INFECTION AND IMMUNITY, Oct. 1985, p. 236–242
0019-9567/85/100236-07$02.00/0

Vol. 50, No. 1

Cloning and Surface Expression in *Escherichia coli* of a Structural Gene Encoding a Surface Protein of *Haemophilus influenzae* Type b

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Received 12 April 1985/Accepted 10 July 1985

Recombinant DNA technology was used to clone a gene coding for a surface protein of *Haemophilus influenzae* type b (Hib) into *Escherichia coli*. Chromosomal DNA from a clinical isolate of Hib was cleaved with *Eco*RI and ligated into plasmid vectors containing three different translational reading frames. *E. coli* carrying recombinant plasmids were screened in a colony blot-radioimmunoassay system by using murine monoclonal antibodies (mabs) directed against cell surface-exposed proteins of Hib. mab 7B2, which is specific for a Hib surface protein with an apparent molecular weight of 27,000 (27K), reacted with several recombinant strains of *E. coli*. Restriction analysis revealed the presence of a 9.1-kilobase DNA insert in each of these recombinant plasmids and also determined that both transcription and translation of the Hib gene(s) coding for the 7B2-reactive antigen were not dependent on the *lac* operator and promoter of the vectors. Radioimmunoprecipitation and Western blot analyses showed that the antigenic determinant recognized by mab 7B2 in these recombinant *E. coli* was present in a 27K protein. In addition, this 27K protein was shown to be both localized on the surface of these *E. coli* cells and accessible to antibody.

The development of an efficacious vaccine for the protection of infants against systemic disease caused by *Haemophilus influenzae* type b (Hib) is the subject of intense research efforts in many laboratories (2, 8, 25–27, 33, 36). We have concentrated on identifying vaccine candidates among the outer membrane proteins of this pathogen. Previous studies from this laboratory have established that certain Hib outer membrane proteins are immunogenic in human infants as well as being both exposed on the Hib cell surface and accessible to antibodies (16–19).

Molecular cloning into *Escherichia coli* may provide a useful system for the production of protective Hib antigens and especially for genetic analysis of these antigens. Specifically, we wished to isolate recombinant clones which express Hib surface antigens recognized by monoclonal antibodies (mabs) which we have previously characterized as being directed against cell surface-exposed proteins. Here we describe the construction of recombinant clones which satisfy this criterion. Moreover, we present data which indicate that the Hib protein encoded by the cloned gene is synthesized in *E. coli* accurately with respect to size and antigenicity and is localized on the surface of *E. coli* cells in which it is expressed.

(A preliminary account of these findings was presented at the 84th Annual Meeting of the American Society for Microbiology [P. L. Holmans, T. A. Loftus, and E. J. Hansen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, D95, p. 66].)

MATERIALS AND METHODS

Bacterial strains, plasmid vectors, and growth conditions. The clinical isolate of Hib (strain DL41) used in this study as the source of chromosomal Hib DNA was obtained from George H. McCracken, Jr., Southwestern Medical School, Dallas, Tex., and has been described previously (14). Hib DL41 was grown at 37°C in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) supplemented with Levinthal base (1) as a source of hemin and NAD. Solid medium was prepared by incorporating 1.5% (wt/vol) agar (Difco) in brain heart infusion supplemented with Levinthal base. Agar plate cultures were incubated at 37°C in a candle extinction jar for 18 to 24 h.

The *E. coli* K-12 derivative HB101 [F$^-$ *hsdS*20($r^-_B m^-_B$) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44* λ^-] (5) was the recipient for all transformation experiments and the host for the vector plasmids pPCϕ1, pPCϕ2, and pPCϕ3 (7). Each of these plasmids possesses a different translational reading frame linked to the *lac* operator and promoter, such that all three possible reading frames expressed from the *lac* UV5 promoter are represented by this set of plasmids. *E. coli* HB101 was grown in L broth (LB) (1 liter contained 10 g of tryptone, 10 g of NaCl, and 5 g of yeast extract) or on LB agar. Plasmid-containing derivatives were grown in LB or on LB agar supplemented with 100 μg of ampicillin trihydrate ml^{-1} (Sigma Chemical Co., St. Louis, Mo.). For large-scale plasmid preparations, *E. coli* HB101 derivatives were grown in medium C (29) supplemented with 0.2% (wt/vol) glucose, 0.1% (wt/vol) Casamino Acids, 0.5 μg of thiamine ml^{-1}, and 100 μg of ampicillin ml^{-1}.

Preparation of DNAs. Plasmid DNA was prepared by the method of Guerry et al. (13). Chromosomal DNA from Hib was prepared from 1 liter of stationary-phase cells by the method of Marmur (30), followed by phenol extraction and dialysis against 50 mM Tris hydrochloride (pH 8.0) containing 5 mM EDTA.

