Long-Range Mapping of the *Streptococcus agalactiae* Phylogenetic Lineage Restriction Digest Pattern Type III-3 Reveals Clustering of Virulence Genes

John F. Bohnsack,1,* April A. Whiting,1 Russell D. Bradford,1 Brenna K. Van Frank,1 Shinji Takahashi,2 and Elisabeth E. Adderson3

Department of Pediatrics, University of Utah Health Sciences Center, Salt Lake City, Utah 84132; Division of Microbiology, Joshi-Eiyoh University, Chiyoda, Sakado, Saitama 350-0288, Japan2; and Department of Infectious Diseases, St. Jude Children’s Research Hospital, Memphis, Tennessee 381053

Received 10 July 2001/Returned for modification 10 September 2001/Accepted 18 October 2001

Human isolates of serotype III *Streptococcus agalactiae* (group B streptococcus [GBS]) can be divided into three separate phylogenetic lineages based on analysis of the restriction digest patterns (RDPs) of chromosomal DNA. Nine DNA sequences that are present in all isolates of the RDP III-3 phylogenetic lineage, but not in the other lineages, were identified by genomic subtractive hybridization. A complete physical map of a III-3 chromosome was constructed. Six of the nine III-3-specific sequences mapped to a 340-kb Sse8387I fragment which contains or is located close to known GBS virulence genes. One of the III-3-specific probes, AW-10, encodes part of GBSi1, a group II intron that is inserted at two sites within the GBS genome. The second chromosomal site for GBSi1 was isolated, sequenced, and mapped to a location near the locus responsible for hemolysin production. These findings suggest that the genetic variation that distinguishes the RDP type III-3 strains from other serotype III strains occurs largely within localized areas of the genome containing known or putative virulence genes.

---

*Corresponding author. Mailing address: Department of Pediatrics, University of Utah Health Sciences Center, 50 North Medical Dr., Salt Lake City, UT 84132. Phone: (801) 581-5319. Fax: (801) 585-9314. E-mail: john.bohnsack@hsc.utah.edu.

**MATERIALS AND METHODS**

Probes. The details of methods used to generate the III-3-specific probes AA3.8, AA3.14, AA3.16, AA4.1, AA4.13, AW-10, DY-1, DY-3, and DY-11 are described elsewhere (5). Probes from several previously described GBS genes were provided by other investigators (Table 1). Probes for *cylA* (14, 18), *lmb* (19), *rrs* (15) were amplified by PCR from genomic DNA of GBS strain 874391. All amplifications were performed in 1× reaction buffer (Qiagen, Valencia, Calif.) containing 0.2 mM deoxynucleoside triphosphates, 3 mM MgCl2, 5 U of DNA polymerase (Qiagen), and 500 ng of genomic DNA. The following primer pairs were used: for *cylA*, 5′ sense GACATTTTCCACAACGTCC and 3′ antisense TCTGATACCAACATCCTTCC; for *lmb*, 5′ sense CCTGATACCACATCTTCC; for *rrs*, 5′ sense GACTTGCTGATCTTTA; and extension at 72°C for 1 min. A

---

*S. agalactiae* bacteria (group B streptococci [GBS]) are the most common cause of serious bacterial disease in neonates and an important pathogen in pregnant women and adults with underlying illnesses (3). GBS are subclassified into nine serotypes based on the immunologic reactivity of the polysaccharide capsule. Types I, II, III, and V GBS cause the majority of neonatal human GBS diseases (3, 4). Serotype III GBS are of particular importance because these bacteria cause considerable early-onset (within the first week of life) and most late-onset (after the first week of life) disease in human neonates and the vast majority of neonatal GBS meningitis (3).

It was previously reported that serotype III GBS isolates from Tokyo, Japan, and Salt Lake City, Utah, can be subclassified by analysis of HindIII and Sse8387I restriction digest patterns (RDPs) of chromosomal DNA into three major phylogenetic lineages, or RDP types III-1, III-2, and III-3 (21, 22). The overwhelming majority (91%) of invasive isolates from neonates were RDP type III-3, suggesting that these strains are more virulent than the other RDP types (21).

