Identification of Staphylococcal Enterotoxin B Domains Involved in Binding to Cultured Human Kidney Proximal Tubular Cells: Imparting Proliferation and Death

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Studies suggest that staphylococcal enterotoxin B (SEB) is initially harbored in the kidney by binding to digalactosylceramide molecules in the proximal tubular cells. However, little is known in regard to the peptide motif within SEB that binds to these cells and imparts toxic effects. Herein, using human kidney proximal tubular cells (PTs) we have performed a systematic study on the binding of various peptides and peptide analogs of SEB and demonstrate a structure-functional relationship. Using [^{125}I]labeled SEB peptides, we show a high affinity and displaceable binding of SEB 191–220 to human PT cells. Binding was mitigated by the use of antibody against SEB, by digalactosylceramide (the putative receptor), and by the use of endoglycoceramidase, which selectively removes the oligosaccharide backbones from glycosphingolipids. Our structure/functional studies revealed that peptide 130–160 induces a concentration-dependent increase in programmed cell death/apoptosis in human proximal tubular cells. Mechanistic studies further suggest that SEB/SEB peptide (130–160) impart apoptosis *via* the activation of neutral sphingomyelinase, which hydrolizes sphingomyelin to ceramide and phosphocholine. SEB 130–160 mediated apoptosis was mitigated by preincubation of cells with antibody against SEB and an SEB 130–160 antibody. Exp Biol Med 232:1142–1151, 2007

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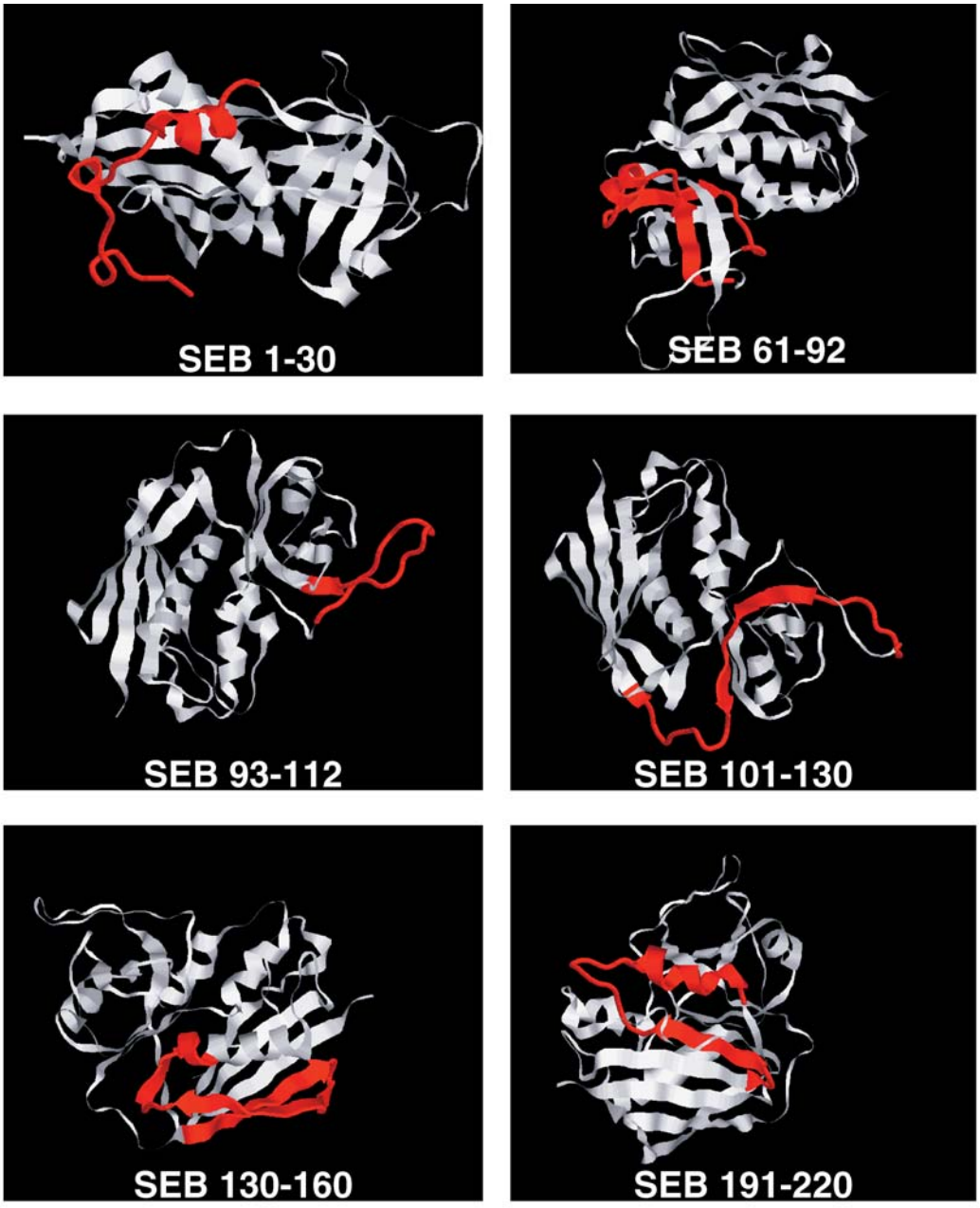
Introduction