Production of mab 7B2. Eight-week-old female BALB/c mice (Cumberland Laboratories, Clinton, Tenn.) were immunized by intraperitoneal injection with 10^7 CFU of Hib DL41. One month later, these animals received a second injection of 10^8 CFU of this same strain, and 3 weeks later they received a third and final injection identical to the second injection. Three days after this last injection, the spleens were removed from two animals and used in the standard hybridoma production system described in detail previously (35). The whole-cell radioimmunoprecipitation (WC-RIP) system (18) was used to screen culture supernatant fluids (500 μl) from the resultant hybridomas for the

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presence of mabs directed against cell surface-exposed and antibody-accessible Hib proteins as previously described (25). Murine lymphocyte hybridoma 7B2 was identified as producing an immunoglobulin G mab directed against a cell surface-exposed outer membrane protein of Hib with an apparent molecular weight of 27,000 (27K) and was cloned by limiting dilution analysis as previously described (35).

Antisera. Immune rat serum was produced in adult Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) by immunization of these animals with viable Hib cells. The rats received intraperitoneal injections with 10^7 CFU of Hib DL41 at 3- to 4-week intervals for 4 months. Immune serum was prepared from the blood of animals exsanguinated by cardiac puncture.

AA-RIA. The antibody-accessibility (AA)-radioimmunoassay (RIA) procedure was used to assess cell surface exposure and AA of the antigenic determinant recognized by mab 7B2 in different strains of bacteria. *E. coli* strains grown overnight on LB agar were suspended in phosphate-buffered saline (PBS) at 4°C to a final concentration of 10^8 CFU/ml. A 1-ml portion of the *E. coli* suspension was incubated with 500 μl of hybridoma culture supernatant containing mab 7B2 for 2 h at 4°C with gentle agitation. The cells were centrifuged at 12,000 × *g* for 2 min and then suspended in 1 ml of PBS containing 10% fetal calf serum (PBS-FCS). The cells were then centrifuged again and suspended in 1 ml of the same buffer. To detect mab 7B2 bound to the bacterial cells, 5×10^5 cpm of affinity-purified and radioiodinated rabbit anti-mouse immunoglobulin (specific activity, 10^7 cpm per μg of protein) was added to the cell suspension. After incubation for 1 h at 4°C with gentle agitation, the bacterial suspension was washed five times with 1-ml quantitites of PBS-FCS and suspended in 500 μl of solubilization buffer (18). Radioactivity in the final washed cell pellet was measured with a Searle model 1195 gamma counter (Searle Analytic Inc., Chicago, Ill.). The results are expressed as counts per minute of antibody probe bound to the test cells. All experiments included a negative control with PBS in place of the 7B2 hybridoma culture supernatant. All data represent the average of duplicate samples.

Construction and screening of genomic libraries. The vector DNAs pPCϕ1, pPCϕ2, and pPCϕ3 were digested with *Eco*RI (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as described previously (22). Partial *Eco*RI digests of chromosomal DNA from Hib DL41 were also prepared. The vector DNAs were then treated with calf intestine alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), followed by phenol and ether extractions (29). The phosphatase-treated, *Eco*RI-digested plasmid DNAs were individually combined with the cleaved Hib DL41 DNA, and T4 DNA ligase (Boehringer Mannheim Biochemicals) was added, along with ATP and dithiothreitol to final concentrations of 0.1 and 1 mM, respectively. The ratio of phosphatase-treated vector DNA to Hib DNA was 20:1, and the ligation reactions were performed at 4°C overnight. The ligated DNA was used to transform *E. coli* HB101 made competent with calcium chloride treatment (28). After incubation at 37°C for 40 min to allow recovery and expression, the transformed cells were plated on LB agar containing ampicillin and incubated overnight at 37°C. The resultant colonies were collectively suspended in LB containing ampicillin and 20% glycerol and stored at −20°C in multiple portions.

Immunoscreening of genomic libraries with mab probes. The genomic libraries were plated at 2×10^2 to 5×10^2 colonies plate^{-1} and grown overnight to prepare them for screening with specific mabs directed against cell surface-exposed proteins. Clones which produced antigens recognized by the mabs were detected by a modification of the colony blot RIA of Henning et al. (21). Colonies were lifted on sterile Whatman no. 40 (ashless) filter paper (8.26-cm diameter). The filters were placed in chloroform for 5 min, dried at 37°C for 30 min, and then probed with a pool of mabs as previously described (14). mab 4C11, which is directed against the 100K cell surface-exposed protein of Hib (17), and mab 6G12, which is specific for the 98K cell surface-exposed protein of Hib (25), were used together with mab 7B2 in this screening system. The presence of mabs attached to the filter-bound lysed cells was detected with the affinity-purified and radioiodinated rabbit anti-mouse immunoglobulin. All incubations were performed with gentle agitation at 4°C. Clones which reacted with the mabs were identified by autoradiographic analysis with Fuji RX safety film and a DuPont Cronex intensifying screen. The original plates were incubated 4 to 6 h at 37°C, and colony blot-positive colonies were then picked and rescreened with the individual mabs.