Characterization of the genetic differences between virulent III-3 and less virulent III-2 strains will help us understand the phylogenetic relationships between different GBS strains. We used genomic subtractive hybridization to identify nine short DNA sequences that are found in all III-3 strains, but not in II-3 or II-1 strains, and thus appear to be markers of the RDP type III-3 phylogenetic lineage (5). In the present study, we constructed a physical map of the III-3 GBS chromosome and identified the location of the III-3-specific probes and other previously described GBS genes. (This work was presented in part at the 14th Lancefield International Symposium on Streptococci and Streptococcal Diseases, 11 to 15 October 1999, Auckland, New Zealand.)

---

**REFERENCES**

AA3.8, AA3.14, AA3.16, AA4.1, AA4.13, AW-10, DY-1, DY-3, and DY-11 were described elsewhere (5). Probes from several previously described GBS genes were provided by other investigators (Table 1). Probes for *cylA* (14, 18), *lmb* (19), *rrs* (15) were amplified by PCR from genomic DNA of GBS strain 874391. All amplifications were performed in 1× reaction buffer (Qiagen, Valencia, Calif.) containing 0.2 mM deoxynucleoside triphosphates, 3 mM MgCl2, 5 U of DNA polymerase (Qiagen), and 500 ng of genomic DNA. The following primer pairs were used: for *cylA*, 5′ sense GACATTTTCCACAACGTCC and 3′ antisense TCTGATACCAACATCCTTCC; for *lmb*, 5′ sense CCTGATACCACATCTTCC; for *rrs*, 5′ sense GACTTGCTGATCTTTA; and extension at 72°C for 1 min. A

---

**Figures**

**Fig. 1.** Summary of physical map of the GBS chromosome. Genes that are known virulence factors or that map in the virulence region are indicated with a triangle. A physical map of the III-3 GBS chromosome and identified the location of the III-3-specific probes and other previously described GBS genes. (This work was presented in part at the 14th Lancefield International Symposium on Streptococci and Streptococcal Diseases, 11 to 15 October 1999, Auckland, New Zealand.)

**Fig. 2.** Bacterial DNA restriction patterns. DNA isolates were separated by PFGE as previously described (22). Restriction fragments were separated by PFGE as previously described (22).

---

**Tables**

**Table 1.** Probes. The details of methods used to generate the III-3-specific probes AA3.8, AA3.14, AA3.16, AA4.1, AA4.13, AW-10, DY-1, DY-3, and DY-11 are described elsewhere (5). Probes from several previously described GBS genes were provided by other investigators (Table 1). Probes for *cylA* (14, 18), *lmb* (19), *rrs* (15) were amplified by PCR from genomic DNA of GBS strain 874391. All amplifications were performed in 1× reaction buffer (Qiagen, Valencia, Calif.) containing 0.2 mM deoxynucleoside triphosphates, 3 mM MgCl2, 5 U of DNA polymerase (Qiagen), and 500 ng of genomic DNA. The following primer pairs were used: for *cylA*, 5′ sense GACATTTTCCACAACGTCC and 3′ antisense TCTGATACCAACATCCTTCC; for *lmb*, 5′ sense CCTGATACCACATCTTCC; for *rrs*, 5′ sense GACTTGCTGATCTTTA; and extension at 72°C for 1 min. A

---

**Legends**

**Fig. 1.** Summary of physical map of the GBS chromosome. Genes that are known virulence factors or that map in the virulence region are indicated with a triangle. A physical map of the III-3 GBS chromosome and identified the location of the III-3-specific probes and other previously described GBS genes. (This work was presented in part at the 14th Lancefield International Symposium on Streptococci and Streptococcal Diseases, 11 to 15 October 1999, Auckland, New Zealand.)

**Fig. 2.** Bacterial DNA restriction patterns. DNA isolates were separated by PFGE as previously described (22). Restriction fragments were separated by PFGE as previously described (22).
Isolation of the AW-10 genomic clone. A GBS RDP type III-3 874391 genomic DNA library was constructed in Lambda FIX II phage (Stratagene) according to the manufacturer’s protocol. Plaques that hybridized with the AW-10 sequence tag were purified. Two HindIII fragments of the AW-10 phage DNA clone, approximately 4 and 5 kb in size, were subcloned and sequenced by chromosome walking.

