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Virulent Human Strains of Group G Streptococci Express a C5a Peptidase Enzyme Similar to That Produced by Group A Streptococci

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Specific proteolytic destruction of the human chemotaxin, C5a, is a property of group A and B streptococcal pathogens. Here we show that virulent group G streptococci from human sources also express C5a peptidase activity. The enzyme responsible for this activity is approximately the same size as and is antigenically similar to that produced by group A streptococci. On the basis of Southern hybridization analysis with an internal fragment of the group A C5a peptidase gene (*scpA*) as a probe, a copy of this gene was found in the genome of all group G human isolates tested. Comparison of partial restriction maps of *scpA* and *scpG* revealed significant similarity between the two genes. Group G strains isolated from dogs and cows were found to lack C5a peptidase activity and did not hybridize to the *scpA*-specific probe. The association of this activity with three streptococcal species suggests that elimination of phagocyte chemotactic attractants is a more universal virulence mechanism than originally anticipated.

Bacterial pathogens have evolved numerous ways to avoid their host's immunological defenses. Group A streptococci resist the phagocytic defenses at two levels. First, a surface peptidase (SCP) specifically cleaves C5a, a major mediator of phagocyte recruitment to the site of infection (26). Second, M protein limits the deposition of C3b opsonin, which is required for efficient uptake of the organism by phagocytes (11, 12). These proteins are associated with the cell surface by anchorage to the protoplasmic membrane. There, they are strategically poised to eliminate their respective complement targets as they are formed by activation of the complement pathway. SCP, a 128-kDa endopeptidase, is produced by most if not all M^+ strains of *Streptococcus pyogenes*. The SCP structural gene (*scpA*) is located adjacent to M-protein genes (4, 9) and is transcriptionally controlled in coordination with this gene (22).

The most compelling evidence that SCP contributes to the pathogenic potential of group A streptococci was reported by O'Connor and Cleary (17). They showed that this peptidase has the capacity to limit recruitment of polymorphonuclear leukocytes (PMNLs) into the mouse peritoneum and that antibody directed against SCP stimulated the migration of PMNLs into the peritoneum in response to injection of M^+ SCP^+ streptococci. The discovery that human isolates of group B streptococci also elicit an inhibitor of chemotaxis with activity similar to that of SCP (10) suggested that proteolytic elimination of chemotactic signals may be a universal virulence mechanism of gram-positive mucosal pathogens. The peptidase produced by group B streptococci, although antigenically distinct from that produced by group A streptococci, was shown to cleave C5a into a functionally inert peptide (10).

Group G streptococci are a taxonomically diverse group of organisms, possibly representing two or more species which have also been associated with a variety of human and animal infections (8, 13). Studies from our laboratory suggest a clear taxonomic distinction between animal and human isolates. The latter are known to express M-like proteins on their surfaces (2) and to have M-protein genes which are nearly identical to those of group A streptococci (23). Here we report that group G streptococci of human origin produce an inhibitor of chemotaxis which is antigenically and genetically similar to SCP produced by group A bacteria. We again present data which is consistent with the taxonomic division of group G streptococci by host range. In addition, this study corroborates the conclusion that specific proteolytic destruction of C5a is a universal virulence mechanism of streptococci pathogenic for humans.

MATERIALS AND METHODS

Bacterial strains. All streptococcal cultures were grown in Todd-Hewitt broth supplemented with 2% neopeptone at 37°C and were grouped and M typed as previously described (15). The group G strains were described by Simpson et al. (23). Strains CS24 and CS101 have also been described elsewhere (12, 21). The *scpA*-specific probe, originally cloned from strain CS24 (24), is a DNA fragment internal to *scpA* and subcloned into plasmid pUC9 to form plasmid pTT45. Cultures of *Escherichia coli* were grown in L broth containing ampicillin at a final concentration of 50 μg/ml.

Extraction and partial purification of SCP protein. SCP was extracted from group G streptococcal cultures following their enrichment for M^+ cells by three passages in phagocytically active human whole blood (1). Cells from 2.5-liter overnight cultures of strain CS246, grown in Todd-Hewitt broth supplemented with 2% neopeptone (Difco Laboratories, Detroit, Mich.), were washed and then concentrated 10-fold into 0.02 M sodium phosphate buffer containing 0.14 M NaCl, 1 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 0.01 mg of aprotinin per ml, and 0.01 mg of RNase per ml. Phage-associated lysin was prepared by the method of Cohen et al. (7). The lysate was concentrated by filtration with an Amicon cell by using a YM100 membrane (Amicon Inc., Danvers, Mass.). Protein in the retentate was further fractionated by gel filtration over a P200 column (Bio-Rad Laboratories, Rockville Centre, N.Y.).