Characterization of recombinant plasmids. Plasmid DNA from the clones of interest was isolated from individual colonies or from 1-ml liquid cultures (medium C) by the method of Holmes and Quigley (23). The use of supplemented minimal medium rather than LB resulted in a better plasmid yield with much less rRNA contamination (P. L. Holmans, unpublished data). The resultant plasmid DNA was used for restriction endonuclease analysis and subsequent transformations.

Immunochemical identification of cloned gene products. Identification of gene products recognized by specific mabs was performed by use of the WC-RIP system, as described previously (15, 17), or by Western blot analysis, by a modification of the method of Towbin et al. (41). In the latter procedure, proteins resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis in a 10% (wt/vol) polyacrylamide separating gel were electrophoretically transferred to nitrocellulose strips overnight at 4°C, by using either 165 mA or 75V, in 20 mM Tris containing 150 mM glycine and 20% (vol/vol) methanol. The nitrocellulose strips were then probed with mab 7B2 by a procedure described previously (25). Detection of antigen-mab complexes on the nitrocellulose strips was achieved by using horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (diluted 10^{-3} in PBS containing 0.1% [vol/vol] Tween 20), sometimes followed by peroxidase-conjugated rabbit anti-goat immunoglobulin G (both from Cappel Laboratories, Cochranville, Pa.) and subsequent reaction with 4-chloro-1-naphthol (Sigma) as described by Hawkes et al. (20).

Electron microscopy. Visualization of immune complexes on bacterial cell surfaces was performed as follows. Cells from a freshly grown agar plate were suspended to approximately 10^9 ml^{-1} in PBS-FCS, pelleted by centrifugation, and resuspended in PBS-FCS. A 0.25-ml portion of the suspended cells was mixed with 1.0 ml of hybridoma culture supernatant containing mab 7B2 and was rotated at 4°C for 60 to 120 min. After three washes in PBS-FCS, the cells were suspended in PBS-FCS (1.0 ml), and a suspension of staphylococcal protein A-colloidal gold particles prepared by the method of Geohegan and Ackerman (11) was added such that the final mixture was light pink. This suspension was rotated at 4°C for 60 to 120 min, after which the cells were washed three times in PBS-FCS and once in PBS and finally suspended in PBS (0.5 ml). Formaldehyde was added to a final concentration of 1.2%; after 10 min, the cells were applied to carbon-coated grids (10 to 15 μl grid^{-1}). Excess

