

A Functional Analysis of the Antigenicity of Streptokinase Using Monoclonal Antibody Mapping and Recombinant Streptokinase Fragments¹

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ABSTRACT. Streptokinase (SK), a bacterial product of pathogenic *Streptococcus species*, is now widely used as an effective therapy for the treatment of heart attacks. Because naturally occurring antibody to SK is ubiquitous, serious allergic reactions to SK therapy are common. To begin to identify regions of the molecule that are important for the antigenicity of SK we performed studies using a panel of 51 hybridomas producing anti-SK antibodies, recombinant SK fragments, and assays of SK activity. Antibodies generated from mice hyperimmunized with wild-type SK were shown to fall into six distinct complementation groups by competitive binding studies. Recombinant SK fragments were used to determine the peptide regions recognized by these complementation groups. Correlation of the effects of the mAb on SK function, with knowledge of their SK fragment-binding pattern, suggested regions of the SK molecule that are important for the construction and the catalytic function of the SK-plasminogen activator complex. *Journal of Immunology*, 1993, 150: 4407.

Clinical studies over the last 10 years have conclusively shown that SK³ decreases the morbidity and mortality of patients suffering an acute myocardial infarction (1, 2). SK is a bacterial protein derived from pathogenic strains of the *Streptococcus* family (3). Although devoid of intrinsic activity, SK avidly binds to plasminogens (from many mammalian species) and generates a SK-PAC (4, 5). This SK-PAC catalyzes the con-

version of the zymogen—plasminogen—to the active enzyme plasmin. Plasmin then cleaves fibrin, the protein backbone of the thrombus, resulting in thrombolysis.

Shortly after discovering the fibrinolytic effects of streptokinase, Tillett et al. (6) also noted its immunogenicity in humans. They found that SK was inactivated in blood samples from patients with recent streptococcal infections as a result of neutralizing Ab. Indeed measurable levels of Ab to SK are nearly ubiquitous in the population as a consequence of the frequency of streptococcal infection (7). This predictable Ab response has been used by immunologists to gauge humoral immunity in patients suspected of immunodeficiency (8). Moreover, in patients receiving streptokinase as a therapy for thrombotic disease, antibody titer to streptokinase rises exponentially after treatment (9, 10). These high antibody titers to streptokinase will frequently neutralize the activity of SK if it is administered a second time in therapy (9). Not surprisingly, one of the most common side effects of SK therapy is allergic reactions, which have been noted in up to 15% (11) of patients treated. Although most allergic reactions are mild, cases of anaphy-

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³ Abbreviations used in this paper: SK, streptokinase; Ab, antibody; SK-PAC, streptokinase-plasminogen activator complex; MBP, maltose binding protein; PCR, polymerase chain reaction.

laxis, immune complex disease and other severe allergic responses to SK have been reported (12–15).

Despite this rich clinical information on the immunogenicity of SK, little is known about the structural basis for its antigenicity. Specifically, it is not known whether certain regions of the molecule are more immunogenic than others, nor have there been studies of the molecular mechanisms responsible for antibody-mediated neutralization of SK activity. To begin to address these questions, we have derived high affinity mAb to SK. We have sought to map the antigenic surface of SK delineated by these mAb by traditional competitive binding analysis and by studying the binding of the mAb to SK fragments generated by the PCR. Finally, we have studied the functional effect of these mAb on the activation of plasminogen by SK to begin to dissect the antigenic and functional structure of SK.

Materials and Methods

mAb production

SK (Hoechst Roussel, Somerville, NJ) was further purified by ammonium sulfate precipitation (40%) followed by ion exchange chromatography on DEAE Affigel Blue (Bio-Rad, Richmond, CA) with an NaCl gradient from 0 to 100 mM. The resulting SK was homogeneous by SDS-PAGE (16). Female BALB/C mice (Charles River, Wilmington, MA) were immunized s.c. with 33 µg of SK in Freund's adjuvant. They were again immunized 2 wk later and hyperimmunized 3 wk later with 3 µg of SK i.v. 2 days before fusion.

After fusion, cells were distributed into 10 96-well microtiter plates. Hybridoma growth was detected in nearly all wells. Hybridoma supernatants were tested for the presence of anti-SK mAb in a reverse solid phase assay (17). Wells of a microtiter plate were coated with goat anti-mouse antibody (25 µl, 3 µg/ml). After blocking nonspecific binding sites with 1% BSA, 25 µl of hybridoma supernatant were added for 1 h. The wells were washed, and [^{125} I]SK (100,000 cpm/25 µl) was added. After 1 h the wells were again washed and the amount of bound SK was determined by gamma counting. The SK was radioiodinated using the Iodogen method (Pierce, Rockford, IL) (18) to a specific activity of $\sim 2 \times 10^6$ cpm/µg.