Staphylococcal enterotoxin B (SEB), a 28-kDa exoprotein produced by gram-positive *Staphylococcus aureus,* has been well studied for its potent role as a T-lymphocyte mitogen (1). SEB, the other enterotoxins, toxic shock syndrome toxin-1, and a host of other viral and bacterial proteins have been termed *superantigens* (sAg). These molecules are able to bypass conventional antigen presentation and stimulate up to 20% of the host T-cell repertoire (1). SEB achieves this by extracellularly binding to major histocompatability complex II (MHC-II) on antigen-presenting cells while subsequently binding specific variable regions on the T-cell receptor (TcR; Ref. 2). This interaction initiates T-cell proliferation, with concomitant cytokine production. Mutational analysis of SEB suggests that the regions of SEB implicated in MHC-II binding are residues 13–17 and 44–52 (3).

Much of the pathophysiology after SEB exposure has been thought to occur *via* the massive production of inflammatory cytokines, tumor necrosis factor-α (TNF-α), and interleukin-2 (IL-2; Ref. 1). Interestingly, the emetic actions of the SE have been found to occur independently of mitogenic events. In fact, a histidine substitution in SEA at position 61 showed that superantigenic and emetic activities can be separated (4). Further, the active region for emesis has been speculated to correspond to amino acid residues 113–126 (5), an area remote from the immunologically active regions. In addition to the discrepancy in explanation of emetic events, there is an increasing body of evidence suggesting that SEB has the ability to interact and induce



SEB 1-30 – ESQPDPKPDELHKSSKFTGLMENMKVLYDN

SEB 61-92 – YDNVRVEFKNKDLADKYKDKYVDVFGANYYYQ

SEB 93-112 – CVFSKKTNDINSHQTDKRKT

SEB 101-130 – DINSHQTDKRKTCMYGGVTEHNGNQLDKYR

SEB 130-160 – RSITVRVFEDGKNLLSFDVQTNKKKVTAQEL

SEB 191-220 – ENENSFWYAMMPAPGDKFDQSKYLMMYNDN

Figure 1. Location of SEB peptides. The red amino acid residues highlight the locations of the experimental peptides used in this study. The sequence of amino acids can be found in Table 1. All structures were obtained from the Protein Data Bank of the Research Collaboratory for Structural Bioinformatics. Downloaded files were subsequently manipulated using the RasMol program. The coordinates are based upon the original publication by Papageorgiou *et al.* (13).