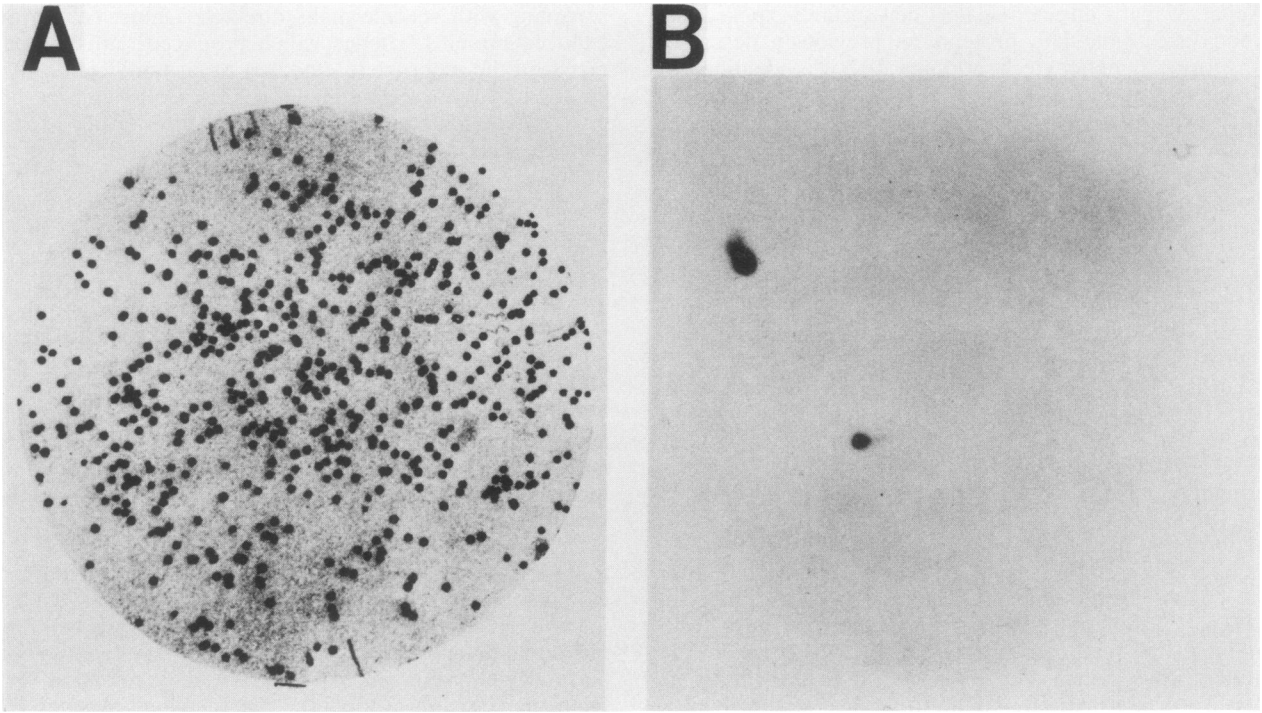

FIG. 1. Colony blot-RIA of *E. coli* HB101 recombinant clones. Colonies were transferred to Whatman no. 40 paper and probed with mab 7B2 in the colony blot-RIA described in Materials and Methods. Panel A shows a filter to which colonies were transferred and assayed by colony blot-RIA, followed by staining with methylene blue to facilitate localization of reactive colonies. Panel B is an autoradiograph of the same filter after colony blot-RIA.

liquid was removed with a wedge of filter paper, and a drop of 2% (wt/vol) neutral aqueous phosphotungstic acid was applied to each grid. The phosphotungstic acid was removed with a wedge of filter paper, and a drop of distilled H_2O was applied to each grid and then removed. The grids were examined in a Philips 301 electron microscope at 60 kV accelerating voltage and photographed at a magnification of ×34,000.

RESULTS

Construction and screening of genomic libraries. A total of 6,700 pPCϕ1 clones, 1,870 pPCϕ2 clones, and 2,000 pPCϕ3 clones were obtained in this study. The three libraries were plated at 200 to 500 colonies plate^{-1} and screened with a mixture of mabs previously found to be specific for Hib surface proteins. A representative filter from an experiment utilizing mab 7B2 that was stained with methylene blue to reveal the position of the bacterial colonies and the matching autoradiograph with two positive reactions is shown in Fig. 1. Of the colonies tested in one experiment, 4/2,477, 6/749, and 3/990 from the pPCϕ1, pPCϕ2, and pPCϕ3 libraries, respectively, reacted with mab 7B2. Assuming that each clone was an independent isolate, mab 7B2-positive recombinant clones were obtained at a frequency of approximately 3×10^{-3}. No recombinant clones reactive with mab 4C11 or 6G12 were obtained in these experiments.

Characterization of recombinant plasmids. Because mab 7B2-positive clones were found in each library, the expression of the mab 7B2-reactive antigen was apparently independent of the *lac* operator and promoter of the vector plasmids for transcription. We therefore addressed the possibility that transcription initiated within the cloned segment of Hib DNA. Four clones were picked at random and analyzed to determine whether the DNA insert encoding the mab 7B2-reactive antigen was present in only one orientation or in both orientations, the latter situation being evidence for expression from a promoter within the cloned Hib DNA segment. The recombinant plasmids were cleaved with *Pst*I (which does not cut within the DNA inserts) and *Xba*I (which does not cut within the vectors) (Fig. 2). The resulting asymmetry of the cleavage products was used in the analysis of the restriction profiles. *Eco*RI cleavage of the four recombinant plasmids examined in this experiment yielded an insert of 9.1 kilobases (Fig. 3, lanes a to d). However, based on the sizes of the restriction fragments resulting from cleavage with *Pst*I and *Xba*I, clones pPLH401 (Fig. 3, lane f) and pPLH402 (lane g) contained inserts whose polarity is directly opposite that of the inserts in clones pPLH403 and pPLH404 (lanes h and i, respectively). Because cleavage of the plasmids with the combination of *Eco*RI, *Pst*I, and *Xba*I resulted in an identical restriction pattern from all four plasmids (Fig. 3, lanes k to n), the inserts were assumed to be identical, with either orientation sufficient for expression of the mab 7B2-reactive antigen (Fig. 2). Therefore, both transcription and translation presumably initiated within the cloned Hib DNA and from the authentic (Hib) promoter.