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Fractions containing SCP antigen were detected by immunoblot, pooled, and lyophilized (25). Resuspended protein was desalted by using a CENTRICON 30 microconcentrator (Amicon) prior to testing for biological activity.

EIA quantitation of SCP-specific antigen. SCP antigen associated with intact cells was quantified by an enzyme-linked immunosorbent inhibition assay (EIA) which employed rabbit anti-SCP serum (16). A standard dilution of rabbit antiserum was incubated either with dilutions of whole cells or with protein extracts before it was added to microtiter wells containing bound, highly purified SCP protein from group A streptococci (16). The amount of residual, unbound rabbit immunoglobulin G (IgG) is inversely proportional to the amount of antigen present in the preincubation mixture. A standard curve with known concentrations of SCP antigen was simultaneously developed for comparison to unknowns. The quantity of bound rabbit IgG was determined as previously described (16).

Chemotaxis inhibition assay for SCP activity. The enzymatic activity of SCP was measured by its capacity to inactivate C5a in human serum. Human serum was activated with yeast zymosan to produce C5a (zymosan-activated serum [ZAS]) prior to its exposure to whole streptococcal cells or protein extracts thought to contain SCP enzyme. After 1 h of incubation of activated serum with SCP or bacterial cells, the residual chemotactic activity was quantitated by the underagarose method described in detail elsewhere (26). Inhibition was considered significant when it was greater than 20% and when it was dependent on the bacterial cell concentration.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) Western blot analysis. Partially purified protein extracts were fractionated on continuous 7% polyacrylamide gels as described previously and were electrophoretically transferred to nitrocellulose membranes (5). Blots were treated with rabbit anti-SCP sera raised against highly purified SCP derived from a group A streptococcal culture (25) and then with goat anti-rabbit IgG coupled to alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.). Bound conjugate was visualized with the chromogenic substrate Nitro Blue Tetrazolium and 5-bromo-4-chloro-indolyl-phosphate (5).

Southern blotting and DNA hybridization. Genomic DNA was extracted and cleaved with restriction endonuclease as previously described, and fragments were resolved on 0.6 or 0.7% agarose gels and then transferred to nitrocellulose sheets (5). Probe DNA was purified from agarose gels and then labeled with [^{32}P]dCTP by using a nick translation kit from Bethesda Research Laboratories (Bethesda, Md.). Alternatively, probe DNA was labeled with digoxigenin-UTP by using the Genius nonradioactive labeling kit (Boehringer Mannheim, Inc., Indianapolis, Ind.). Hybridization conditions were adjusted to allow 7 to 11% base pair mismatch, depending on the experiment.

RESULTS

Inhibition of C5a-directed chemotaxis by group G streptococci. The association of C5a peptidase activity with virulent strains of group A and B streptococci prompted us to search for this enzyme among group G streptococcal strains isolated from human infections. First we examined group G cultures for the capacity to destroy C5a in ZAS. Dilutions of washed cells were preincubated with ZAS, after which residual chemotaxin was assayed by the underagarose method (26) (Table 1). C5a peptidase activity was detected on all group G strains isolated from human infections. Compared with the results from group A streptococci, however, high cell concentrations of group G bacteria were required. The number of cells required to eliminate 50% of the chemotactic activity was strain dependent (ranging from 6×10^7 to 1×10^9), but in all cases a minimum of 5×10^7 cells was required to reliably measure inhibitory activity. Group G strains isolated from dogs and a cow were completely devoid of activity, since activity was not detected even when ZAS was exposed to 1×10^9 bacterial cells of animal origin.

TABLE 1. Quantitation of SCP activity and antigen expressed by streptococcal strains

Strain	Origin	Cell concn required for 50% inhibition in[a]:	
		Chemotaxis assay	EIA
CS138	Human	3.5×10^8	1.5×10^8
CS140	Human	1.0×10^9 (25)	1.9×10^8
GT8864	Human	1.5×10^8	7.6×10^7
GT8499	Human	$<1.0 \times 10^8$	2.4×10^8
74-436	Human	2.0×10^8	6.4×10^7
74-530	Human	1.0×10^9 (40)	2.0×10^7
74-446[b]	Human	7.0×10^8	2.5×10^8
CS530	Human	1.0×10^8 (25)	ND
CS101	Human (group A)	6.0×10^7	2.0×10^7
76-423	Dog	ND	$>10^9$
86-026	Dog	$>10^9$	ND
86-185	Cow	$>10^9$	$>10^9$
86-179	Dog	$>10^9$	$>10^9$
76-426	Dog	ND	$>10^9$
76-428	Dog	$>10^9$	$>10^9$
86-089	Dog	$>10^9$	$>10^9$

[a] Values indicate the concentration of bacterial cells per milliliter of ZAS. Numbers in parentheses indicate the percent inhibition when less than 50% inhibition was achieved. Assays were routinely performed in duplicate. ND, not done.

