

FIG. 1. Structure of plasmid vector pFab1-PhoA-H used for expression of the fusion protein of Fab and alkaline phosphatase. Genes encoding the light chain and the Fd region of the heavy chain are ligated into the *NheI/AscI* and *SfiI/NotI* sites, respectively. *tacSD*, tac promoter Shine-Dalgarno sequence; *pelB*, signal sequence of peptate lyase of *Erwinia carotovora*; *PhoA*, gene for alkaline phosphatase; *His-tag*, gene for hexahistidine tag; *amp*, gene for ampicillin resistance.

tent *E. coli* JM109. The bacteria were spread on Luria-Bertani plates containing 50  $\mu$ g of ampicillin per ml, and the vector with the inserts was selected. The positive clone was cultured in 1 liter of super broth (30 g of tryptone, 20 g of yeast extract, 10 g of MOPS [morpholinepropanesulfonic acid] per liter [pH 7]) containing ampicillin at 37°C until an optical density at 600 nm of 0.5 was achieved. Isopropyl- $\beta$ -D-thiogalactopyranoside was added to the cultures to a final concentration of 100  $\mu$ M, and the cultures were then incubated at 30°C for 12 h to achieve optimal expression. The bacteria were pelleted by centrifugation at 6,000  $\times$  g for 20 min, suspended in 20 ml of phosphate-buffered saline (PBS) containing 1 mM phenylmethylsulfonyl fluoride, and then sonicated. The lysates were centrifuged at 12,000  $\times$  g for 30 min, and the supernatant was filtered through 0.2- $\mu$ m-pore-size syringe filters (Iwaki, Tokyo, Japan). Purification of the fusion protein from the supernatant was performed by affinity chromatography with His•Bind resin (Novagen, Madison, Wis.) in accordance with the manufacturer's instructions. Purified fusion protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (22). Western immunoblot analysis was also performed as previously described (22). The horseradish peroxidase (HRP)-conjugated goat immunoglobulin G (IgG) fraction specific to the human kappa chain (Organon Teknica, Durham, N.C.) and HRP-conjugated rabbit IgG fraction specific to alkaline phosphatase (Rockland, Gilbertsville, Pa.) were used for detection.

Approximately  $2 \times 10^5$  trophozoites of *E. histolytica* HM-1:IMSS cultured axenically in BI-S-33 medium (10) were incubated on acetone-washed coverslips at 37°C for 30 min. The trophozoites were fixed with 4% paraformaldehyde in PBS for 30 min and then washed three times with PBS. After blocking with 5% bovine serum albumin was conducted for 15 min, the

cells were incubated with the recombinant protein (50  $\mu$ g/ml) for 30 min. After the cells were washed with PBS, development was conducted with a Vector red alkaline phosphatase substrate kit I (Vector Laboratories, Burlingame, Calif.) for 30 min in accordance with the manufacturer's instructions. Microscopic observation of the cells was performed under bright-field and fluorescent conditions by using a Nikon (Tokyo, Japan) XF-EFD2 fluorescence microscope.

SDS-PAGE analysis of the purified fusion protein of CP33 and PhoA revealed the expected sizes of two bands with apparent molecular masses of 25 and 75 kDa, although minor bands with apparent molecular masses of 50 kDa were also present (Fig. 2A). With Western immunoblot analysis, the 25-kDa band was recognized by an anti-human kappa chain goat antibody (Fig. 2B, lane 1). On the other hand, the 75-kDa band was detected by an anti-PhoA rabbit antibody, indicating that the molecule was a fusion protein of the Fd fragment and PhoA (Fig. 2B, lane 2). When the fusion protein of Fab-PhoA was incubated with paraformaldehyde-fixed trophozoites of *E. histolytica* and developed with the substrate, the surfaces of the trophozoites were stained clearly under both bright-field and fluorescent conditions (Fig. 3).

