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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

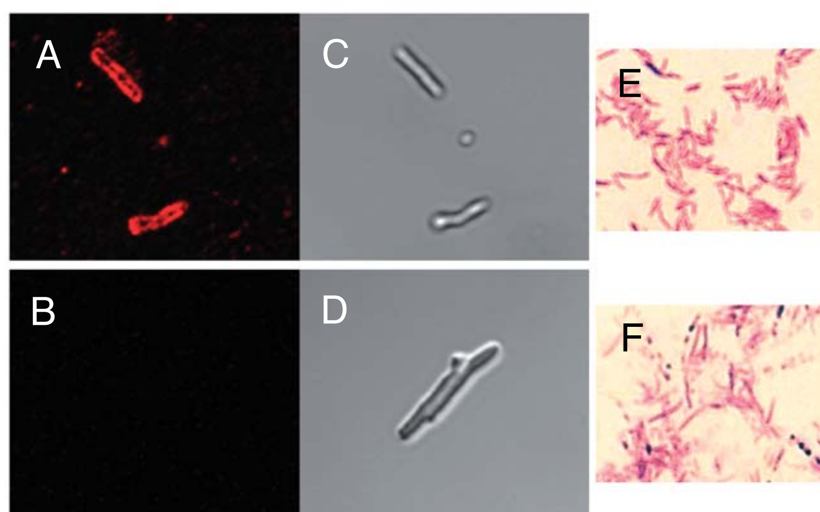

Fig. 1. Immunofluorescence staining of BCG (A, C, E) and *M. smegmatis* (B, D, F) strains (×1000).

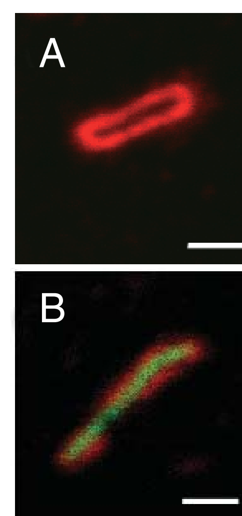

Fig. 2. Labeling of BCG (A) and GFP-expressed BCG (B) with anti-BCG antiserum and QD-conjugated anti-rabbit IgG. Scale bar, 1 μm.

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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.

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