[b] 74-446 cells were passed through phagocytic blood.

These preliminary experiments suggest that group G cultures generally produce less inhibitory activity than group A strains. One culture of human origin, strain 74-446, was virtually devoid of activity upon first testing the culture (Fig. 1). However, following three passages in fresh, phagocytically active human blood, this strain gained the capacity to express peptidase activity (Fig. 1). These results suggest that stored laboratory cultures may be a mixture of cells, unstable with regard to expression of SCP. Growth in phagocytic human blood presumably selected for cells which harbor M protein and SCP on their surfaces. Likewise, the passage of group A streptococci in human blood or through mice has also been shown to enrich cultures for cells which express antiphagocytic M protein (1, 21) and SCP (17; unpublished data).

Passage of an animal isolate which had undetectable levels of peptidase activity, strain 86-026, through human blood did not alter its capacity to produce SCP activity (Fig. 1). Although this strain survived blood passage, cells which grew out of the passed culture still lacked measurable C5a peptidase activity. These results confirmed that animal isolates truly lack the ability to produce a C5a peptidase activity.

C5a peptidase from group A and group G streptococci are antigenically related. The next question of interest was whether the C5a peptidase activity associated with group G streptococci was antigenically related to that produced by

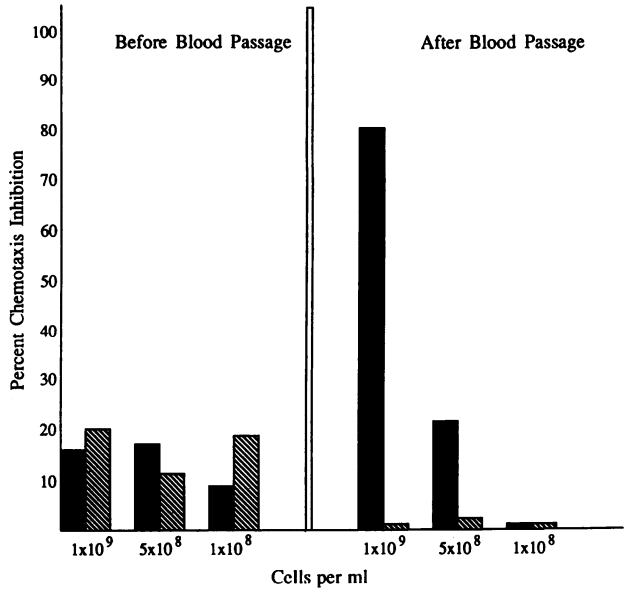

FIG. 1. Growth in phagocytic human blood enriches for SCP production. Fresh human blood was inoculated with 10^4 CFU of the human isolate strain 74-446 (■) or a dog isolate strain, 86-026 (▨). After three transfers in blood (1), the survivors were grown and tested for SCP activity. Different concentrations of the original cultures (at left) and the blood-passaged cultures (at right) were tested. Although the unpassed 86-026 cells produced 12 to 20% inhibition in this experiment, this inhibition was not dependent on the bacterial concentration and was not reproducible.

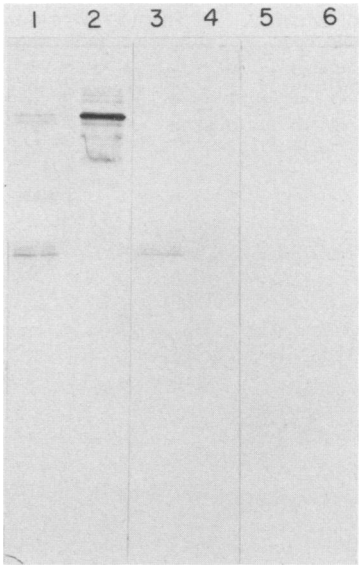

FIG. 2. Western blot analysis of protein extracted from group G streptococci. After electrophoresis, proteins were blotted onto nitrocellulose and the membranes were treated with primary antibody. Lanes: 1, 3, and 5, partially purified protein from the group G strain 74-446; 2, 4, and 6, purified SCP from group A streptococci (25). Lanes 1 and 2 were exposed to anti-SCP serum, lanes 3 and 4 were exposed to preimmune rabbit serum, and lanes 5 and 6 were exposed to the alkaline phosphatase conjugate only. Sizes of the various bands were estimated from the mobility of molecular mass standards (kilodaltons): 116, 87, 66, 45, and 29 (data not shown).