Nucleotide sequence accession number. The sequence of the AW-10 genomic clone is available from GenBank under accession no. AF380672.

RESULTS

Restriction fragments used to generate the physical map. The genome of GBS strain 874391 was digested with Sse8387I, SmaI, and EagI in order to construct a physical map of the III-3 GBS strain 874391 genome (Fig. 1). Complete digestion with Sse8387I produced nine fragments of 374, 340, 318, 289, 220 (two fragments), 194, 162, and 48.5 kb, as previously reported (Table 2) (21). Digestion with EagI produced 16 fragments, and SmaI produced 13 fragments (Table 2). Smaller fragments than those shown in Table 2 may have been generated but were not systematically identified. The sum of the fragments ranged from 1,996 kb for the EagI fragments to 2,174 kb for the Sse8387I fragments, a genome size consistent with that previously reported by Dmitriev et al. (7).

![Diagram of physical and genetic map of the RDP type III-3 GBS isolate 874391.](http://iai.asm.org/article/pii/S0099224097014811)

**FIG. 1.** Physical and genetic map of the RDP type III-3 GBS isolate 874391. The sizes of the restriction fragments are shown. The hatched areas represent areas of the genome that contain additional restriction fragments not definitively mapped, including the area containing the six 16S rRNA operons. See text for details.
TABLE 2. Restriction fragments of GBS 874391 chromosome

<table>
<thead>
<tr>
<th>Fragment length (kb) produced by digestion with:</th>
<th>Sse8387I</th>
<th>Smal</th>
<th>Eagli</th>
<th>SgrAl</th>
</tr>
</thead>
<tbody>
<tr>
<td>440</td>
<td>460</td>
<td>400</td>
<td>1,700</td>
<td></td>
</tr>
<tr>
<td>318</td>
<td>460</td>
<td>235</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>340</td>
<td>350</td>
<td>140</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>289</td>
<td>190</td>
<td>130</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>150</td>
<td>120</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>100</td>
<td>115</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>195</td>
<td>78</td>
<td>110</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>162</td>
<td>64</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>61</td>
<td>98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total 2,174  2,100  1,921  2,135

Physical mapping of known GBS genes. Partial digestion with Sse8387I resulted in larger bands comprised of two or more Sse8387I fragments, enabling most segments to be definitively aligned. The rs probe mapped to three separate Sse8387I fragments, six Smal fragments, at least three Eagli fragments, and six SgrAl fragments (the only other SgrAl fragment was approximately 1,700 bp; Table 2). Two probes were used to identify the location of the cps locus. One gene, cpsF, maps to a 15-kb Smal fragment, while the other gene, cpsA, maps to the same 460-kb Smal fragment to which hylB, scpB, and lmb hybridize (Fig. 1). This 460-kb Smal fragment is therefore adjacent to the 15-kb Smal fragment. The 140-kb Eagli fragment, which contains hylB, scpB, and lmb, is next to the cps locus since hylB hybridizes to the 480-kb Sse8387I fragment, which also hybridizes with the cps genes (Fig. 2). The cylA and rib genes and the cps and recA genes mapped to two distinct areas of the genome, both removed from the cps locus (Fig. 2).

MAPPING OF THE III-3 SPECIFIC SEQUENCES. Southern hybridization demonstrated that the 874391 genome contains single copies of each of the III-3-specific probes except AW-10, which maps to two separate areas of the chromosome (Fig. 2). The nine III-3-specific probes mapped to three distinct areas of the chromosome. AA 4.13 and AA 3.14 mapped to a 400-kb Eagli fragment which also hybridizes with the cps gene. This 400-kb fragment is juxtaposed to the region containing the rRNA operons. AA 4.1 maps to a 374-kb Sse8387I fragment proximal to the cyl locus and to the rib gene. The remaining III-3-specific probes DY-1, DY-3, AA 3.8, AA 3.16, and DY-11 and one copy of AW-10 hybridize with the 450-kb Smal fragment that is adjacent to the cps locus. AW-10 and DY-3 hybridize to the 140-kb Smal fragment that contains scpB, lmb, and hylB, while DY-11, AA 3.8, and AA 3.16 hybridize to a 98-kb Eagli fragment. The 98-kb Eagli fragment appears to be in the center of the 460-kb Smal fragment because all of the sequences hybridizing to the 98-kb Eagli fragment also hybridize with the 340-kb Sse8387I fragment, and not with the 440-kb Sse8387I fragment.