Of the 960 wells tested 13% produced anti-SK antibody capable of capturing greater than 2000 cpm of [^{125} I]SK (2.5 times control binding). Fifty-one hybridomas produced Ab that consistently captured $>10,000$ cpm (>10 times control values—apparent $K_d \leq 10^{-8}$ M); these were selected for further study. Hybridomas were cloned by limiting dilution and serotyped using Zymed reagents (San Francisco, CA). The isotype of the representative, complementation group mAb (see below) was Ig γ 1 κ .

Ab-binding assays

A competitive RIA was used to determine whether hybridomas produced Ab that bound to the same region of SK. Wells of a microtiter plate were coated with affinity-purified goat anti-mouse Ab (3 µg/ml) for 90 min. Nonspecific protein-binding sites were then blocked by incubation with 100 µl of 1% BSA in Tris-buffered saline. The BSA solution was removed and 25 µl of a given hybridoma culture supernatant were added to a series of wells and incubated for 90 min. After washing, culture supernatants from other hybridomas, the same hybridoma, or 1% BSA was added to these wells as competitors. (The culture supernatant from the same hybridoma was a positive control for inhibition; 1% BSA was a negative control.) Then 25 µl of [^{125} I]SK ($\sim 50,000$ cpm) were added to the culture supernatants in each well. After 1 h of incubation the unbound radioactivity was aspirated and the wells were washed, cut, and counted.

If the Ab in the culture supernatant from the second hybridoma inhibited the binding of the [^{125} I]SK to the immobilized Ab (first hybridoma culture supernatant) by more than 50% (typically $>90\%$), the Ab were considered to bind to the same region of SK and to be members of the same "complementation group." If no inhibition was seen, the Ab were considered to bind to sterically distinct or minimally overlapping regions of SK, and thus the Ab were classified as members of different complementation groups. All hybridomas could be classified into six complementation groups on the basis of these experiments. Based upon this classification, one representative mAb was selected from each complementation group for expansion into ascites. These representative mAb were purified by chromatography on protein A-agarose as described (17). Competition-binding studies were repeated using the purified mAb representing each of the six different complementation groups. Plates were coated with 20 µg/ml of purified mAb (25 µl) for 1 h. The plates were washed and nonspecific protein-binding sites were blocked with 1% BSA for 30 min. Then purified mAb (diluted to a final concentration of 10 µg/ml) were mixed with [^{125} I]SK and 25 µl of the mixture (50,000 cpm) were added to the wells containing the immobilized mAb. After 60 min of incubation the reaction mixtures were aspirated. The wells were washed and gamma counted to determine the amount of bound SK.

Binding of mAb to SK fragments

Wells of a microtiter plate were coated with purified SK fragments in a concentration of 20 µg/ml or no Ag. After 90 min the wells were washed and nonspecific binding sites were blocked with 1% BSA. The wells were washed and incubated with culture media for 1 h. After washing, bound antibody was detected with ^{125} I-goat anti-mouse antibody. The competition between human plasminogen and the anti-

SK mAb for binding to [^{125} I]SK was measured in a reverse solid phase assay. Microtiter plates were coated with 20 μ g/ml (25 μ l) of purified anti-SK mAb or no mAb for 2 h in duplicate. After washing, the plates were blocked with 150 μ l of 1% BSA for 1 h. Then 25 μ l of purified human plasminogen (19) (concentrations ranging from 0 to 1.0 mg/ml) in 1% BSA and aprotinin (1000U/ml) were added to the wells followed immediately by [^{125} I]SK (100,000 cpm/25 μ l). After 1 h of incubation the wells were washed, cut, and counted. The amount of bound [^{125} I]SK was determined by gamma counting. The percent inhibition of binding of [^{125} I]SK to the anti-SK mAb by different concentrations of plasminogen was computed by reference to the amount bound to the mAb in wells containing no plasminogen (defined as 0% inhibition) and no mAb (defined as 100% inhibition).