Characterization of the 7B2 gene product. We wished to determine whether the cloned gene product which reacted with mab 7B2 was identical to the protein recognized by mab 7B2 in Hib. We addressed this question first with RIP analysis of one of the recombinant clones, by using a WC-RIP method which has been shown to be specific for the identification of surface antigens (15, 18). The protein which was immunoprecipitated from radiolabeled Hib cells by mab 7B2 (Fig. 4, lane c) has an apparent molecular weight of

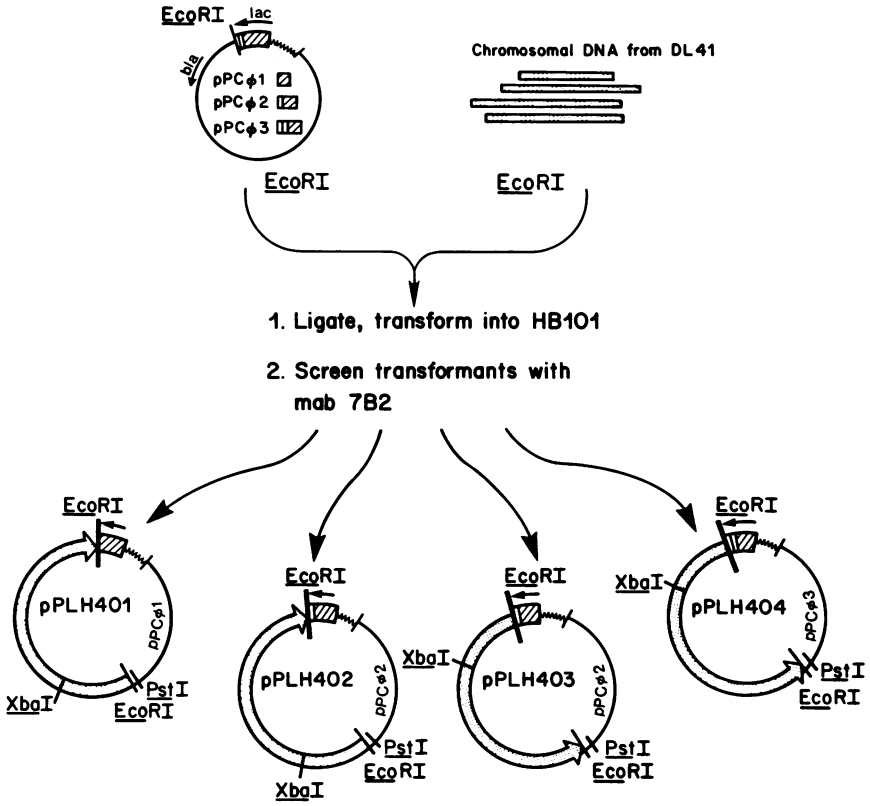

FIG. 2. Schematic diagram of the cloning approach used to generate the recombinant plasmids pPLH401, pPLH402, pPLH403, and pPLH404. The arrows on the Hib DNA inserts reflect the relative orientation of each insert and do not necessarily indicate the direction of transcription. The cleavage sites for *Eco*RI, *Pst*I, and *Xba*I are indicated on each recombinant plasmid.

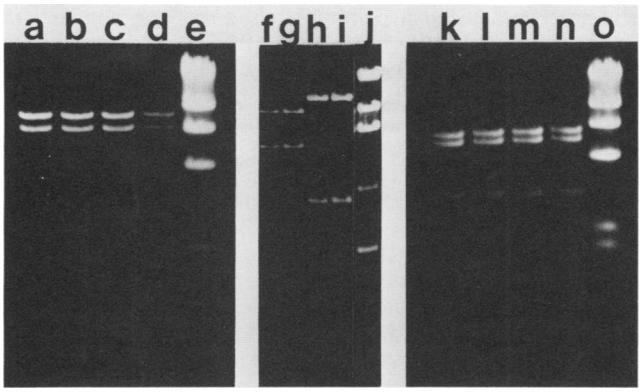

FIG. 3. Restriction endonuclease analysis of recombinant plasmids. Plasmid DNAs were isolated by a minipreparation method (23) and cleaved with the restriction enzymes indicated, followed by electrophoretic separation of the products in 1% agarose containing 0.5 μg of ethidium bromide ml^{-1}. Electrophoresis was performed in a Bio-Rad minisubmarine apparatus. The samples were as follows. *Eco*RI digests: pPLH401 (lane a), pPLH402 (lane b), pPLH403 (lane c), pPLH404 (lane d). *Pst*I and *Xba*I digests: pPLH401 (lane f), pPLH402 (lane g), pPLH403 (lane h), pPLH404 (lane i). *Eco*RI, *Pst*I, and *Xba*I digests: pPLH401 (lane k), pPLH402 (lane l), pPLH403 (lane m), pPLH404 (lane n). *Hin*dIII fragments (23.1, 9.6, 6.6, 4.4, 2.3, and 2.01 kilobases) of bacteriophage lambda DNA (lanes e and o) and *Eco*RI fragments (19.4, 9.15, 7.16, 3.98, and 2.39 kilobases) of bacteriophage P22 DNA (lane j) were used as molecular mass markers. The upper band in lanes a to d represents the cloned Hib DNA insert. The very small DNA fragment resulting from cleavage by *Eco*RI and *Pst*I at two closely adjacent sites cannot be seen in lanes k, l, m, and n in this particular gel because of the relatively small quantity of plasmid DNA used in these lanes.