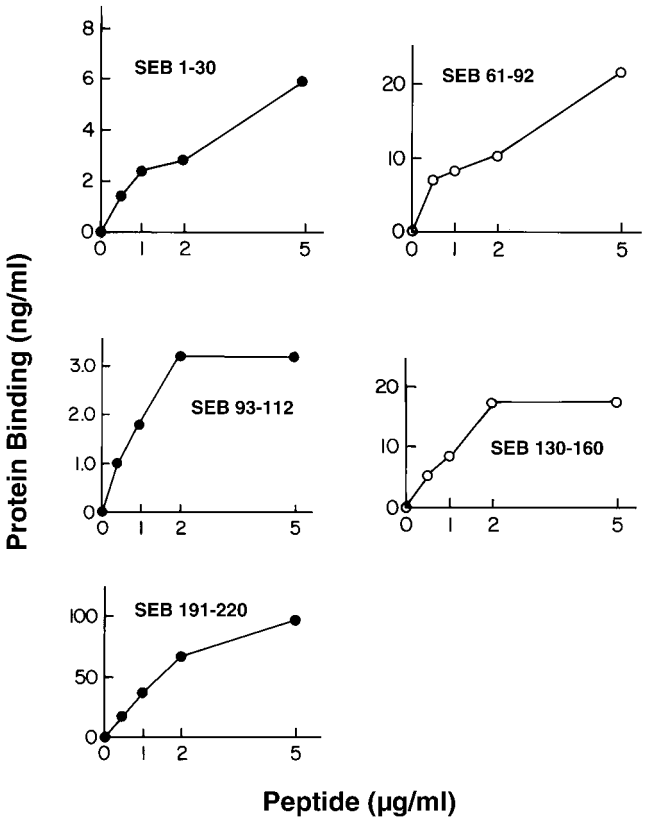


Figure 2. Binding of ^{125}I-SEB peptides by cultured human PT cells. PT cells grown as described in *Materials and Methods*. On the sixth day of culture, medium was replaced with that containing LPDS and incubated for 24 hrs. Subsequently, fresh medium containing LPDS and 0–5 μg/ml ^{125}I-SEB peptide was added. To another set of dishes, prior to the addition of ^{125}I-toxin, 20-fold excess of corresponding unlabeled toxin was added, and incubation was continued for 2 hrs at 37°C. Next, medium was removed, and the cells were washed 10 times with ice-cold PBS. Samples were then solubilized overnight with 1 *N* NaOH, and cell-associated radioactivity and protein content were measured. All assays were performed in duplicate dishes from two batches of PT cells and analyzed in duplicate. Specific binding (binding in the absence of unlabeled toxin-binding in the presence of unlabeled toxin) was calculated and plotted.

lesions in several nonimmunologic mammalian tissues (1, 6, 7, 8).

Localization and metabolic turnover studies in experimental animals reveal that the kidney is a major site of toxin sequestration (9, 10–12). *In vivo* studies show that the clearance of ^{125}I-SEB is markedly altered by perturbations in renal blood flow (10, 14). Other studies indicate that SEB is predominantly confined (more than 75% of the injected dose) to proximal tubular (PT) cells (11). Later, it was determined that SEB bound digalactosylceramide, a neutral glycosphingolipid found on human renal proximal tubular cells (15, 16).

Here we have investigated a series of synthetic SEB peptides (Fig. 1) to evaluate their binding to the putative receptors on PT cells and establish structure/function relationship in regard to peptide motif specific to induce cell proliferation and apoptosis. Our preliminary mechanistic studies suggest that apoptotic cell death induced by one of the synthetic peptides (SEB 130–160) may be due to the activation of neutral sphingomyelinase/ceramide generation. This phenotypic change may be mitigated by the use of SEB and/or SEB 130–160–specific antibodies. In SEB there is clear dichotomy between SEB peptide 93–112, which promotes cell proliferation, versus peptide 130–160, which induces apoptosis. In this article we present evidence that synthetic peptides can bind to PT cells and augment cell proliferation and apoptosis.

Materials and Methods

Isotopes and Chemicals. ^{125}I (specific activity: 644 MBq/μg iodine) was purchased from Dupont, New England Nuclear (Waltham, MA). All other biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Rhodococcus endoglycoceramidase was purchased from Genzyme Corp. (Boston, MA). Human kidney neutral glycosphingolipids were prepared in our laboratory (17) and characterized employing high-performance thin layer chromatography and high-performance liquid chromatography techniques (16). Lipoprotein-deficient serum (LPDS) was prepared from lipoprotein-deficient plasma by precipitation with thrombin as described and was determined to be free from glycosphingolipids and cholesterol (18).

Radiolabeling of SEB Peptides with ^{125}I. SEB synthetic peptides were prepared by Peninsula Labs (now a subsidiary of Bachem, Torrance, CA). Such peptides were classified based upon the SEB amino acid sequence they represented. SEB peptides were labeled with ^{125}I using iodogen (19), were solubilized in sample buffer, and were subjected to polyacrylamide gel electrophoresis on phorcast gels at 12.5 mA/gel for 24 hrs at room temperature. Appropriate standard proteins of known molecular weights were also electrophoresed, and a portion of the gel including the standard molecular weight proteins was excised and stained with Coomassie blue at 60°C for 5–10 mins. The gel area corresponding to individual SEB was excised, eluted, and dialyzed. The material was freeze dried, solubilized, and assessed for purity by SDS-PAGE. Such preparations were free from contaminating proteins.

Cells. Cultured human PT cells were prepared from autopsy kidney as described previously (20). Cells were trypsinized and seeded (1×10^5) in 60×15-mm plastic Petri dishes and grown for 6 days in minimum essential medium containing heat-inactivated, dialyzed 10% fetal calf serum and no antibiotics. On the sixth day, medium was removed, cells were washed with phosphate-buffered saline (PBS), and incubation continued for 24 hrs in medium containing LPDS (1 mg protein/ml).