Production of recombinant SK fragments

Bacterial genomic DNA was prepared from *Streptococcus equisimilis*, strain H46A (Lancefield's group C, American Type Culture Collection no. 12449, Bethesda, MD) and the SK gene was cloned using PCR (20). Two synthetic oligonucleotide primers were designed based upon a previously published SK nucleotide sequence (21). The upstream primer was designed to recapitulate the codons of the N terminus leader sequence of SK. The downstream primer corresponded to the antisense sequence of the C terminus of the SK gene and contained two stop codons. A restriction endonuclease site was incorporated at the 5' end of each primer in order to facilitate cloning. The oligonucleotide primers used for amplification and cloning of the SK gene were: SK leader peptide, [5'-d(CCC AGA TCT ATG AAA AAT TAC TTA TCT TTT GG)]; SK carboxyl terminus, [5'-d(CCC GGA TCC TCA TTA TTT GTC GTT AGG GTT ATC AG)]. The cloned SK DNA was sequenced (22) and found to be identical to the published sequence. Truncated portions of the SK gene were generated in a similar manner using PCR and specific internal primers. The following primers were used for amplification and cloning of SK fragments (numbers refer to the amino acid residues): SK amino terminus, [5'-d(GC GAT ATC GCT GGA CCT GAG TGG)]; SK carboxyl terminus, [5'-d(GC CTG CAG TCA TTA TTT GTC GTT AGG)]; SK 244–248, [5'-d(GC GAT ATC CGT GTT AAA AAT CGG G)]; SK 127–122, [5'-d(GC CTG CAG TCA TTA CAA GGT TAC CGA ACC ATC)]; SK 253–248, [5'-d(GC CTG CAG TCA TTA CCT ATA AGC TTG TTC CCG)]; SK 352–347, [5'-d(GC CTG CAG TCA TTA TCC AGT TAA GGT ATA GTC)]. The primer restriction endonuclease sites are underlined (*EcoRV* or *PstI*). An internal *HincII* site in the SK gene was also used in order to generate a protein fragment that began with a valine residue, the amino acid in position 14 of full length SK.

After the DNA sequence corresponding to full length SK, or the desired SK fragment was cloned, it was ligated into the pMAL expression vector (New England Biolabs, Beverly, MA) and expressed in *Escherichia coli* as a fusion protein (with maltose binding protein, MBP) (23). This vector also contained the Lac repressor allowing induced synthesis by isopropyl-b-D-thiogalactoside. The SK fusion proteins were purified by affinity chromatography on an amylose resin as described by the supplier, or by chromatography on a DEAE Affigel Blue agarose (Bio-Rad, Hayward, CA) and the purity was assessed by SDS-PAGE (16). After SDS-PAGE, SK proteins were stained with Coomassie blue dye or transferred to polyvinylidene difluoride membranes by semi-dry electroblotting as described (24). The membranes were blocked in 5% nonfat milk and probed with pooled anti-SK hybridoma supernatants for 1 h. After washing six times, the membranes were incubated in [^{125} I]-goat anti-mouse antibody (1,000,000 cpm) for 1 h. After additional washing, the membranes were exposed to Kodak X-Omat film (Rochester, NY) at -70°C .

Effects of mAb on SK activity

Purified mAb (25 μ l, 1 mg/ml) was mixed with native SK (25 μ l, 1 μ g/ml) for 20 min at room temperature and then added to wells of a microtiter plate containing 25 μ l each of 0.1% BSA in Tris-buffered saline, human plasminogen (100 μ g/ml), and CBS 33.08 substrate (AcOH.H-D-NLeu-CHA-Arg-pNA, 1.9 mM, Diagnostica Stago, Asnieres-Sur-Seine, France). The rate of plasminogen activation to plasmin was determined by the cleavage of the plasmin substrate CBS 33.08 and subsequent release of the paranitroanilide product. The rate of paranitroanilide production was recorded continuously at 405 nm in triplicate for each of these samples in a microtiter plate reader (Thermomax, Molecular Devices, Palo Alto, CA). The rate of paranitroanilide product formation in the presence of a given anti-SK mAb was divided by the mean rate of product formation in the presence of a purified, inactive, control antidigoxin mAb to determine the percent residual SK activity.

In other experiments the effect of the mAb was studied on the preformed SK-PAC. SK (25 μ l, 1 μ g/ml) was mixed with human plasminogen (25 μ l, 2 μ g/ml) for 5 min and added to wells containing 25 μ l each of mAb (1 mg/ml), human plasminogen (1 mg/ml) and CBS substrate. The rate of plasminogen activation and percent residual activity were determined in triplicate as described above.

Results

From the fused splenocytes of one immunized mouse, 121 hybridomas were found to produce antibody that specifically bound to SK at levels greater than 2.5 times background in a reverse solid phase RIA. Of these, 51 hybridomas consistently "captured" [^{125} I]SK at levels exceeding

