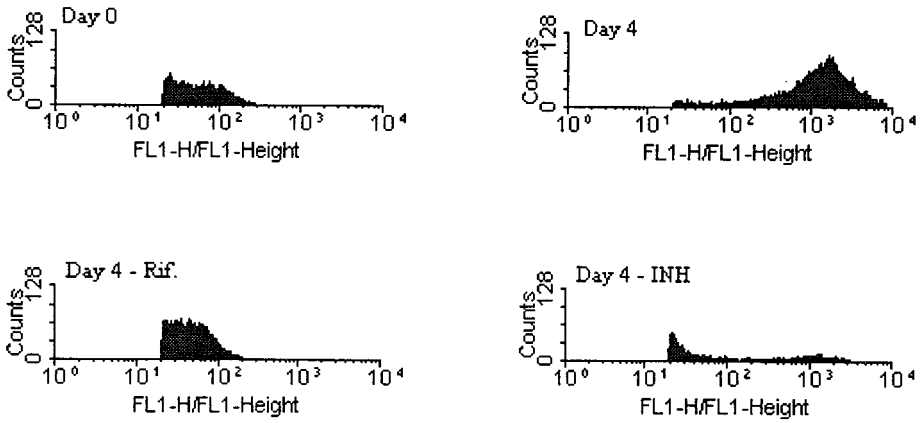

FIG. 4. Detection of a 10% antibiotic-resistant population of mycobacteria. The encapsulated population of about 10^7 mycobacteria contained approximately 90% *M. bovis* BCG 734 and 10% *M. bovis* BCG 747. Analysis after 4 days of culture in the absence of antibiotics showed growth (mean fluorescence increased from 60 to 1,655 units), which was completely inhibited by 1 μg of rifampin (Rif.) per ml (mean of 69 units). In the presence of 5 μg of INH per ml, growth of the resistant BCG 747 cells can be seen (mean of 468 units).

encapsulated cell population contained approximately 10^7 mycobacteria and was composed of 10% INH-resistant *M. bovis* BCG 747 and 90% antibiotic-susceptible *M. bovis* BCG 734. As anticipated, growth was seen in the absence of antibiotics and no growth was detectable in the presence of rifampin. However, in the presence of INH, growth of the 10% antibiotic-resistant population of *M. bovis* BCG 747 was readily detectable.

Similar results from experiments in which the encapsulated mycobacterial population was made up of 3% INH-resistant *M. bovis* BCG 747 and 97% *M. bovis* BCG 734 are shown in Fig. 5. Comparison of GMD-associated fluorescence intensities at day zero and day 4 shows growth in the absence of antibiotic and inhibition of growth by rifampin. In the presence of INH, however, the 3% INH-resistant *M. bovis* BCG 747 population shows measurable growth. The mean fluorescence of 180 ± 6 units (mean ± standard error) measured for this population is significantly different from those measured at day 0 and day 4 with INH (81 ± 3 and 50 ± 1 units, respectively). In preliminary experiments, growth of a 1% antibiotic-resistant population has been detected by microscopic examination, although the flow cytometry analysis did not show a statistically significant signal. Studies designed to optimize staining, signal detection, and encapsulation in order to increase the assay sensitivity and achieve this level of detection by flow cytometry are under way.

Identification of mycobacteria. By using the BBL Quick Stain kit, a simplified modification of the traditional Ziehl-Neelsen and Kinyoun techniques, the purity of our mycobacterial cultures was evaluated every several weeks. In addition, the acid-fast auramine staining is also considered to be fairly specific for mycobacteria. However, neither of these methods can differentiate between strains of mycobacteria and acid-fast auramine staining of other nonmycobacterial species has been reported (19). Therefore, the use of specific nucleic acid hybridization probes such as the Accuprobe Culture Identification kits in conjunction with GMD encapsulation is also being investigated to determine the feasibility of using encapsulated mycobacteria for both growth measurements and identification. The incubation at 95°C used in the Accuprobe protocol to inactivate the mycobacteria had to be modified in order to maintain microdrop integrity. However, preliminary results indicate that the hybridization reaction, which requires only a 60°C incubation, can be carried out within the GMD.