^{125}I-SEB Peptide Binding Assay. Unless otherwise described in the text, the following assay was adopted to measure the binding of ^{125}I-SEB peptide to PT cells. Medium was removed from cells primed with LPDS. Next, fresh medium (1 ml) and ^{125}I-SEB peptide (1 μg/ml) plus a

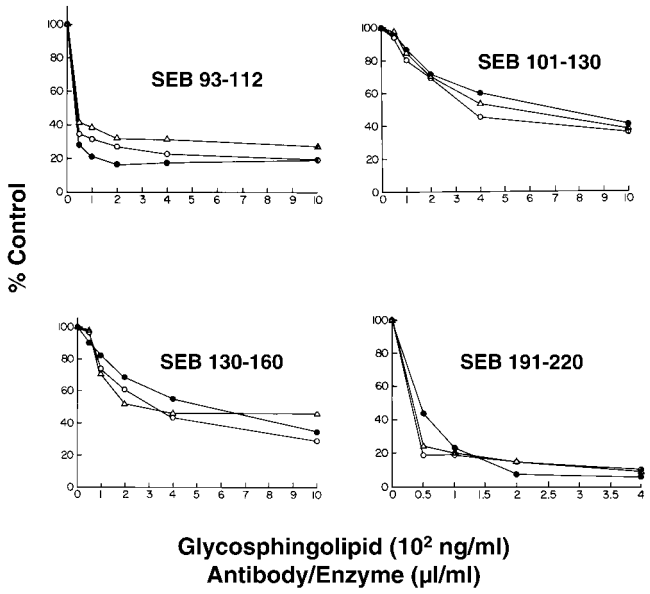


Figure 3. Effect of antibody against SEB peptides, human kidney glycosphingolipids, and endoglycoceramidase on the binding of ^{125}I-SEB in PT cells. Increasing concentrations of SEB peptide antibodies (closed circle), glycosphingolipids (open circle), and endoglycoceramidase (open triangle) were added to cultured PT cells prior to the addition of 1 µg/ml ^{125}I-SEB peptide. Incubation was carried out for 4 hrs at 37°C, and the specific binding of ^{125}I-SEB peptide to PT cells was measured. The data represent average values obtained from duplicate dishes from two batches of PT cells analyzed in duplicate.

20-fold excess of unlabeled SEB/peptide were added, and incubation was continued for 2 hrs at 37°C. Next, the medium was discarded and the cells washed with 5 ml PBS containing 0.2% bovine serum albumin (maintained at 4°C) and five times with PBS. The monolayer was solubilized in 1 *N* NaOH, protein and radioactivity were measured according to Lowry *et al.* (12) and scintillation spectrometry, respectively. Specific binding of ^{125}I toxin peptide was calculated by subtracting the data obtained in the absence of unlabeled toxin/peptide from the data obtained in the presence of 20-fold excess of unlabeled toxin/peptide (15, 16).

Incubation of Cells with Endoglycoceramidase. Cells preincubated with medium containing LPDS were further incubated with endoglycoceramidase (0.15–0.6 mU/ml) for 1 hr at 37°C and washed, and the binding of ^{125}I-SEB peptide was pursued as described above.

Incubation of Cells with Glycosphingolipids and SEB Peptide Antibodies. Glycosphingolipids were taken into a sterile glass tube and dried in N_2 atmosphere. Then, medium containing LPDS was added and sonicated, and suitable aliquots were added to the assay mixture. Cells preincubated with medium containing LPDS were further incubated with fresh medium containing human kidney neutral glycosphingolipids and ^{125}I-SEB peptide. After incubation for 2 hrs at 37°C, the assay was terminated and the binding of toxin to PT cells measured. Similarly, cells were incubated with SEB peptide antibodies for 1 hr prior to the ^{125}I-SEB peptide binding assay.