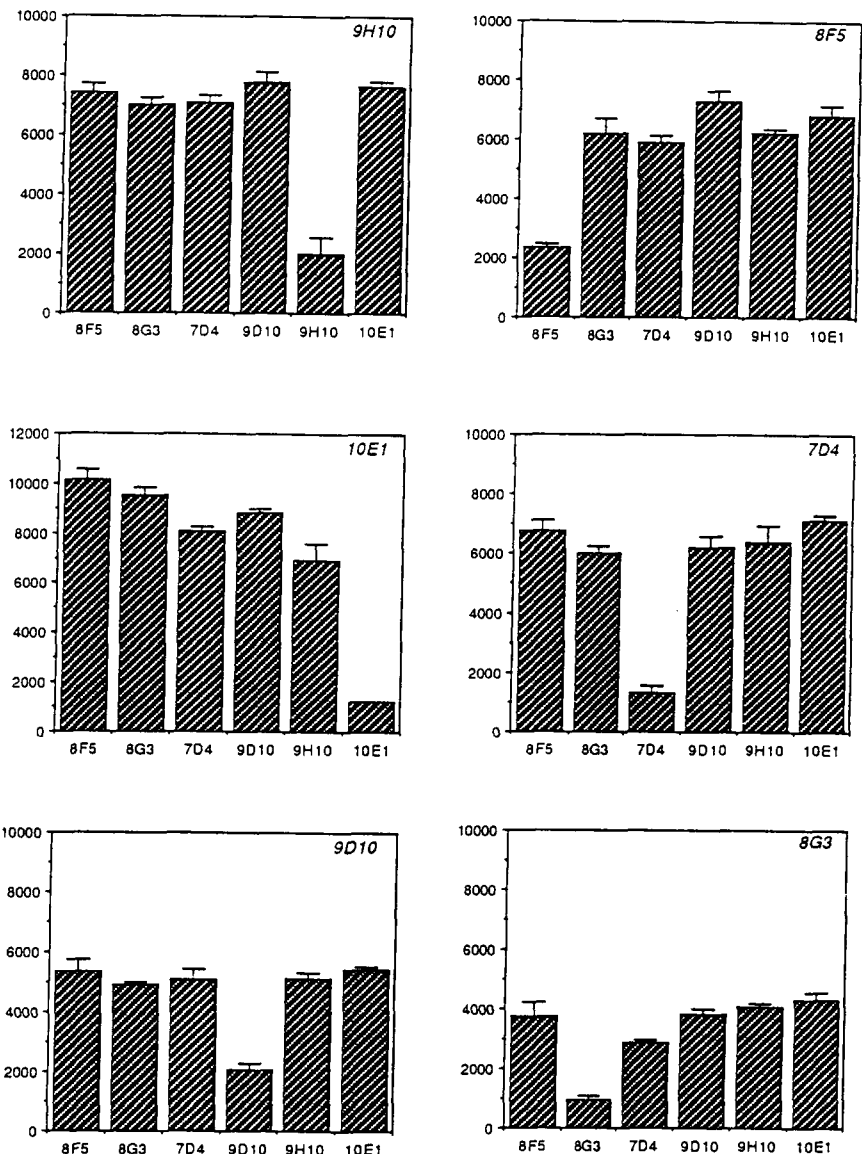


FIGURE 1. Competitive binding assays for representative mAb from each complementation group. Wells of a microtiter plate were coated with the purified mAb indicated in the upper right corner of each graph. Then different purified mAb (indicated on the abscissa of each graph) were added as inhibitors to the wells in duplicate with [^{125} I]SK. The resulting amount of [^{125} I]SK bound to the immobilized mAb (shown on the ordinate) was determined by gamma counting. The mean and SEM of duplicate observations is shown.

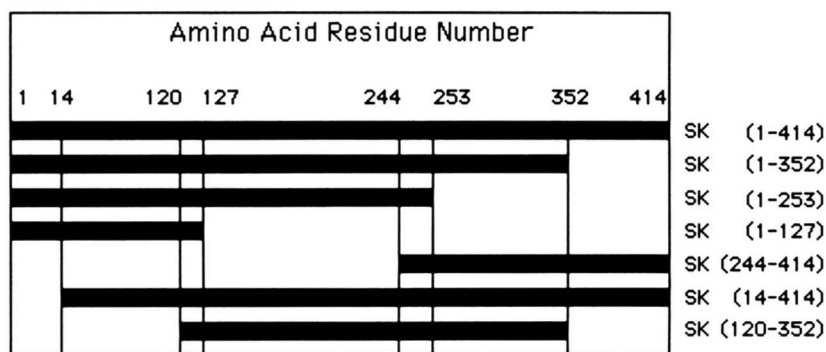
10 times control. The regional binding specificities of these hybridomas were determined by competitive binding experiments. In this assay an Ab from one hybridoma was immobilized in the wells of a microtiter plate. Then culture supernatant from another hybridoma was added to compete with the immobilized Ab for binding to [^{125} I]SK. Virgin culture media and culture supernatants from the same hybridoma were used as negative and positive controls for inhibition, respectively. Ab were considered to bind to the same region of SK, and thus to be members of the same complementation group, if the Ab in the culture supernatant inhibited the binding of the [^{125} I]SK to the immobilized Ab by more than 50%. (Typically the inhibition was >90%). These competitive inhibition assays revealed that the hybridomas could be classified into six major complementation groups.

