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Detection of Mycobacterium bovis Bacillus Calmette-Guerin Using Quantum Dot Immuno-Conjugates

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Luminescent quantum dots (QDs) are a novel and promising class of fluorophores for cellular imaging (1,2). The benefits of QDs include their photostability, high brightness, multi-target labeling with several colors, and single-source excitation for QDs of all colors. We have developed procedures for using QDs to detect mycobacteria in a species-specific manner.

Mycobacterium bovis BCG strain 172 was obtained from Japan BCG Laboratory, Tokyo, Japan. A green fluorescent protein (GFP) expressing M. bovis BCG, containing plasmid pGFM-11, was supplied by C. Locht, Institut Pasteur de Lille, France. The BCG strains were grown in liquid Middlebrook 7H9 medium (Difco Laboratories, Detroit, Mich., USA) supplemented with 10% oleic acid-albumin-dextrose-catalase enrichment (OADC, Difco) and incubated at 37°C. Ten microliters of liquid medium was mounted on a glass coverslip beneath a hole in a plastic petri dish bottom (Matsunami Glass Industry., Ltd., Tokyo, Japan; code. D110100) and were subsequently air dried. Two percent glutaraldehyde in PBS was applied for 1 h at room temperature. After several rinses with PBS, the 1% bovine serum albumin (BSA) in PBS (BSA/PBS) was applied for 20 min at room temperature to block nonspecific binding. Antiserum obtained from rabbits immunized with heat-killed BCG was applied at a dilution of 1:4000 with BSA/PBS, and the dishes were incubated for 1 h at room temperature. After several rinses with 0.02% Tween 20 in PBS (PBS/Tween 20), Qdot™ 655 goat F(ab')2 anti-rabbit IgG conjugate (H+L) highly cross-absorbed (antibodies QD-conjugate: Quantum Dot Corp., Hayward, Calf., USA) was applied at a dilution of 1:1000 with 1% BSA for 1 h at room temperature. The dishes were then rinsed three times with PBS/ Tween 20, and microscopic examinations were conducted with a confocal laser scanning microscope (LSM 510, Carl Zeiss, Oberkochen, Germany) equipped with a ×100/1.40 oil immersion objective, an HBO 50 illuminator, and an FITC/Rhodamine dual-band filter set.

The results of immunofluorescent staining (A, B), conventional mycobacterial staining (C, D), and Ziehl-Neelsen staining (E, F) are shown in Fig. 1. BCG strains were labeled in red when treated with anti-BCG antibodies (Fig. 1A), whereas Mycobacterium smegmatis (Fig. 1B) was not labeled when treated with anti-BCG antibodies, indicating that these antibodies was specific to M. bovis BCG.

As shown by the confocal image in Fig. 2A, the surface of
BCG strain 172 was labeled with red-colored QD-conjugated anti-rabbit IgG when treated with antiserum against BCG. The size of the labeled BCG was 3.5 (SD: 0.4) × 0.5 (SD: 0.1) μm (n = 4). The microorganisms were not labeled when treated with pre-immune serum. GFP-expressing BCG was stained using the same procedure (Fig. 2B). GFP was detected in the bacteria’s intracellular region and was labeled only negligibly by QD-conjugate. The anti-BCG antibodies in combination with the QD-conjugated anti-IgG antibodies labeled the surface of BCG in a specific manner.

Acid-fast staining, such as Ziehl-Neelsen stain and auramine-rhodamine stain, are well-established procedures for detecting *Mycobacterium tuberculosis* and other mycobacterial spp. The immunostaining using QD-conjugates may be useful for identification of mycobacterial-specific antigen.

**REFERENCES**