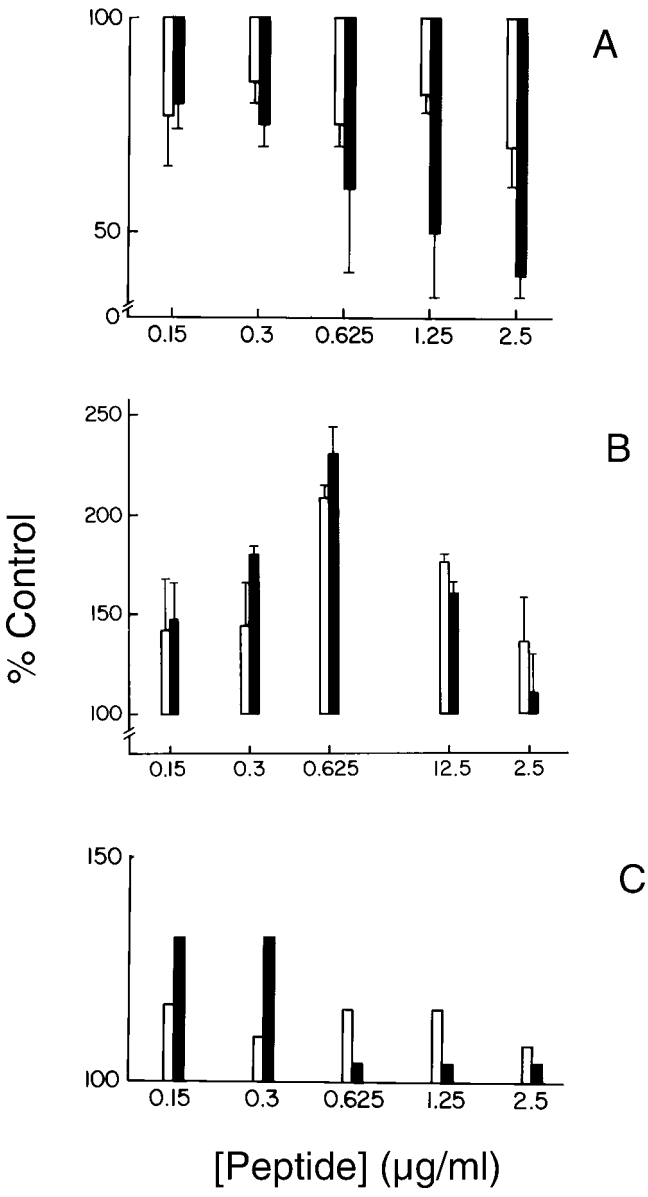


Figure 4. Effect of SEB peptide concentration on PT cell proliferation. Confluent culture of PT cells grown in 96-well trays was incubated in serum-free medium for 24 hrs. Next, medium containing 1 mg/ml LPDS and increasing concentrations of SEB peptides (0.15–2.5 µg/ml) were added. Following incubation for 22 hrs, [^{3}H]thymidine (5 µCi/ml) was added, and incubation continued for another 2 hrs. Next, the cells were washed five times with PBS, and incorporation of [^{3}H]thymidine into DNA was measured. A parallel set of dishes was trypsinized, stained with trypan blue, and subject to viable cell counting employing a hemocytometer and a light microscope. The data obtained from three separate experiments and six microtiter wells each were analyzed. Open bars indicate [^{3}H]thymidine incorporation; solid bars, viable cell count. (A) SEB 130–160. (B) SEB 93–112. (C) SEB 191–220. Proliferation data from SEB alone (two separate sources): source 1 SEB = 6894 ± 416; source 2 SEB = 8987 ± 2698.

Measurement of Cell Proliferation. [^{3}H]Thymidine incorporation into DNA and trypan blue exclusion assay were employed to measure the effect of SEB, and SEB peptides on cell proliferation. Further details are described in the legend to individual figures.