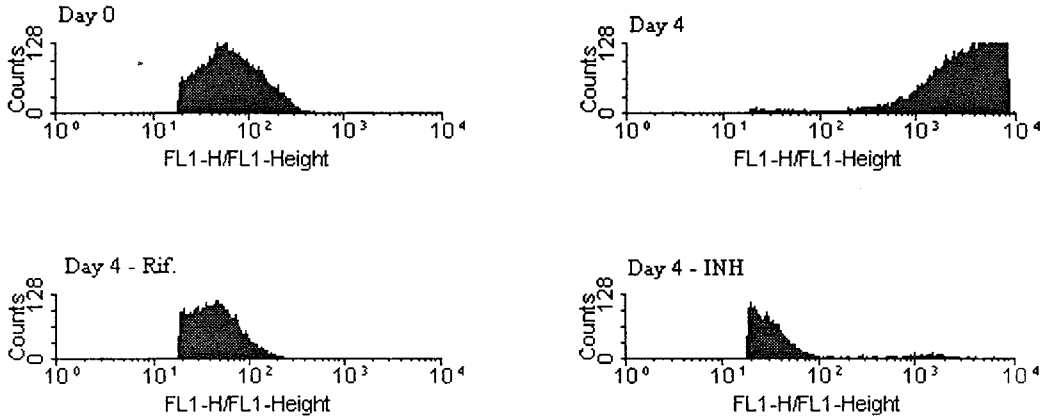

FIG. 5. Detection of a 3% antibiotic-resistant population of mycobacteria. In this experiment, the encapsulated mycobacterial population contained 3% *M. bovis* BCG 747 and 97% *M. bovis* BCG 734. Analysis after 4 days of culture in the absence of antibiotics showed growth (mean fluorescence increased from 81 to 4,068 units), which was completely inhibited by 1 μg of rifampin (Rif.) per ml (mean of 51 units). In the presence of 5 μg of INH per ml, growth of the resistant BCG 747 cells can be seen (mean of 180 units).

DISCUSSION

The recent increase in *M. tuberculosis* and *M. avium* complex infections has generated intense interest in development of diagnostic tools for the rapid identification of pathogenic mycobacteria and antimicrobial drug susceptibility. The studies presented in this report have demonstrated the potential of a novel method to measure growth of mycobacteria and antimicrobial susceptibility by using flow cytometry. Flow cytometry has not been routinely used as an analytical tool for clinical mycobacteriology, in part because of the technical challenges associated with cell aggregation. However, encapsulation in agarose microspheres provides a means to analyze colony growth within a microdrop by using standard flow cytometric analysis.

Six different strains of mycobacteria maintained viability and growth after encapsulation in 25-μm-diameter GMDs. In initial experiments, PI staining was used as a fluorescent indicator of colony growth. However, since the nucleic acid content of microorganisms is significantly less than that of eukaryotic cells, fluorescence signals from microorganisms stained with PI, 4′,6-diamidino-2-phenylindole (DAPI), and other nucleic acid dyes are relatively weak (22). Although auramine has not been widely used in flow cytometry, auramine staining of mycobacteria proved to be very useful as a means of quantifying colony growth within the GMDs. Analysis by either fluorescence microscopy or flow cytometry indicated that growth of encapsulated *M. smegmatis* and *M. bovis* could be detected within 1 to 3 days of culture. Furthermore, inhibition of mycobacterial growth in the presence of the antimicrobial agents rifampin and INH could be quantified by flow cytometry. These studies demonstrated that the presence of an antibiotic-resistant strain which represented 3% of the total population of approximately 10^7 mycobacteria could be detected within 3 or 4 days of culture. These results are consistent with previous studies using GMD encapsulation of *E. coli*, in which growth of a population containing 3% penicillin-resistant organisms could be detected by flow cytometry (28). Improvements in the assay, such as further optimization of the staining and encapsulation protocols, should make it possible to detect a 1% resistant population. This level of sensitivity would be consistent with the clinical and bacteriological data on TB which indicate that a 1% antibiotic-resistant population is clinically relevant (15).