group A bacteria. To address this question, we examined both human and animal isolates for surface antigen by EIA by using rabbit antibody raised against highly purified C5a peptidase from group A streptococcus strain CS101. The number of cells which were able to inhibit binding of rabbit anti-SCP to microtiter plates coated with purified SCP is shown in Table 1. All group G human isolates tested reacted with anti-SCP immunoglobulin directed against the group A antigen from strain CS101. The number of cells required to inhibit 50% binding of antibody ranged from 6.4×10^7 to 2.5×10^8. In contrast, fewer group A streptococcal cells were required to achieve 50% inhibition (Table 1), again suggesting that the group G cultures are either mixtures of SCP^+ and SCP^- cells, that they have lower concentrations of antigen on their surface, or that the group G antigen is less active than that of group A bacteria. Consistent with activity assays, all human group G strains expressed antigen whereas none of the animal isolates had measurable antigen.

The protein responsible for chemotaxis inhibition activity was partially purified from group G strain 74-446 and was compared with the group A antigen by SDS-PAGE analysis. Antigen was extracted by incubation of washed concentrated cells with phage-associated lysin. Extracted protein was first concentrated by ultrafiltration with an Amicon YM100, and the retentate was further fractionated by gel filtration on a P200 column. P200 fractions which reacted positively in immunoblot assays with SCP-specific serum were pooled and lyophilized. Resuspended samples were then analyzed by SDS-PAGE (5). As previously reported (25), SCP purified from group A streptococci formed a major band of 137 kDa and minor bands representing aggregates and smaller degradation products (Fig. 2, lane 2). SDS-PAGE has recently been shown to exaggerate the molecular mass of this protein, because the size of the SCP protein deduced from the nucleic acid sequence is 128.5 kDa (5). Protein extracted from the group G strain 74-446 exhibited an antigen of a size similar to SCP from group A streptococci. In addition to these protein bands, protein preparations from strain 74-446 also produced two sets of bands which are smaller in size (lane 1). These proteins most likely represent various forms of an IgG Fc receptor known to be associated with group G bacteria (3). This conclusion is based on their sizes, 57 and 54 kDa, and their capacity to bind preimmune rabbit IgG, known to lack SCP-specific antibody (lane 3). Purified SCP from group A streptococci did not react with preimmune rabbit serum as expected (lane 4). Neither SCP preparation reacted with the goat anti-rabbit conjugate (lanes 5 and 6).

C5a peptidase genes of group G and group A streptococci are conserved. The above results indicate that group G streptococci produce a C5a peptidase which is very similar in size, if not identical, to that produced by group A cultures. We next tested whether animal isolates of group G streptococci lack the SCP gene (*scpG*) and compared the *scpG* gene of human isolates to *scpA* harbored by group A streptococci. This was accomplished by Southern hybridization analysis, employing a 1.3-kb *Hin*dIII-*Bgl*II fragment contained in plasmid pTT45 as the probe (5) (see Fig. 4). Total DNAs from 13 group G cultures and from the group A strain CS24, the origin of the probe insert, were digested with *Hin*dIII, electrophoresed, blotted onto nitrocellulose, and hybridized to probe under conditions which would allow 11% base pair mismatch (Fig. 3). DNA from human isolates contained a single strongly hybridizing *Hin*dIII fragment, which ranged in size from 7 to 8 kb. This size is considerably larger than the corresponding *Hin*dIII fragment present in DNA from

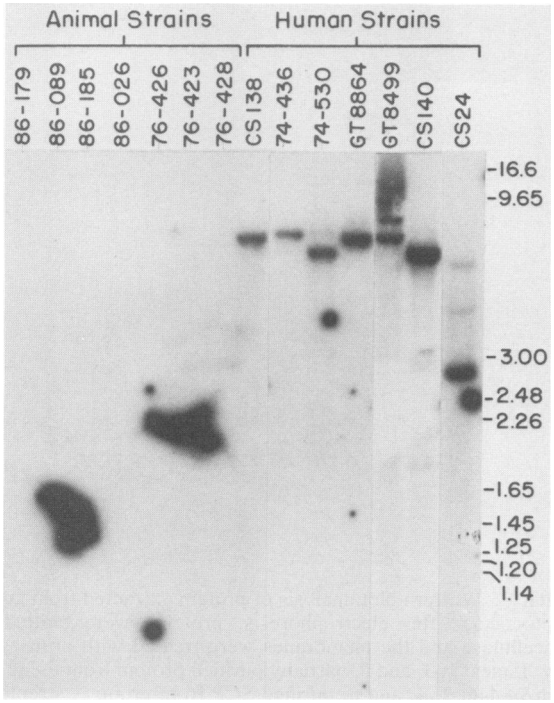

FIG. 3. Southern hybridization analysis of group G streptococcal genomic DNA. Chromosomal DNA was isolated from both human- and animal-associated strains, cleaved with *Hin*dIII, electrophoresed, blotted onto nitrocellulose, and hybridized to the *scpA*-specific probe, plasmid pTT45 (Fig. 4). Wash conditions permitted 11% base pair mismatch. Strain designations are shown at the top. The positions of molecular markers in kilobases are shown at the right.

the group A cultures (Fig. 3, lane CS24) and suggests that *scpG* contains an insertion or deletion or that it is located at a different chromosomal location in group G streptococci (5).