Identification of the second GBSi1 locus. The nucleotide sequence of AW-10, which was previously reported to be homologous to a maturase-related protein of Streptococcus pneumoniae (5, 7), is identical at the nucleotide level to GBSi1, the GBS group II intron recently described by Granlund et al. (9). These investigators reported that there are at least two copies of GBSi1 in the GBS chromosome and demonstrated that one copy lies between the scpB and lmb genes, the same site to which AW-10 maps. The other GBSi1 locus was not identified.

A 15-kb genomic DNA clone hybridizing with the AW-10 probe was isolated from a III-3 genomic lambda library, and 4- and 5-kb HindIII fragments hybridizing with the AW-10 probe were subcloned and sequenced. These subclones contain neither lmb nor scpB, demonstrating that this genomic clone contains the hitherto unidentified second GBSi1 locus. The 4-kb clone contained 100 bp of the 5’ terminus of GBSi1, while the 5-kb fragment contained 953 bp of the 3’ terminus. The intervening 475 bp region of GBSi1 was amplified by PCR using primers from the 4- and 5-kb clones.

The 9,331-bp region of the chromosome comprising the 4- and 5-kb and 475-bp HindIII fragments contains 10 open reading frames (ORFs), one of which is GBSi1 (Fig. 2 and Table 3). Eight ORFs were homologous to hypothetical genes of type M1 Streptococcus pyogenes that are located within 50 kb of each other on the S. pyogenes chromosome (6). Seven of these S. pyogenes ORFs (the homologs of ORFs 2, 3, 4, 6, 7, 8, and 10) are located within a 12-kb interval in the S. pyogenes chromo-

FIG. 2. Map of the AW-10 genomic clone. The arrowheads indicate the positions of HindIII restriction sites. The putative insertion site for the GBSi1 group II intron, consisting of an inverted repeat and poly(T) tail, is shown.
some (Table 3). Thus, both copies of GBSi1 are found within areas of the GBS genome that share significant homology with that of *S. pyogenes*. Granlund and coworkers (9) suggested that an inverted repeat and poly(T) sequences upstream of GBSi1 might be the target motif for streptococcal group II introns. Prior to the studies reported here, analyses of RDPs of chromosomal DNA to identify the locations of genomic DNA unique to virulent RDP III-3 strains. The resulting map resembles, in some respects, the only previously published map of the type III GBS chromosome, which was constructed by Dmitriev et al. using *SmaI* and *SgrI* digests of serotype 1/6586 (7). In that study, the size of the GBS genome was estimated to be approximately 2,200 kb, similar to our figure of 1,921 to 2,174 kb. As in Dmitriev’s map, *SgrI* digestion of strain 874391 identifies six rRNA operons grouped in one area of the chromosome, an organization also shared by a serotype II strain, 78/471 (7). We identified two *SmaI* fragments of approximately 460 kb and show that *scpB* maps to one of these fragments. In contrast, the previous map indicated that the *scpB* probe resided on a 945-kb restriction fragment. The studies reported here also mapped the *rib* gene, which encodes the Rib protein, a member of the family of alpha-like proteins expressed on many serotype III GBS (12, 20, 23). The location of *rib* near *rrs* corresponds to the site to which the alpha protein gene maps in serotype II 78/471 GBS (7), a finding consistent with the assertion made by Lachenauer et al. that members of the alpha protein family are encoded by variant genes at a single location in the GBS genome (12). Other than *scpB*, *rib*, and *rrs* genes, no other probes were used in both Dmitriev’s or our mapping studies, making it difficult to draw further conclusions about the similarity or differences of the chromosomes of these strains.