For more detailed studies, one mAb was selected as a

representative from each complementation group. These "representative" mAb were expanded into ascites and purified using affinity chromatography. Competitive binding assays were performed with each of these purified mAb to verify that they bound to different regions of SK. Purified mAb was immobilized in wells of a microtiter plate. After blocking nonspecific binding, each of the purified mAb was added in duplicate to the wells with [^{125} I]SK. Figure 1 shows that the binding of the immobilized mAb to [^{125} I]SK was inhibited when the same mAb was used as competitor (self-inhibition). But when other mAb were used as competitor, they caused little or no effect. Thus these six representative mAb appeared to bind to sterically distinct or minimally overlapping regions of the SK molecule.

To identify the peptide fragments of the SK molecule, which showed cross-reactive binding with these mAb, we generated SK fragments by the PCR and expressed them in

FIGURE 2. Schematic illustrating the recombinant SK fragments. The initial and terminal amino acid numbers of each fragment are shown.



E. coli. Figure 2 displays the expressed rSK fragments in schematic form. The NH₂-terminal and COOH-terminal amino acid residues of the SK fragments are indicated by numbers. Figure 3A shows Coomassie blue-stained gels demonstrating the expression of these induced fusion proteins containing the SK fragments. Figure 3B shows the same material subjected to immunoblotting with pooled mAb against SK. These two figures demonstrate the major protein product in the induced *E. coli* lysates is the SK fusion protein. Some minor degradation of the SK fusion proteins as well as of the native (wild-type) SK proteins is evident. All of the SK fusion proteins are of appropriate molecular mass and are immunoreactive with the SK mAb, whereas the other component of the fusion protein, MBP, is not reactive.

The binding patterns of the representative mAb to these SK fragments were studied in a direct binding assay. mAb were considered to show significant cross-reactive binding to a fragment of SK if their binding was $\geq 15\%$ of their binding to full length SK in the same assay. The results of these binding assays are shown in Figure 4 and summarized in Table I. mAb 9D10 binds to full length SK (SK 1-414) but does not bind to SK 14-414, which lacks the first 13 amino terminal amino acids. This would suggest that mAb 9D10 requires the first 13 amino acids of full length SK for binding. mAb 8G3 binds to SK 244-414, but does not show significant binding to SK 1-352, suggesting that it largely requires amino acids within the region of 353 to 414 for binding. Because mAb 10E1 binds to SK 1-127 and to SK 14-414, but not to SK 120-352, it appears to bind to a region of SK largely formed by amino acids 14-120. Two mAb, 8F5 and 9H10, although they did not compete with each other for binding to SK, had a similar pattern of binding to the SK fragments, as did all of the mAb in their respective complementation groups. Neither 8F5 nor 9H10 bound to the overlapping fragments of SK 1-253 or 244-414. But both mAb did bind to SK 1-352 and to the smaller fragment SK 120-352. Thus it would appear that these mAb require residues in the middle one-third to one-half of the SK molecule for binding to SK. Finally, mAb 7D4 binds to SK 1-253, but not to the smaller fragments SK 1-127 or SK 120-352. Similar direct binding studies were performed

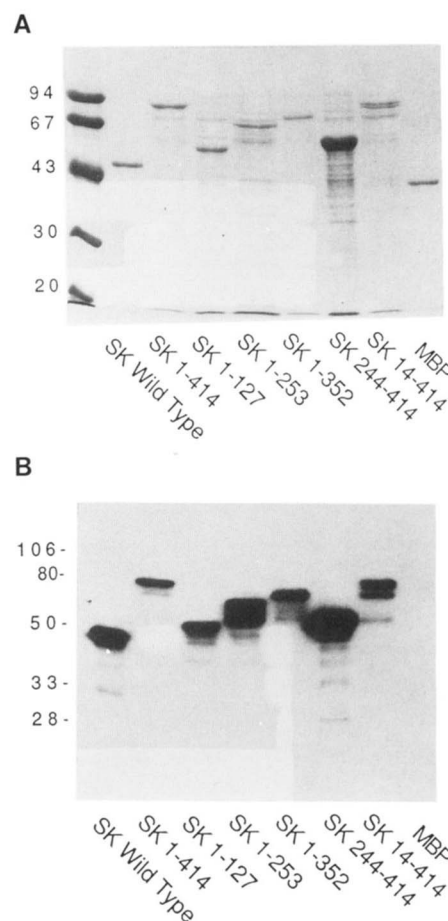


FIGURE 3. SDS-PAGE of recombinant SK fragment fusion proteins. Partially purified MBP-SK fusion proteins were electrophoresed on 12% SDS gels under reducing conditions. A, staining of the proteins with Coomassie brilliant blue; B, immunoblotting of recombinant SK fragments with pooled mAb followed by ¹²⁵I-goat anti-mouse antibody. The contents of each lane are shown. Molecular weight markers ($\times 10^{-3}$) are indicated at left.

with the other 45 mAb raised against SK. These mAb bound to SK fragments in a pattern consistent with the complementation group assignment determined by the competitive binding assays described above (data not shown).