A partial restriction map of *scpG* in a group G culture of strain CS140, known to have an *emm12* gene nearly identical to that of the group A strain CS24, was developed by a series of double-enzyme digests (Fig. 4). Genomic digests were electrophoresed, blotted onto Nytran membranes, and hybridized to the *scpA*-specific probe, plasmid pTT45. Many of the restriction sites of *scpA*, such as *Bgl*II, *Hin*dIII, *Sac*I, *Xba*I, *Cla*I, and some *Hin*f sites, appear to be conserved in *scpG*. There is, however, at least one extra *Hin*f site in the region corresponding to the 5′ end of *scpG*. The precise locations of this *Hin*f site and a second *Hin*dIII site, known to be within *scpA*, have not been determined. In general, the 3′ end of *scpG* is most similar to that of *scpA*.

To determine whether *scpG* is linked to *emm12* in a manner similar to that in M12 group A streptococci (5), genomic DNA was digested with enzymes known to cut outside the *emm12-scpA* linkage group. After detection of *scpG*-specific fragments, blots were stripped and hybridized to an M protein gene-specific probe, plasmid pPC134 (23). Large *Eco*RI and *Bam*HI fragments, greater than 20 kb in size, hybridized to plasmid pTT45 DNA, but the same fragments did not hybridize to the *emm* gene probe, plasmid pPC134. Conversely, large *Eco*RI, *Cla*I, and *Bam*HI fragments which hybridized to plasmid pPC134 did not hybridize to the *scpG* probe, plasmid pTT45. Moreover, a *Sac*I fragment known to overlap *emm12* and *scpA* in group A streptococci was not detected in genomic DNA from group G streptococci. Instead, the *scpA* and *emm* probes hybridized to two distinct *Sac*I fragments. Therefore, we conclude that the *emm12* and *scpG* genes are not closely linked, as they are in group A streptococci.

In contrast to human isolates, DNA from animal group G strains did not hybridize to the *scpA* probe at the stringency used here (Fig. 3). Hybridization signals were not observed even under conditions which allow 30% base pair mismatch. These results are consistent with our failure to detect SCP antigen or activity associated with these cultures.

DISCUSSION

Cellular immune defenses localize invading microorganisms by detecting a variety of chemical stimuli produced by their interaction with tissue and body fluids. The complement cleavage product, C5a, has been shown to be an important mediator of this chemotactic response. Fixation of complement on bacterial cell surfaces is mediated by either the classical or alternative pathway. Soluble C5a is released into the surrounding tissues to attract phagocytic and other cells of the immune system to the site of infection. It is not surprising then that bacteria have evolved a means to abrogate the chemotactic response. Our laboratory discovered that group A streptococci produce a surface bound peptidase, SCP, that specifically cleaves C5a in the PMNL binding site (25). The amino acid sequence of the SCP protein deduced from the nucleotide sequence indicated that SCP is a serine proteinase of 128.5 kDa (4).

By using transposon-induced mutant streptococci, we showed that SCP slows the influx of PMNLs into the mouse peritoneum and that antibody directed against SCP could speed the recruitment of PMNLs in response to injected streptococci (17). The importance of serum and secretory IgG and IgA directed against SCP in limiting streptococcal infections is unknown, but the nearly universal presence of these antibodies in serum and saliva from adults in contrast to that from children is compatible with a protective role (18).

To determine whether destruction of C5a was a more universal virulence strategy, we tested group G streptococci for the capacity to express an inhibitor of chemotaxis. All eight strains, which had originally been isolated from human infections, destroyed the chemotactic activity of ZAS. Moreover, these strains were found to have SCP-like specific antigen on their surfaces. The potential of group G cells to competitively bind rabbit IgG which is directed against SCP purified from group A streptococci showed that the group G protein is antigenically similar if not identical to that expressed by group A streptococci. Similarity between SCP proteins from group A and G streptococci was further substantiated by the facts that they had the same mobility during SDS PAGE and that many of the restriction sites in *scpA* and *scpG* genes are conserved (Fig. 4).