Six of the nine III-3-specific sequences were located on the same 340-kb *Sst* fragment as the *cps*, *hydB*, *scpB*, and *lmb* loci and, therefore, are no further away than 340 kb (and in some cases much closer) from these known or putative virulence genes. One of the III-3-specific sequences, *AW-10*, is located at a second site on another part of the chromosome within 220 kb of another III-3-specific sequence, *AA 4.1*, and of genes encoding two other potential virulence factors, hemolysin, and the Rib protein. The remaining two III-3-specific sequences map to a 400-kb *EagI* fragment in a third part of the chromosome. These data raise the possibility that the genetic differences between the RDP type III-3 phylogenetic lineage and other GBS lineages are limited to relatively circumscribed areas of the bacterial chromosome and that a large part of the genetic differences occur in or near the region that contains *scpB*, *lmb*, *hydB*, and *cps*.

### DISCUSSION

We recently used analysis of RDPs of chromosomal DNA to show that serotype III GBS isolates from Salt Lake City, Utah, and Tokyo, Japan, consist of three clonal populations, called RDP III-1, III-2, and III-3. The overwhelming majority of invasive disease in human neonates in this study were caused by III-3 strains (21). These findings are consistent with other reports that demonstrated that serotype III GBS consist of at least three distinct phylogenetic lineages (11, 13, 16, 17, 21, 22).

We identified nine III-3-specific DNA sequences by genomic subtractive hybridization of a III-2 strain from a III-3 strain, and showed that these III-3-specific sequences are almost exclusively found in RDP type III-3 GBS, and not in strains from other GBS serotypes (5).

In the studies reported here, a physical and genetic map of the III-3 GBS chromosome was constructed in order to iden-

---

### TABLE 3. AW-10 genomic clone ORFs and homologies to other bacterial proteins

<table>
<thead>
<tr>
<th>ORF No.</th>
<th>Position (aa)</th>
<th>Length (aa)</th>
<th>Function</th>
<th>Species</th>
<th>Accession no.</th>
<th>Length (aa)</th>
<th>% Identity (aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 – 491</td>
<td>163</td>
<td>Putative chromosome segregation</td>
<td><em>S. pyogenes</em></td>
<td>AAK33527.1</td>
<td>1,179</td>
<td>82 (159/233)</td>
</tr>
<tr>
<td>2</td>
<td>582 – 1379</td>
<td>265</td>
<td>Conserved hypothetical protein</td>
<td><em>S. pyogenes</em></td>
<td>AAK33552.1</td>
<td>265</td>
<td>52 (138/265)</td>
</tr>
<tr>
<td>3</td>
<td>1379 – 2203</td>
<td>274</td>
<td>Conserved hypothetical protein</td>
<td><em>S. pyogenes</em></td>
<td>AAK33553.1</td>
<td>274</td>
<td>64 (176/274)</td>
</tr>
<tr>
<td>4</td>
<td>2203 – 3813</td>
<td>536</td>
<td>Putative docking protein</td>
<td><em>S. pyogenes</em></td>
<td>AAK33554.1</td>
<td>516</td>
<td>58 (322/550)</td>
</tr>
<tr>
<td>5</td>
<td>4331 – 5641</td>
<td>436</td>
<td>GBSi1</td>
<td><em>S. agalactiae</em></td>
<td>CAC35989.1</td>
<td>436</td>
<td>99.8 (435/436)</td>
</tr>
<tr>
<td>6</td>
<td>5707 – 6405</td>
<td>232</td>
<td>Conserved hypothetical protein</td>
<td><em>S. pyogenes</em></td>
<td>AAK33559.1</td>
<td>271</td>
<td>68 (159/233)</td>
</tr>
<tr>
<td>7</td>
<td>6518 – 7420</td>
<td>300</td>
<td>Conserved hypothetical protein</td>
<td><em>S. pyogenes</em></td>
<td>AAK33560.1</td>
<td>300</td>
<td>82 (248/300)</td>
</tr>
<tr>
<td>8</td>
<td>7424 – 7554</td>
<td>30</td>
<td>Hypothetical protein</td>
<td><em>S. pyogenes</em></td>
<td>AAK33562.1</td>
<td>51</td>
<td>68 (13/19)</td>
</tr>
<tr>
<td>9</td>
<td>7821 – 8732</td>
<td>303</td>
<td>Putative oxidoreductase</td>
<td><em>Staphylococcus carnosus</em></td>
<td>AAB94650.1</td>
<td>301</td>
<td>53 (160/300)</td>
</tr>
<tr>
<td>10</td>
<td>8836 – 9331</td>
<td>165</td>
<td>Conserved hypothetical protein (5’)</td>
<td><em>S. pyogenes</em></td>
<td>AAK33563.1</td>
<td>710</td>
<td>70 (116/165)</td>
</tr>
</tbody>
</table>