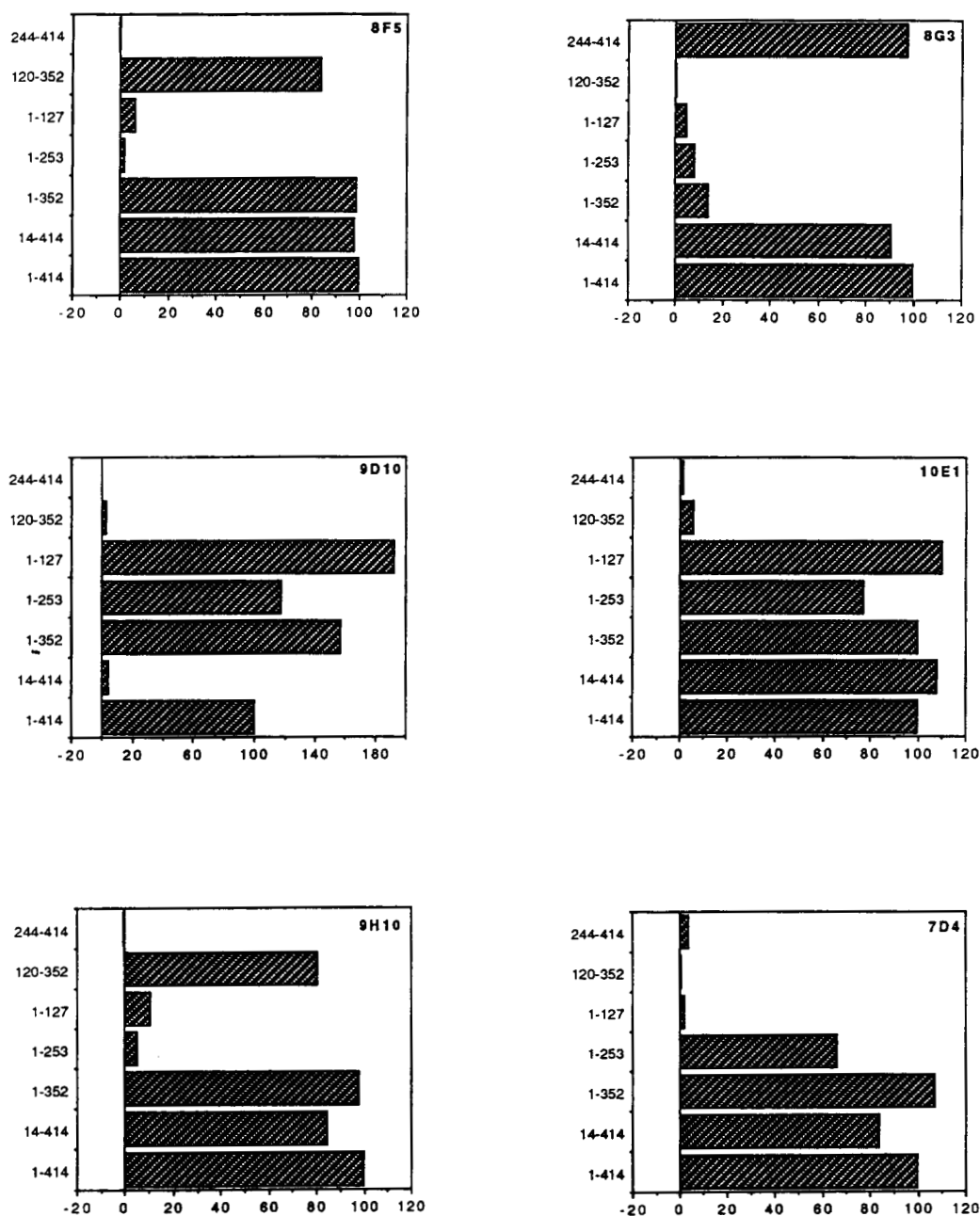


FIGURE 4. Binding of representative mAb to recombinant SK fragments. Wells of a microtiter plate were coated with purified SK fragments. Then representative mAb from each of the six complementation groups were added to the wells. The bound mAb was detected by ^{125}I -goat anti-mouse antibody and subsequent gamma counting. Each bar graph depicts the binding of one representative mAb (indicated in the upper right of each graph) to each of six recombinant SK fragments (*ordinate*) as a percentage of its binding to full length SK 1-414 (*abscissa*) in the same assay. The data represent the means of duplicate observations.