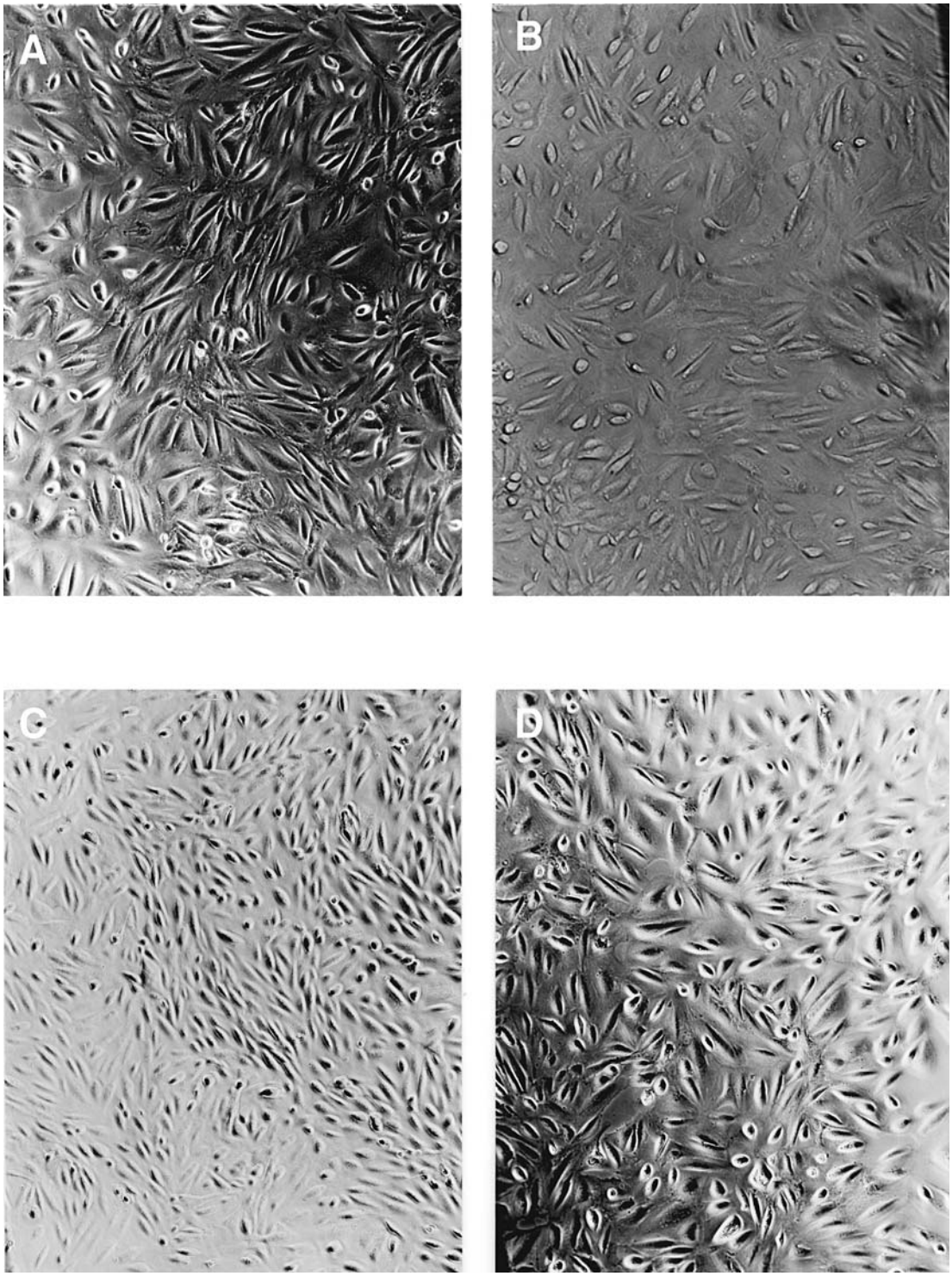


Figure 5. Effect of SEB and SEB peptides on PT cell morphology. The protocol of this experiment was identical to that described in the Figure 4 legend, except that cells were incubated with media alone (A), SEB (B), SEB 93–112 (C), and 130–160 (D) at a concentration of 1 μg/ml for 24 hrs and photographed.

Results

Effect of ^{125}I-SEB Peptide Concentration on Binding to Cultured Human Proximal Tubular Cells. Using cultured human PT cells, we found high affinity binding of ^{125}I-SEB peptides (Fig. 2). SEB 191–220 was bound to PT cells with the highest affinity compared with four other SEB peptides depicted here (Fig. 2).

Effect of SEB Peptide Antibody, Human Kidney Neutral Glycosphingolipids and Endoglycoceramidase on the Binding of ^{125}I-SEB Peptides to Human

Table 1. Effect of Amino Acid Composition in SEB 130–160 on Human PT Cell Proliferation[a]

	Sequence	[^{3}H]Thymidine incorporation (cpm/well, $N = 6$)
SEB 130–160	SITVRVFEDGKNLLSFDVQTNKKKVTAQEL	539 ± 50
SEB 141–161	KNLLSFDVQTNKKKVTAQELD	544 ± 48
SEB 146–161	FDVQTNKKKTAQELD	610 ± 52
SEB 145–175	SFDVQTNKKKVTAQELDYLTRHYLVKNKKLY	588 ± 56
SEB 150–165	TNKKKVTAQELDYLTR	502 ± 90
SEB 154–170	KVTAQELDYLTRHYLVK	485 ± 40
SEB 140–156	GKNLLSFDVQTNKKKVT	4386 ± 350
SEB 100–117	NDINSHQTDKRKTCMYGG	685 ± 46
Control		3151 ± 350

[a] SEB 130–160 and its amino acid substitutes were prepared as described in *Materials and Methods*. These peptides were added to PT cells grown in 96-well trays. [^{3}H]Thymidine incorporation into DNA was measured as described previously. The data were obtained from two separate experiments analyzed in 6-μl wells each.