27,000 (27K). A negative control utilizing *E. coli* HB101 containing the vector (pPCϕ1) showed that mab 7B2 did not immunoprecipitate any radiolabeled native *E. coli* proteins (lane e). In contrast, when *E. coli* HB101 containing recombinant plasmid pPLH401 was assayed in the WC-RIP system, a 27K protein was immunoprecipitated by mab 7B2 (lane g). Thus, the Hib protein recognized by mab 7B2 is apparently synthesized as the authentic polypeptide in *E. coli*.

Western blot analysis was used to determine whether the other three mab 7B2-reactive recombinant clones carrying plasmids pPLH402, pPLH403, and pPLH404 all synthesized a 27K protein. All three of these recombinant clones synthesized 27K proteins which reacted with mab 7B2 and which exhibited electrophoretic mobilities (Fig. 5, lanes b to d) identical to the 27K protein synthesized by Hib DL41 (lane f).

The Hib protein recognized by mab 7B2 is a surface antigen of Hib DL41, and the results of the WC-RIP assay indicated that it was also localized on the surface of the recombinant clones. To confirm this finding, two different approaches were used, including an RIA method which determines the accessibility to antibody of a given antigen in whole bacterial cells and electron microscopy of immune complexes on the bacterial cell surface.

The results obtained with the AA-RIA are shown in Table 1. *E. coli* HB101 containing the recombinant plasmids pPLH401 and pPLH403 bound a significantly greater amount of mab 7B2 than *E. coli* HB101 containing the vector plasmids pPCϕ1 and pPCϕ2. Electron microscopic examination of whole cells of these strains treated with mab 7B2 followed by reaction with protein A-colloidal gold particles

to detect cell surface-bound antibody confirmed this finding (Fig. 6). The antigenic determinant recognized by mab 7B2 was accessible to antibody on the surface of the recombinant clone containing pPHL403 (Fig. 6B), as well as on Hib DL41 (Fig. 6A). In contrast, there was no binding of antibody observed when *E. coli* HB101 carrying only the homologous plasmid vector pPCϕ2 was tested (Fig. 6C). The same results were obtained when the other three mab 7B2-reactive recombinant clones were examined by these methods (data not shown).

Instability of expression of the 27K protein in *E. coli*. In the course of this work, we also observed an instability in the phenotype of the mab 7B2-reactive recombinant clones with regard to the expression of this antigenic determinant. In an effort to isolate clones which stably express this antigenic determinant, we isolated the plasmid DNA from four of the mab 7B2-reactive clones and used it to transform *E. coli* HB101. Of the ampicillin-resistant transformants obtained, 30 to 50% did not react with mab 7B2. There was, however, no apparent change in the size of the plasmids contained in these 7B2-negative phenotypic variants (data not shown).

DISCUSSION

The use of recombinant DNA methodology for the molecular cloning of genes from pathogenic bacteria has been applied to the study of several pathogens (6, 10, 12, 24, 31, 32, 34, 37–39) to isolate genes which encode immunogenic proteins. In some cases, the workers wished to circumvent problems associated with studying fastidious organisms; others wished to study genes whose products are antigenically variable, to understand the mechanisms responsible for this variability.

By using a molecular cloning approach, we produced recombinant plasmids containing the gene encoding a 27K surface protein from Hib DL41. The fact that no differences were observed in the electrophoretic mobilities of the 27K proteins synthesized in Hib and in the recombinant *E. coli* clones (as detected by both WC-RIP [Fig. 4] and Western blot [Fig. 5] analyses) suggests that the 27K protein was synthesized from the correct (Hib) promoter. In addition, the facts that the gene encoding the 27K protein in the 9.1-kilobase insert can be transcribed and translated in either orientation in the vector (Fig. 2) and that the two known promoters (*bla* and *lac*) which flank the insert are both transcribed in the same direction (7) suggest that the Hib promoter is used for transcription.