Effects of mAb on plasminogen activation

Assays were performed to determine the effects of the mAb on the formation of the SK-PAC and on the rate of plasminogen activation once the activator complex was formed. When purified mAb were incubated with SK before the addition of plasminogen, all but one mAb appeared to inhibit the rate of plasmin generation (Fig. 5A). mAb 7D4 and 8F5 inhibited plasminogen activation by >90%. mAb 8G3

and 9D10 also inhibited plasminogen activation by 60 to 75%, whereas mAb 10E1 had minor effects. Only mAb 9H10 had no apparent effect on SK activity. However, when the mAb were added after the SK-PAC was allowed to form, only one of the mAb, 8F5, showed significant inhibition of plasminogen activation (Fig. 5B). This suggested that mAb 8F5 interfered with the catalytic function of the SK-PAC, whereas the other mAb inhibited SK activity by

Table I
Binding of SK MAbs to SK fragments^a

SK fragment	mAb					
	7D4	8F5	8G3	9D10	9H10	10E1
1-127	–	–	–	+	–	+
1-253	+	–	–	+	–	+
1-352	+	+	–	+	+	+
1-414	+	+	+	+	+	+
14-414	+	+	+	–	+	+
244-414	–	–	+	–	–	–
120-352	–	+	–	–	+	–
Binding Region ^b	1-253	120-352	353-414	1-13	120-352	14-120
No. Ab ^c	6	10	3	5	12	15

^a Tabular summary of the binding of anti-SK mAb to purified SK fragments (see Fig. 4). A + indicates that the mAb showed significant binding to the SK fragment; – indicates that the mAb did not show significant binding. A mAb was considered to show significant binding to a SK fragment if its binding to the SK fragment was at least 15% of its binding to full length SK 1-414.

^b The binding region of SK recognized by the antibody is inferred from the pattern of binding indicated in the table.

^c The total number of antibodies that bind to the same region of SK is listed.

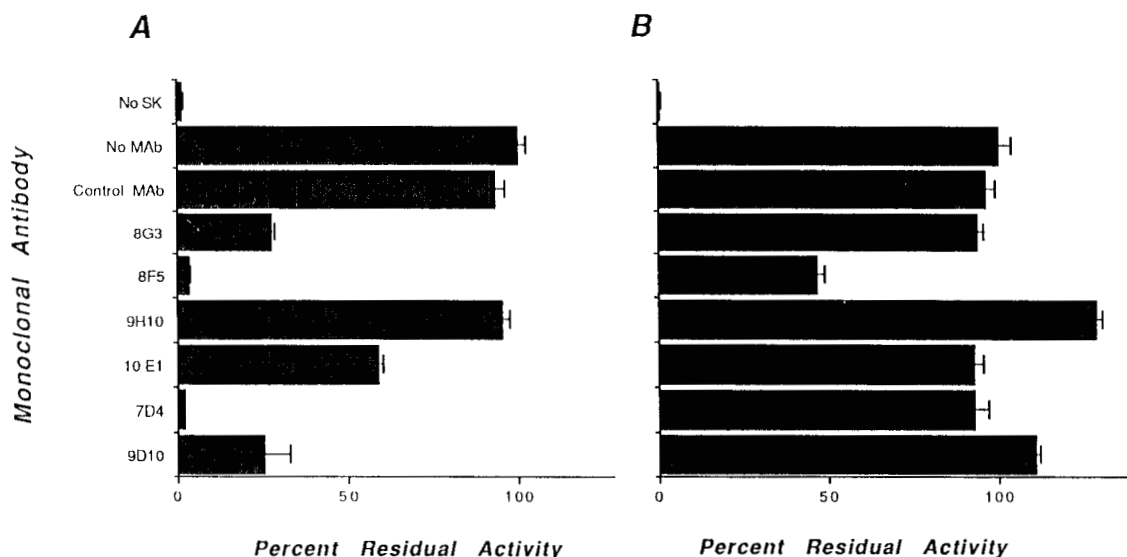


FIGURE 5. Effects of mAb on plasminogen activation by SK. *A*, mAb added before formation of SK-PAC. Purified mAb were premixed with native SK for 20 min and then added to wells containing plasminogen and a chromogenic substrate (CBS 33.08). The generation of plasmin was monitored by the cleavage of the chromogenic substrate. The percentage of residual SK activity was determined as described in *Materials and Methods*. *B*, mAb added after formation of the SK-PAC. SK and plasminogen were preincubated for 5 min and then added to wells of a microtiter plate containing anti-SK mAb, or a control, inert mAb, plasminogen, and chromogenic substrate. The generation of plasmin was monitored and the percentage of residual activity was determined as described. The means and SEM of triplicate observations are shown.

interfering with the binding of SK to plasminogen during the formation of the SK-PAC. To test this hypothesis the binding of [¹²⁵I]SK to plasminogen was measured in the presence and absence of the mAb (Fig. 6). This graph shows that mAb 7D4 and 8F5 were effective inhibitors of the binding of [¹²⁵I]SK to human plasminogen at plasminogen concentrations up to, and slightly in excess of, the concentration of plasminogen in plasma (210 µg/ml). The other mAb showed no significant competition with SK-plasminogen binding at these concentrations.