PT Cells. Peptide 191–220 was bound with the highest affinity (Fig. 1), and this was markedly inhibited by the presence of glycolipids and endoglycoceramidase, albeit less so when an antibody against SEB was used (Fig. 3). These findings suggest that SEB peptide 191–220 represents the major peptide domain that binds to the glycosphigolipid domain in the kidney cells. This finding is in agreement with our previous studies that digalactosylceramide is the receptor for SEB in cultured human kidney cells (15, 16). Although the other peptides in this study did not bind to cells with the same affinity as peptide 191–220, this was nonetheless affected by the use of antibody to SEB, glycolipids, and treatment with endoglyccoceramidease. For example, peptide 93–120 binding was significantly affected by the use of SEB antibody but relatively less so with the use of glycolipids and endoglycoceramidase treatment. The relevance of these findings remains to be explored. However, we can speculate that the clustering of the glycosphigolipid on the lipid rafts and the dissolution/perturbation of the lipid raft due to endoglycoceramidease treatment may have a general adverse effect on the binding of these SEB peptides.

Effect of SEB Peptide Concentration on Human PT Cell Proliferation. Cell proliferation was measured employing multiple criteria. These were [^{3}H]thymidine incorporation into DNA (shown in open bars in Fig. 4) and employment of trypan blue dye exclusion viable cell count assay (solid bars in Fig. 4). As shown in Figure 4A, incubation of PT cells with SEB 130–160 exerted a concentration-dependent decrease in cell proliferation. In sharp contrast, in PT cells, SEB 93–112 exerted a concentration-dependent increase in cell proliferation (Fig. 4B). Maximum increase in cell proliferation (~2.5-fold) occurred with 0.625 μg/ml SEB 93–112. However, further increasing the amount of SEB 93–112 did not increase cell proliferation. SEB peptide 191–220 had a modest increase—~30% in cell proliferation—compared with control at a concentration of 0.15–0.3 μg/ml (Fig. 4C). However, at relatively higher concentrations (0.625–2.5 μg/ml), SEB 191–220 did not alter cell proliferation significantly (Fig. 4C). Light microscopy studies revealed that PT cells incubated with SEB 93–112 were highly densely packed (Fig. 5C) and appeared small in size compared with control, untreated cells, or SEB-treated cells, respectively (Fig. 5A and B). In contrast, PT cells incubated with peptide 130–160 were relatively less dense and had a polygonal morphology (Fig. 5D).

Effect of Amino Acid Composition of SEB Peptide 130–160 on Cell Proliferation. SEB 130–160 exerted a 6-fold decrease in cell proliferation compared with control (Table 1). Amino acid substitution of this sequence, as well as deletion of some amino acids within this sequence, imparted a partial or complete restoration in PT cell proliferation.

Effect of SEB and SEB Peptide 130–160 on Apoptosis. To determine phenotypic changes that may accompany SEB peptide 130–160 in inhibition of cell proliferation, we examined the effect of SEB, SEB peptide 130–160, and SEB peptide 93–112 on apoptosis. The ability of antibody against SEB and SEB peptide 130–160 to mitigate apoptosis was also examined by staining the cells with 4′,6-diamidino-2-phenylindole (DAPI) reagent. DAPI stains intact nucleus blue, whereas fragmented DNA, as in apoptotic cells, is stained white. Figure 6A shows the DAPI-stained cells, and the corresponding quantitative bar chart in Figure 6B represents the percentage of apoptotic cells. We observed that SEB (1–2 μg/ml) exerted a concentration-dependent increase in number in apoptotic cells compared with control (Fig. 6B). SEB peptide 130–160 also markedly induced apoptosis in these kidney cells. However, SEB peptide 93–112 did not alter apoptosis. Moreover, SEB- and/or SEB peptide 130–160–induced apoptosis was completely reversed by the use of ether SEB or SEB 130–160 antibodies (Fig. 6B).

Effect of SEB and SEB Peptide 130–160 on Neutral Sphingomyelinase-Ceramide Pathway. To determine the biochemical mechanism by which SEB and SEB peptide 130–160 may induce apoptosis we examined the effect of these compounds on the activity of neutral sphingomyelinase and ceramide and sphingomyelin mass.