The characteristic slow growth of these microorganisms, as well as the small number found in most clinical samples, has made the direct detection of TB bacilli by biochemical and immunological methods difficult. Use of GMD encapsulation in combination with flow cytometry could reduce the time required to evaluate clinical samples and establish effective treatment regimens. Flow cytometry is an attractive analytical method because it allows rapid analysis of large numbers of particles. Although the cost and operation of a flow cytometer are important considerations, the clinical impact of multidrug-resistant TB together with the rate of TB transmission and treatment costs are compelling forces for development of rapid antibiotic susceptibility assays. Furthermore, colony growth within the GMD could also be quantified by using digital image analysis as an alternative to flow cytometry. With either method, encapsulation of mycobacteria within the GMD is essential in providing a discrete microenvironment for measuring colony growth.

These studies using the *M. bovis* model system have shown the feasibility of this method for detecting mycobacterial growth. However, demonstrating that GMD encapsulation technology is a significantly faster method for analyzing clinical specimens will be the key issue. The BACTEC 460 radiometric detection system is currently an option for more rapid growth measurements, but this method also requires a significant capital expenditure and generates radioactive waste. Other systems such as the Septi-Chek AFB (Becton Dickinson) and BacT/Alert (Organon Teknika, Durham, N.C.) do not use radioisotopes but require a longer time for detection. In a recent comparison of these systems with conventional culture, the average times for detection of mycobacteria from smear-positive samples were decreased from 24 days to 19 and 13 days for the Septi-Chek and BACTEC systems, respectively (21). Many clinical laboratories combine conventional culture and BACTEC detection and can generate drug susceptibility data in approximately 4 weeks for a specimen containing about 10^4 mycobacteria (10). Nucleic acid amplification methods such as those used by GenProbe and Roche Molecular Systems (Somerville, N.J.) are also being investigated for rapid detection of mycobacteria. However, these techniques would still require culture for assessment of drug susceptibility.

In contrast to the model system of a pure culture of mycobacteria, analysis of clinical specimens will be complicated by the variable number of mycobacteria present and the sample processing involved. The use of GMD encapsulation and flow cytometry may facilitate more rapid analysis of mycobacterial growth; however, it is not likely that this method could be used for direct detection from a clinical specimen. Rather, an initial period of culture in liquid medium to expand the population to 10^6 to 10^7 cells would precede the encapsulation and 3-day susceptibility testing. Although as few as 10^4 organisms can be encapsulated, detection of a small (1 to 3%) antibiotic-resistant population by flow cytometry would be difficult since that population would represent only 100 to 300 events. To determine if GMD encapsulation could be a practical alternative to systems such as the BACTEC, analysis of clinical samples by this method will need to be evaluated in terms of speed, sensitivity, and cost.

In addition to GMD encapsulation being used for clinical diagnosis, rapid, automated screening of drug candidates against infectious disease targets could accelerate pharmaceutical drug discovery. Efforts to identify new antibiotics had lessened because of the number of drugs available and a decrease in disease incidence (7, 8). However, antibiotic-resistant microorganisms are becoming increasingly prevalent, prompting renewed interest in new antimicrobial agents. Furthermore, isolation of antibiotic-resistant populations by using GMD encapsulation in conjunction with fluorescence-activated cell sorting would allow the recovery of microorganisms of interest for further analysis, such as gene sequence determination. Although this study has focused on TB, GMD encapsulation technology could be extended to development of rapid assays for other infectious diseases.

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