*aa*, amino acids.
The presence of a group II intron in GBS was suggested to us by the homology of the III-3-specific AW-10 sequence to a putative materase-related protein previously identified in S. pneumoniae (1), and confirmed by Granlund’s definitive identification of GBSI1, which contains the AW-10 probe sequence (9). Granlund reported that there are two copies of GBSI1 in the GBS genome and showed that one copy of GBSI1 was inserted between scpB and lmb. The site of the other copy of GBSI1 is characterized in this report. The sequence of the second copy of GBSI1 is virtually identical to the original GBSI1 insertion sequence (9). It is interesting that both GBSI1 introns are found within areas of the genome that are highly conserved between S. pyogenes and S. agalactiae, even though GBSI1 has not yet been identified in S. pyogenes (8).

Granlund et al. also demonstrated that IS1548, an insertion sequence with multiple chromosomal copies in some GBS strains (10), was present between the scpB and lmb genes 88 bp downstream of the GBSI1 insertion site. The authors showed that GBSI1 is present in three of three invasive GBS isolates analyzed and proposed that GBSI1 is a marker for the ET1 GBS phylogenetic lineage, a population of closely related, virulent GBS originally identified by Musser et al. (13). It was previously shown that AW-10 (GBSI1) is a marker for the RDP type III-3 phylogenetic lineage, and it was proposed that the III-3 and ET1 GBS phylogenetic lineages are identical, based on hyaluronate lyase expression, their genetic distance from other GBS, and their propensity to cause invasive disease in human neonates (5). Granlund also proposed that GBSI1 and IS1548 are present in mutually exclusive GBS populations because the two insertion sequences were never shared among 40 GBS isolates of various serotypes that were analyzed (9). Our finding that insertional inactivation of hylB by IS1548 is a marker for RDP type III-2 GBS further supports the assertion that IS1548 and GBSI1 are found in distinct GBS phylogenetic lineages. Further elucidation of the distribution and origin of these two insertional elements could shed light on the evolutionary origin of GBS population structure.

GBS strains from the ET1/III-3 phylogenetic lineage cause the overwhelming majority of neonatal serotype III GBS disease according to some studies and a large proportion of neonatal invasive serotype III disease according to all published studies on the subject (11, 13, 16, 17, 21, 22). We hypothesized that genomic subtractive hybridization would identify genes that contribute to the increased virulence of ET1/III-3 GBS, in addition to defining genetic differences between the serotype III GBS populations. Additional genomic clones that hybridize with the other III-3-specific sequences have been isolated and are being sequenced. We therefore believe that the approach we have taken will identify candidate virulence genes and provide insights into the evolutionary origin of pathogenic GBS populations. Complete delineation of the genetic differences between GBS populations will be greatly facilitated by comparative genomics based on complete GBS genomic sequences and will result in understanding of the genetic basis for the difference in virulence between GBS phylogenetic lineages.

ACKNOWLEDGMENTS

This work was supported by USPHS NIH grant RO1 AI 40918 (to J.F.B. and E.E.A.), Cancer Center Support CORE Grant P30 CA 21765 (to E.E.A.), and a grant from the American Lebanese Syrian Associated Charities (to E.E.A.). E. E. Adderson is an Established Investigator of the American Heart Association.

REFERENCES


