Cloning of the Gene, *speB*, for Streptococcal Pyrogenic Exotoxin Type B in *Escherichia coli*

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The structural gene encoding streptococcal pyrogenic exotoxin type B, designated *speB*, was cloned in *Escherichia coli* and localized onto a 4.5-kilobase BamHI-BglII DNA fragment. Streptococcal pyrogenic exotoxin type B, partially purified from *E. coli* clones, was immunologically related to streptococcus-derived toxin. Also, toxin derived from either *E. coli* or *Streptococcus pyogenes* had similar lymphocyte mitogenic activity and molecular weight (29,300) and displayed comparable microheterogeneity when evaluated by isoelectric focusing.

*Streptococcus pyogenes* and *Staphylococcus aureus* produce a family of related pyrogenic toxins (PTs) with similar biological and biochemical properties. These include the streptococcal enterotoxins (ENTS) A through E (2), pyrogenic exotoxins A and B (20) toxic shock syndrome toxin-1 (TSST-1) (3), and the streptococcal pyrogenic exotoxins (SPEs) A through C (1). All of these toxins induce lymphocyte mitogenicity, immunosuppression, and fever and enhance lethal endotoxin shock (10, 13, 20, 25, 29). In addition to these shared biological properties, ENTs have the additional ability to induce emesis and diarrhea after oral administration (11). Likewise, SPEs are unique for their capacity to induce damage to heart tissue (24).

The study of streptococcal and streptococcal PTs has been facilitated by molecular cloning and sequencing of toxin genes. Thus far, genes for TSST-1 (15), ENT A (5), ENT B (22), ENT C1 (7), ENT D (K. W. Bayles and J. J. Landolo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, B-187, p. 56), SPE A (13), and SPE C (S. C. Goshorn, G. A. Bohach, and P. M. Schlievert, submitted for publication) have been cloned. We report in this paper the cloning of *speB*, the gene encoding SPE B.

DNA for construction of a genomic library was obtained from *S. pyogenes* 86-858 (M-type 12), a pharyngitis isolate that produces SPE B but not SPE A or C. The procedure used for obtaining the DNA involved cell lysis by treatment with mutanolysin (Sigma Chemical Co., St. Louis, Mo.) and sodium dodecyl sulfate (SDS) as described by Spanier and Cleary (28). For cloning, a partial digest of the genome was prepared by incubating the DNA (45 min) with *Sau3A* (0.1 U/μg of DNA). The resulting DNA fragments were separated by agarose gel electrophoresis (13), and those in the 4- to 10-kilobase (kb) size range were recovered by the rapid freezing method of Smith (26). T4 DNA ligase was used to ligate the DNA fragments to pBR328 (27) previously linearized with *BamHI* and dephosphorylated by using calf intestinal phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Recombinant plasmids were transformed into *Escherichia coli* RR1 (9) by the method of Kushner (16), and transformants were selected on LB agar (4) containing ampicillin (40 μg/ml).

Approximately 2,000 transformants were screened for SPE B production with anti-SPE B hyperimmune rabbit antiserum by using a colony immunoblot assay (7). Two reactive colonies were detected. To confirm that these colonies were producing SPE B, the two *E. coli* clones were grown in broth culture, lysed and precipitated with 4 volumes of ethanol (14), redissolved in water (50-fold concentration), and tested by Ouchterlony immunodiffusion (19) against SPE B rabbit antiserum. Lysates from the clones produced precipitin lines of identity with streptococcus-derived SPE B (Fig. 1). The antiserum was specific for SPE B in that it did not react with purified streptococcus-derived SPE A or SPE C or with lysates from control cultures of *E. coli* RR1 (pBR328). The SPEB-containing recombinant plasmids carried by these clones were designated pUMN701 and pUMN702.

The 12.3-kb plasmid pUMN701, containing a 7.4-kb insert, was chosen for subcloning *speB* (Fig. 2). Digestion of pUMN701 with *BglII* yielded a 10.5-kb fragment containing pBR328 and a portion (5.6 kb) of streptococcal DNA. This 10.5-kb fragment, when ligated with pUC13 (30) and transformed into *E. coli* JM83 (18), resulted in transformants expressing SPE B (designated *E. coli* JM83(pUMN711)). DNA flanking *speB* was removed from pUMN711 by digestion with *EcoRI* or *BamHI*. This yielded *EcoRI*-BglII and *BamHI*-BglII DNA fragments of 7.8 and 4.5 kb, respectively. Both fragments were ligated to pUC13 and transformed into *E. coli* JM83. Each transformant expressed SPE B. Although we identified and mapped numerous additional restriction sites on pUMN701 (Fig. 2), all attempts to localize *speB* onto fragments smaller than 4.5 kb were unsuccessful. Since approximately 1 kb of DNA should contain the entire structural gene for SPE B, we suspect that additional regulatory sequences contained within the 4.5-kb *BamHI*-BglII insert but not adjacent to the *speB* structural gene may be required for its expression.

A molecular weight comparison of streptococcus-derived and *E. coli*-derived SPE B was made by SDS-polyacrylamide gel electrophoresis. Toxin from both sources was first purified by thin-layer isoelectric focusing (23) in a pH gradient of 7.0 to 9.0. As observed previously (1), SPE B displayed microheterogeneity, and multiple charged forms (pl 8.0 to 9.0) were recovered in preparations from both sources. The biologically active pl-8.4 fractions (1) were recovered and analyzed by electrophoresis through 15% acrylamide vertical gel slabs by using the discontinuous system of Laemmli (17). Toxin preparations from *S. pyogenes* and *E. coli* RR1(pUMN701) contained a single protein (M<sub>r</sub> 29,300) (Fig. 3). Both proteins also reacted with SPE B.

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antiserum in a Western blot assay (6, 7). The molecular weight of SPE B was originally reported to be 17,500 (1), which is smaller than the value obtained in this study. This discrepancy is most likely explained by the extreme sensitivity of the protein to proteolysis (1).

To determine whether E. coli-derived SPE B (isoelectric focusing purified) was biologically active, its mitogenic ability as a representative property was compared to that of streptococcus-derived toxin, using rabbit (American Dutch belted) splenocytes as indicators of activity. Proliferation of the splenocyte cultures was evaluated by measuring the incorporation of [3H]thymidine into cellular DNA as described by Poindexter and Schlievert (21). Both toxin preparations stimulated similar lymphocyte proliferative responses. Mitogenic capacity was measured in SPE B-stimulated and unstimulated cultures (2 x 10⁵ cells per culture) after 4 days. The streptococcus-derived toxin (1 µg) stimulated [3H]thy-midine incorporation at the rate of 50,200 ± 3,300 cpm, the E. coli-derived toxin (1 µg) stimulated incorporation at 46,500 ± 2,500 cpm, and the unstimulated culture exhibited incorporation at 2,000 ± 200 cpm. All of the incorporation rates are the means of four test cultures plus or minus the standard error of the mean.

The results of cloning the structural gene for SPE B presented in this paper are important for future studies of the structure-function relationship of the staphylcoccal and streptococcal PTs. At least three other PTs (SPE A, ENT C1, and ENT B) have structural genes which share extensive sequence homology (8, 12, 31). Therefore, the shared biological and biochemical properties of some PTs may be the result of structural similarities. Whether or not SPE B is related to other PTs at the molecular level remains to be determined.

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