Discussion

Streptokinase is now widely used as an effective treatment for dissolving the thrombus that causes heart attacks. Although its immunogenicity has been evident since the 1930s, the regions of the molecule responsible for its antigenicity have not been identified. We have generated a panel of 51 murine hybridomas producing Ab against the native protein as probes to begin to analyze the humoral immune response to SK. These 51 hybridomas were ini-

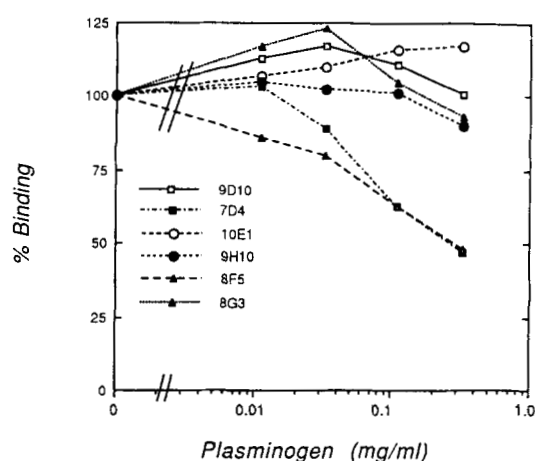


FIGURE 6. Effects of anti-SK mAb on the binding of SK to plasminogen. Wells of a microtiter plate were coated with purified anti-SK mAb or no mAb. Various amounts of plasminogen (0 to 1.0 mg/ml), aprotinin (1000 U/ml), and [^{125} I]SK (100,000 cpm) were added to the wells. After 1 h of incubation the wells were washed and the amount of bound [^{125} I]SK was determined by gamma counting. The percent inhibition of binding of [^{125} I]SK to the mAb was computed by reference to wells containing no plasminogen as inhibitor (100% binding) and no mAb (0% binding).

tially selected at somatic cell fusion because of their avid binding to [^{125} I]SK in a reverse solid phase assay. Because this type of assay avoids solid phase immobilization of SK, it should allow all solution-phase epitopes of the protein to be available for binding to the mAb. Competition binding assays (performed in the same fashion) demonstrated that these mAb could be grouped into six major complementation groups that bind to spatially or sterically distinct regions of SK. The assignment of these mAb to complementation groups by competition-binding studies was confirmed by their characteristic pattern of binding to SK fragments generated by rDNA techniques. The regions of SK recognized by most of the representative mAb could be localized to a specific SK fragment as noted in Table I.

As an additional means of probing the antigenic regions of SK we studied the effects of these mAb on the binding of native SK to plasminogen and on the catalytic activity of SK-PAC. By correlating the mAb-binding data (Table I) with the functional effects of the mAb we may begin to formulate some hypotheses about the functional regions of SK. For example, mAb 7D4 is a strong inhibitor of the binding of SK to plasminogen but does not appear to affect the catalytic activity of the SK-PAC once it is formed. This suggests that the region recognized by mAb 7D4, amino acids 1–253, may contain a plasminogen-binding site. Similarly, mAb 8F5 inhibits the binding of plasminogen to SK as well as inhibiting the catalytic activity of the SK-PAC. This suggests that amino acids critical to the function of the SK-PAC reside in the region of amino acids 120–352 of SK. Further studies using SK fragments can build on these find-

ings to more precisely delineate the structural regions responsible for SK function.

Although there is a growing clinical literature on allergic reactions to SK, this report represents the first attempt to identify regions of SK that are responsible for its antigenicity and function. SK is unique among previously studied model Ag because humans are naturally immunized with it as a result of streptococcal infections. More recently, immunization has occurred as a consequence of the use of SK as a treatment for thrombosis. This natural immunization process facilitates the analysis of the immune response to SK in humans as well as other mammals. SK also has other advantages as a model Ag. Its complete amino acid and DNA sequence are known (21, 25). It has no carbohydrate chains or cysteine amino acids, thus recombinant SK produced in bacteria should be antigenically similar to the native molecule. Furthermore, the several naturally occurring variations in SK sequence from different streptococcal strains may allow additional insights into the antigenicity and function of the molecule. Finally, lessons learned from the study of SK in the laboratory may be tested and applied to humans treated with SK as well as those suffering from streptococcal disease. Ultimately it may be possible to uncouple the antigenic and functional structure of SK in order to design a nonimmunogenic, but still effective, plasminogen activator.

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