To obtain 50% chemotaxis inhibition with group G cells, it generally required 10 to 50 times more bacteria than that required for group A streptococci. In fact, activity was not detectable with some cultures until they had been selectively passed through phagocytic human blood. Purification of the enzyme from group G bacteria was hindered by the scarcity of activity associated with these cultures. Typically, less than 500 μg of enzyme could be extracted from 5-liter cultures (unpublished observation). Variation in the amount SCP activity and antigen associated with group A cells has also been observed (unpublished observation). Like group G cultures, group A cultures can also be enriched for SCP

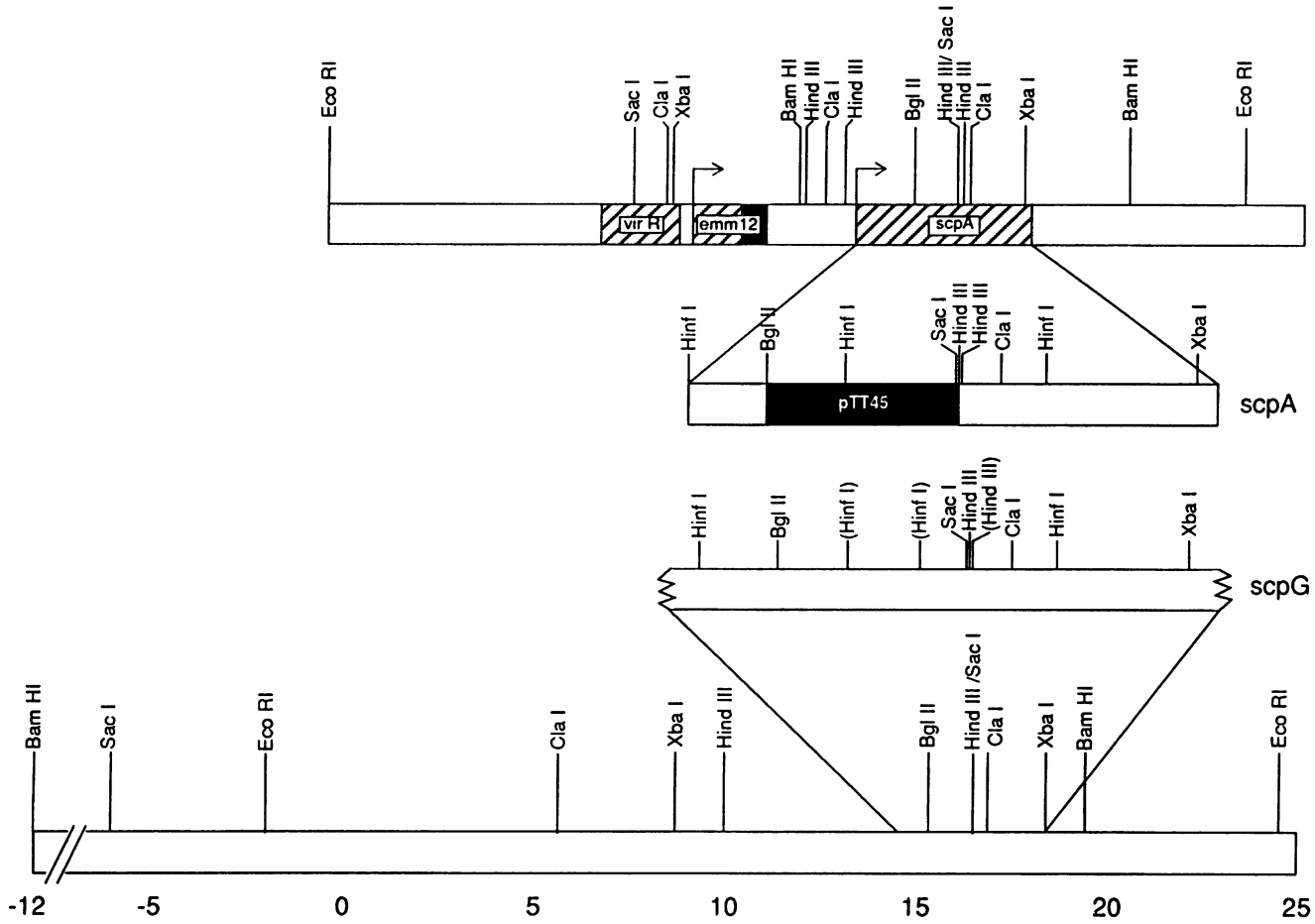

FIG. 4. Comparison of restriction enzyme sites in and adjacent to *scpA* and *scpG*. The map shown at the bottom was obtained by Southern blot analysis of genomic DNA from the M12 group G strain 74-446, by using a series of single and double restriction digests. The map shown at the top was partially derived from nucleotide sequence (4, 20) and Southern blot analyses of genomic DNA from an M12 group A strain, CS24 (5). Expanded portions of the *scpA* and *scpG* maps are shown in the middle. The solid regions correspond to the *emm12*-specific probe, plasmid pPC134 (23), and to the *scpA*-specific probe, plasmid pTT45 (5), used in the mapping experiments. Numbers at the bottom correspond to distances in kilobases. *virR* designates a gene required for transcription of *emm12* and *scpA* (22). Arrows indicate the direction of transcription. Parentheses around restriction sites indicate that their precise locations relative to other sites are unknown.