It is of interest to note that the 27K protein is accessible to mab 7B2 on the surface of *E. coli* cells in which it is expressed, as it is in Hib DL41. A similar situation was recently described in which surface antigens of *Legionella pneumophila* are expressed on the surface of *E. coli* (10). The availability of recombinant *E. coli* clones bearing Hib proteins on their cell surfaces will facilitate the production of

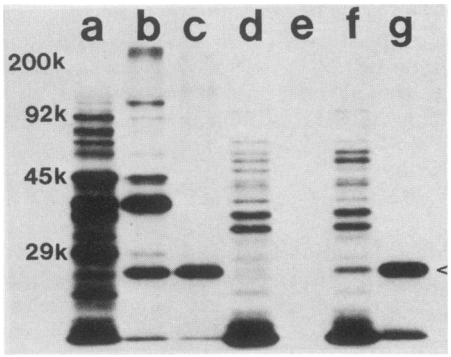

FIG. 4. RIP analysis of Hib DL41 and *E. coli* cells with mab 7B2. The Hib and *E. coli* cells were radioiodinated and used in the WC-RIP system together with mab 7B2, as described in Materials and Methods. Lane a: Radioiodinated Hib cells (solubilized in 2% SDS for SDS-polyacrylamide gel electrophoresis). Lane b: Immune precipitate obtained when immune rat serum raised against Hib DL41 was used in the WC-RIP system with Hib DL41 cells as antigen. Lane c: Immune precipitate obtained when mab 7B2 was used in the WC-RIP system with Hib DL41 cells as antigen. Lane d: Radioiodinated *E. coli* HB101 cells containing the plasmid vector pPCϕ1. Lane e: Immune precipitate obtained when mab 7B2 was used in the WC-RIP system with *E. coli* HB101 (pPCϕ1) cells as antigen. Lane f: Radioiodinated *E. coli* HB101 cells containing the recombinant plasmid pPLH401. Lane g: Immune precipitate obtained when mab 7B2 was used in the WC-RIP system with *E. coli* HB101 (pPLH401) cells as antigen. The arrowhead to the right of lane g indicates the position of the 27K protein. Molecular weight reference markers are provided on the left.

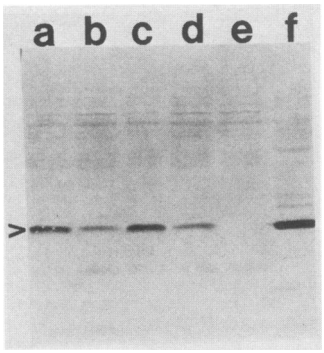

FIG. 5. Western blot analysis of mab 7B2-reactive recombinant *E. coli* clones. Bacterial cells were suspended in PBS to a density of 10^9 CFU ml^{-1}. Suspended cells (2 volumes) were mixed with 1 volume of 0.1875 M Tris hydrochloride (pH 6.8) containing 6% (wt/vol) SDS, 30% (wt/vol) glycerol, and a trace amount of pyronin Y. This mixture was heated at 100°C for 1 min and then mixed vigorously. Portions of this mixture representing 10^7 CFU were subjected to SDS-polyacrylamide gel electrophoresis in a 12.5% (wt/vol) polyacrylamide separating gel. The electrophoretically resolved proteins were transferred to nitrocellulose and subjected to Western blot analysis with mab 7B2 as described in Materials and Methods. *E. coli* HB101 clones carrying the recombinant plasmids pPLH401 (lane a), pPLH402 (lane b), pPLH403 (lane c), and pPLH404 (lane d) are shown. Lane e: *E. coli* HB101 carrying the vector pPCϕ1 (negative control). Lane f: Hib DL41 (positive control). The arrowhead on the left indicates the position of the 27K protein.

TABLE 1. Antibody-accessibility of the antigenic determinant recognized by mab 7B2 in *E. coli* strains carrying vector or recombinant plasmids[a]

Plasmid carried by *E. coli* HB101	^{125}I-goat anti-mouse immunoglobulin (amt bound [cpm])[b]
pPCϕ1 (vector)	1,241
pPLH401 (recombinant)	44,025
pPCϕ2 (vector)	1,108
pPLH403 (recombinant)	81,766

[a] The AA-RIA was performed as described in Materials and Methods.
[b] Counts per minute of radioiodinated goat anti-mouse immunoglobulin probe bound to mab 7B2 on the surface of *E. coli* cells.