Recombinant human antibodies have been developed re-

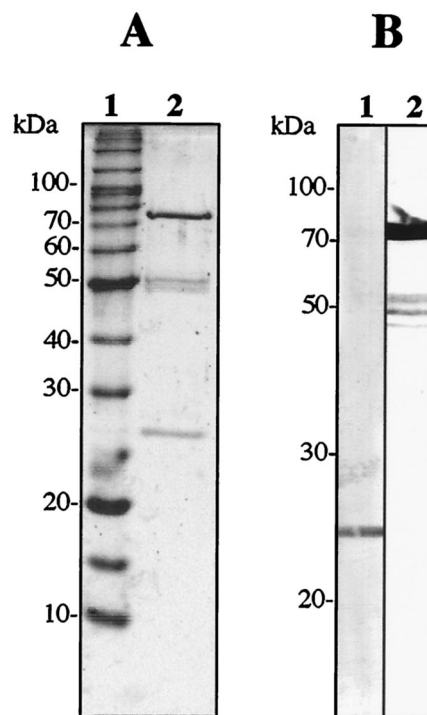


FIG. 2. SDS-PAGE (A) and Western immunoblot (B) analyses of a purified fusion protein of human Fab CP33 and alkaline phosphatase. (A) Two micrograms of the protein was subjected to analysis in 10% gel under reducing conditions and then stained with Coomassie brilliant blue. Lane 1, molecular size markers (BenchMark protein ladder; Life Technologies, Gaithersburg, Md.); lane 2, purified CP33-PhoA. Numbers to the left indicate molecular masses of the markers (in kilodaltons). (B) Protein bands were transferred to a polyvinylidene difluoride membrane. Lane 1 was treated with HRP-labeled anti-human kappa chain goat antibody. Lane 2 was treated with HRP-labeled anti-PhoA rabbit antibody.

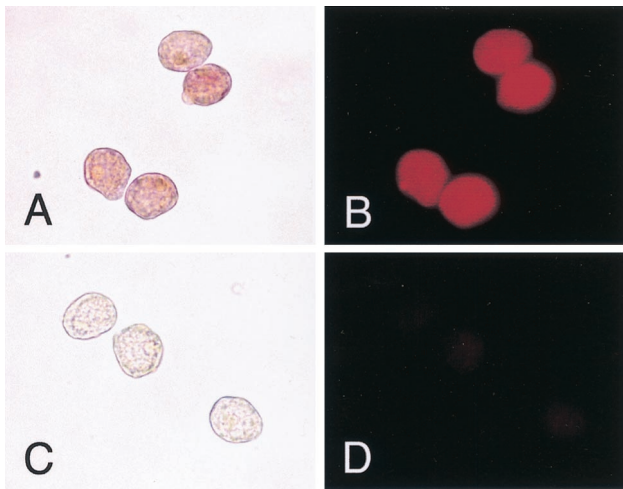


FIG. 3. Immunocytochemistry of *E. histolytica* with a fusion protein of human Fab CP33 and alkaline phosphatase. Paraformaldehyde-fixed trophozoites were treated with the fusion protein (A and B) and then with the substrate Vector red. As the controls (C and D), trophozoites were treated with a supernatant of *E. coli* lysates (vector control). (A and C) Bright-field microscopy; (B and D) fluorescence microscopy with a green filter. Magnification,  $\times 360$ .

cently for application in immunoprophylaxis, or the treatment of infectious diseases. Although such human antibodies would also be useful for diagnostic purposes, one of the disadvantages of the use of human antibodies for the detection of pathogens in human samples might be reactivity of endogenous immunoglobulins with the secondary anti-human antibodies used in indirect methods. Therefore, direct labeling of the human antibody with enzymes is needed to reduce nonspecific binding of the second antibody. It was reported recently that immunoglobulin genes derived from murine hybridoma cells could be expressed in *E. coli* as fusion protein Fab-PhoA (7, 25) or scFv-PhoA (5, 7, 13, 17). The present study demonstrates that the bacterial expression of a human MAb-PhoA conjugate specific for *E. histolytica* is also possible. In addition to the advantage of using the antibody to detect the *E. histolytica* antigen without the need for chemically conjugated secondary antibodies, there is no requirement for experimental animals or reagents and equipment for the culture and cryopreservation of hybridoma cells. Accordingly, the use of this human recombinant antibody also provides an economic benefit.

The antigen recognized with CP33 was the heavy subunit of the galactose- and *N*-acetyl-D-galactosamine-inhibitable lectin of *E. histolytica* (23). It is well known that this lectin molecule is suitable as a target antigen for the detection of *E. histolytica* in fecal and serum samples (1, 2, 11, 12). In conclusion, we propose here that the human Fab-PhoA fusion protein can be used in the diagnosis of amebiasis.

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