activity by passage through mice or phagocytic human blood (17). We have previously shown that SCP expression is coordinately controlled and undergoes phase variation with that of M protein in the latter species (22), and therefore we surmised that blood passage merely selects for M^+ SCP^+ cells in cultures which continually segregate M^- SCP^- cells. The low levels of SCP associated with the group G cultures examined here could, likewise, be due to an inherent genetic instability of SCP expression in stored laboratory cultures. This instability could also account for the observation that group G cultures are less able to resist phagocytosis relative to group A streptococci (6), even though they are known to genetically encode (23) and express M proteins (2).

Group G streptococci are increasingly recognized as potential human pathogens. Epidemic pharyngitis caused by this species is associated with food contamination and has been associated with respiratory droplet exposure (8, 13). Determinants of virulence have not been well defined: some strains produce M proteins (2, 14), and all human isolates tested were shown by Simpson et al. to possess M-protein genes which are homologous to those of group A streptococci (23). In contrast, group G isolates of animal origin lacked homology to M-protein gene probes (23). The fact that animal isolates also lack SCP genetic information is consistent with the findings of Simpson et al. with regard to M proteins (23) and further supports the notion that grouping these streptococci together by using a single serological marker is misleading and is a taxonomic mistake.

The discovery of the M-protein gene and SCP gene in human isolates of group G streptococci suggests that at least some strains are more closely related to group A bacteria than their taxonomic separation indicates. Alternatively, the genetic determinants of M and SCP proteins could be transmissible between these species. Indeed, Simpson et al. have shown that the *emm12* gene of strain CS140 is nearly identical to that of group A bacteria and for this reason suggested that CS140 was a derived group A bacterium (23). The *emm12* and *emm49* genes were shown to be 2 to 3 kb upstream from *scpA* (4, 9). This orientation is not conserved in the group G strain CS140. In this strain, *emm12* and *scpG* reside on separate *Eco*RI fragments, greater than 20 kb in size. Genomic digestions with other enzymes which did not cut within *emm12* or *scpA* supported our conclusion that the genes are unlinked. Therefore, the acquisition of these

virulence factors by group G bacteria was probably due to independent genetic events. Alternatively, this genetic information was acquired in the distant evolutionary past and DNA rearrangements in the group G chromosome have since separated the two genes.

It is becoming increasingly clear that bacterial mucosal pathogens use extracellular proteases specifically targeted toward key components of local immune defenses to assist their colonization of mucosal surfaces (10, 17, 19). In a previous report, we were unable to reliably detect SCP activity associated with laboratory strains used as prototype cultures for serological grouping. However, the discovery by Hill et al. of C5a peptidase activity associated with many strains of group B streptococci (10) and this discovery of SCP enzyme associated with human group G streptococcal strains support the proposal that disruption of C5a-mediated chemotaxis is a more universal virulence mechanism for streptococcal pathogens than we originally anticipated. Experiments are now in progress to screen other streptococcal species for SCP-like activity.