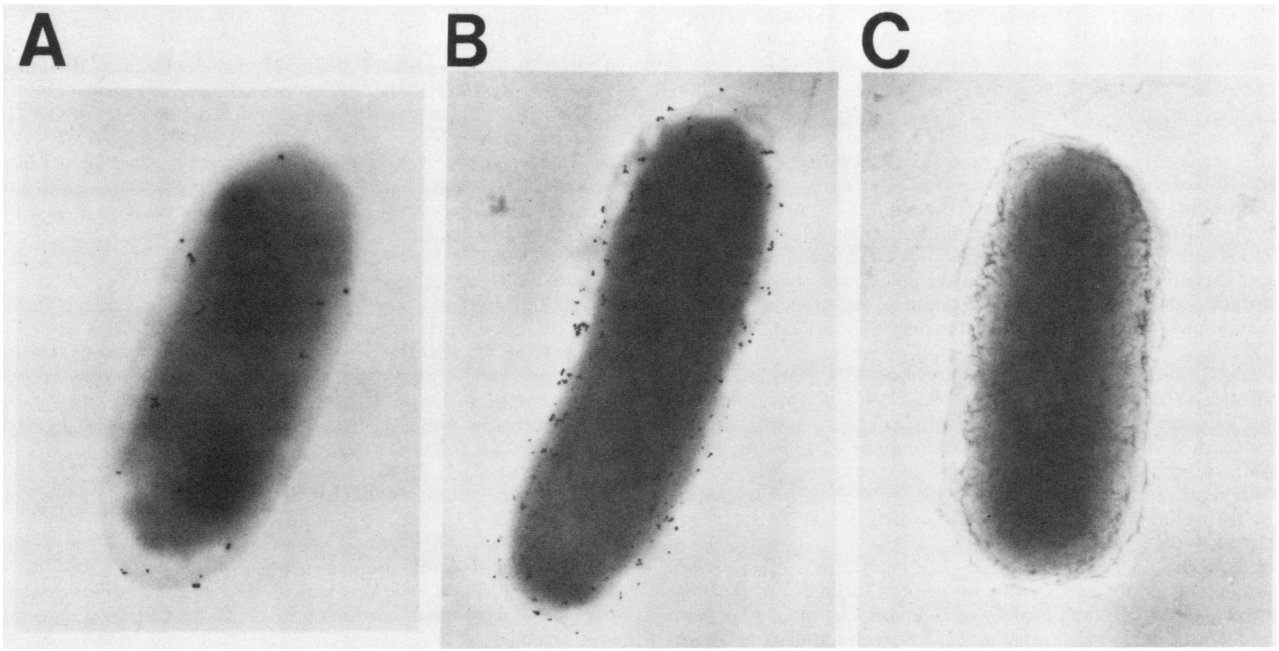

FIG. 6. Electron microscopic visualization of the interaction of mab 7B2 with bacterial cell-surface antigens, as detected by the use of the protein A-colloidal gold method. Cells of Hib DL41 (A), the mab 7B2-reactive recombinant *E. coli* strain carrying the recombinant plasmid pPLH403 (B), and the host *E. coli* HB101 carrying the homologous vector plasmid pPCϕ2 (C) were incubated with mab 7B2 and then processed as described in Materials and Methods.

highly specific polyclonal antisera to such proteins. These polyclonal antisera will be especially useful in protection tests designed to determine whether a given Hib protein represents a protective antigen. This is an important consideration, because the protective ability of any mab is very dependent on its isotype or immunoglobulin G subclass, such that a negative result in a protection test involving a mab can be difficult to interpret correctly.

The observed instability of expression of the 27K protein by *E. coli* recombinants is apparently not related to the potential problems inherent in the synthesis of a foreign membrane protein in *E. coli*. Recent studies with Hib DL41 indicate that not every cell in the population expresses this antigen (data not shown). Because antigenic variability of surface components has been reported in a variety of pathogenic organisms (3, 4, 9, 40), it is possible that we observed an example of this type of phenomenon. A better understanding of the mechanism(s) involved in this phenomenon is important when proteinacious vaccine candidates are considered, and the recombinant clones which exhibit this behavior will facilitate such analyses.

The successful cloning of the gene encoding the 27K cell surface-exposed protein of Hib into *E. coli* indicates that it may be possible to use this approach with other Hib surface proteins. We are especially interested in the 98K outer membrane protein which we have shown to be both immunogenic in human infants and a target for antibodies protective against experimental Hib disease (25). The 98K protein is a very minor component, in a quantitative sense, of the Hib outer membrane, and molecular cloning of the gene encoding this protein into appropriate expression vectors might permit the production and purification of substantial quantities of this protein from recombinant *E. coli*. The use of recombinant DNA techniques should facilitate the molecular and genetic characterization of both this and other immunogenic Hib surface protein molecules.

ACKNOWLEDGMENTS

We thank Michael Norgard, Jacob Kupersztoch, and Robert Munford for their comments concerning this manuscript which was expertly typed by Cindy Baselski.

This research was supported by a Robert A. Welch Foundation research grant (I-933) and by Public Health Service grant AI-17621 from the National Institute of Allergy and Infectious Diseases to E.J.H.

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