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REFERENCES

1. **Becker, C. G.** 1964. Selection of group A streptococci rich in M-protein from populations poor in M-protein. Am. J. Pathol. **44**:51–60.
2. **Bisno, A. L., D. E. Craven, and W. R. McCabe.** 1986. M proteins of group G streptococci isolated from bacteremic human infections. Infect. Immun. **26**:1172–1176.
3. **Bjorck, L., W. Kastern, G. Lindahl, and K. Wideback.** 1987. Streptococcal protein G expressed by streptococci or by *Escherichia coli*, has separate binding sites for human albumin and IgG. Mol. Immunol. **24**:1113–1122.
4. **Chen, C., and P. Cleary.** 1990. Complete nucleotide sequence of the streptococcal C5a peptidase gene of *Streptococcus pyogenes*. J. Biol. Chem. **265**:3161–3167.
5. **Chen, C. C., and P. P. Cleary.** 1989. Cloning and expression of the streptococcal C5a peptidase gene in *Escherichia coli*: linkage to the type 12 M protein gene. Infect. Immun. **57**:1740–1745.
6. **Cleary, P. P.** Unpublished results.
7. **Cohen, J. O., H. Gross, and W. K. Harrell.** 1977. Immunogenicity and characteristics of M protein released by phage-associated lysin from group A streptococci types 1 and 23. J. Med. Microbiol. **10**:179–194.
8. **Gerber, M. A., M. F. Randoph, N. J. Martin, P. P. Cleary, E. L. Kaplan, and E. M. Ayoub.** Pediatrics, in press.
9. **Haanes, E., and P. Cleary.** 1990. Identification of a divergent M protein gene and an M protein-related gene family in *Streptococcus pyogenes* serotype 49. J. Bacteriol. **171**:6397–6408.
10. **Hill, H. R., J. F. Bohnsack, E. Z. Morris, N. H. Augustine, C. J. Parker, P. P. Cleary, and J. T. Wu.** 1988. Group B streptococci inhibit the chemotactic activity of the fifth component of complement. J. Immunol. **141**:3551–3556.
11. **Horstmann, R. D., H. J. Sievertsen, J. Knobloch, and V. A. Fischetti.** 1988. Antiphagocytic activity of streptococcal M protein: selective binding of complement control protein factor H. Proc. Natl. Acad. Sci. USA **85**:1657–1661.
12. **Jacks-Weis, J., Y. Kim, and P. P. Cleary.** 1982. Restricted deposition of C3 on M^+ group A streptococci: correlation with resistance to phagocytosis. J. Immunol. **128**:1897–1902.
13. **Martin, N. J., E. L. Kaplan, M. A. Gerber, M. A. Menegus, M. Randolph, K. Bell, and P. Cleary.** 1990. Comparison of epidemic and endemic group G streptococci by restriction enzyme analysis. J. Clin. Microbiol. **28**:1881–1886.
14. **Maxted, W. R., and E. V. Potter.** 1967. The presence of type 12 M-protein antigen in group G streptococci. J. Med. Microbiol. **49**:119–125.
15. **Moody, M. D., J. Padula, D. Lizana, and C. T. Hall.** 1965. Epidemiologic characterization of group A streptococci by T-agglutination and M-precipitation tests in the public health laboratory. Health Lab. Sci. **2**:149–162.
16. **O'Connor, S. P., and P. Cleary.** 1986. Localization of the streptococcal C5a peptidase to the surface of group A streptococci. Infect. Immun. **53**:432–434.
17. **O'Connor, S. P., and P. Cleary.** 1987. *In vivo Streptococcus pyogenes* C5a peptidase activity: analysis using transposon- and nitrosoguanidine-induced mutants. J. Infect. Dis. **156**:495–504.
18. **O'Connor, S. P., D. Darip, K. Fraley, C. M. Nelson, E. L. Kaplan, and P. P. Cleary.** 1991. The human antibody response to streptococcal C5a peptidase. J. Infect. Dis. **163**:109–116.
19. **Plaut, A. G.** 1983. The IgA proteases of pathogenic bacteria. Annu. Rev. Microbiol. **37**:603–622.
20. **Robbins, J. C., J. G. Spanier, S. J. Jones, W. J. Simpson, and P. P. Cleary.** 1987. *Streptococcus pyogenes* type 12 M protein gene regulation by upstream sequences. J. Bacteriol. **169**:5633–5640.
21. **Simpson, W. J., and P. P. Cleary.** 1987. Expression of M type 12 protein by a group A streptococcus exhibits phaselike variation: evidence for coregulation of colony opacity determinants and M protein. Infect. Immun. **55**:2448–2455.
22. **Simpson, W. J., D. LaPenta, C. Chen, and P. Cleary.** 1990. Coregulation of type 12 M protein and streptococcal C5a peptidase genes in group A streptococci: evidence for a virulence regulon controlled by the *virR* locus. J. Bacteriol. **172**:696–700.
23. **Simpson, W. J., J. C. Robbins, and P. P. Cleary.** 1987. Evidence for group A-related M protein genes in human but not animal associated group G streptococcal pathogens. Microb. Pathog. **3**:339–350.
24. **Spanier, J. G., S. C. Jones, and P. P. Cleary.** 1984. Small DNA deletions creating avirulence in *Streptococcus pyogenes*. Science **225**:935–938.
25. **Wexler, D. E., and P. P. Cleary.** 1985. Purification and characteristics of the streptococcal chemotactic factor inactivator. Infect. Immun. **50**:757–764.
26. **Wexler, D. E., R. D. Nelson, and P. P. Cleary.** 1983. Human neutrophil chemotactic response to group A streptococci: bacteria-mediated interference with complement-derived factors. Infect. Immun. **39**:239–246.