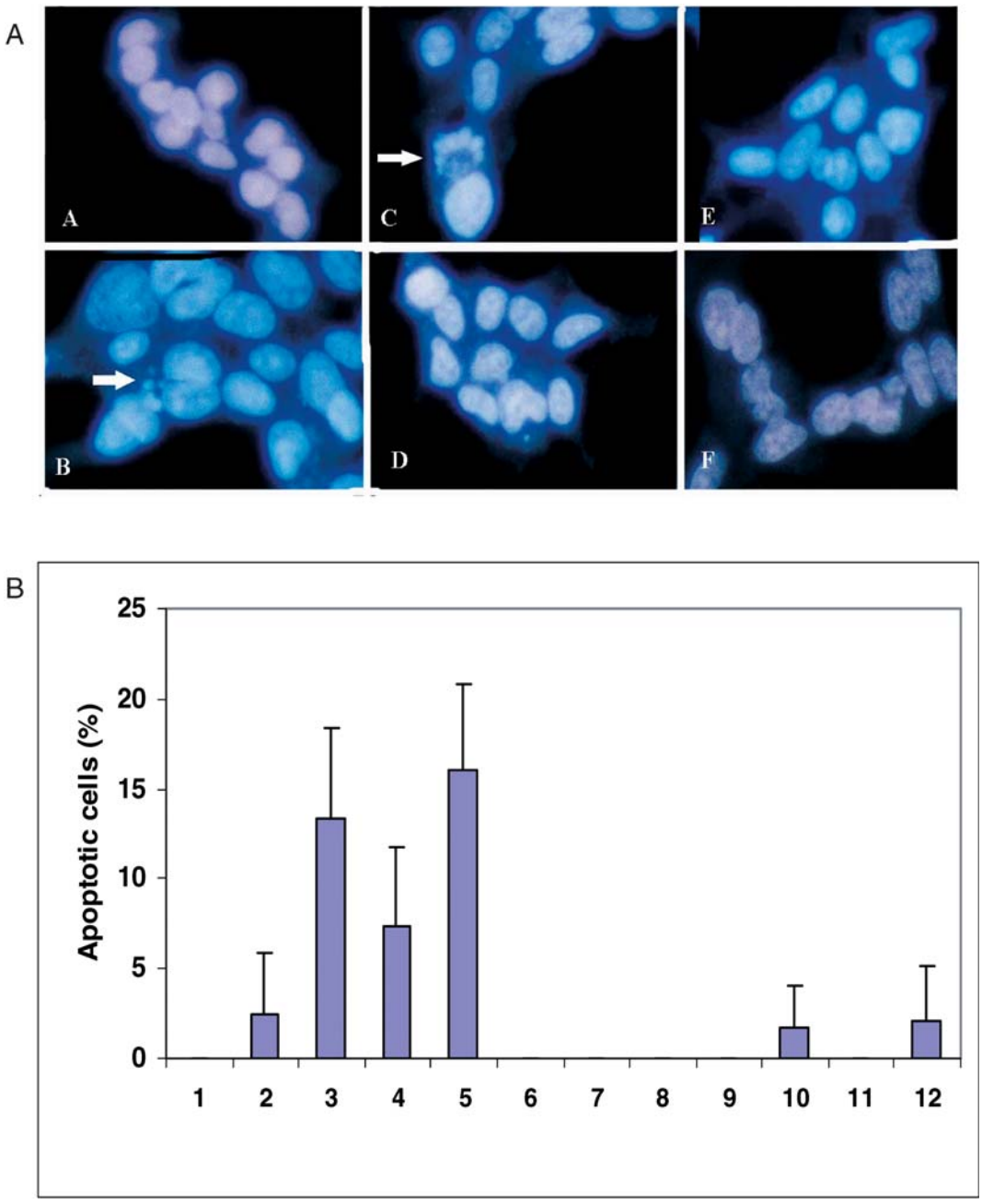


Figure 6. Effect of SEB and SEB peptides on apoptosis. Confluent culture of human kidney proximal tubular cells grown on glass cover slips were switched to 1% serum containing medium. SEB, SEB peptide 130–160 (1 and 2 μg/ml medium), and SEB peptide 93–112 (2 μg/ml) were added. To other dishes, SEB and SEB peptide 130–160 antibodies (2 μl/ml) were added first, followed by the addition of SEB and SEB peptide 130–160. After incubation for 24 hrs the number of cells shed in the medium was counted following staining with trypan blue. Cells on the glass cover slips were fixed with ethanol–acetic acid (3:2 v/v) for 10 mins, washed with PBS, and stained with DAPI reagent. Representative areas were photographed (Panel A). A – Control (no apoptotic cells were observed); B – SEB (2 μg/ml); C – SEB 130–160 (1 μg/ml); D – SEB 93–112; E – SEB 30–160 (1 μg/ml) + SEB Ab; F – SEB 130–160 (2 μg/ml) + SEB130–160 Ab. Five different areas on the microscope field were counted for apoptotic and nonapoptotic cells and percentage of apoptotic cells plotted (Panel B). (1) Control, (2) SEB (1 μg/ml), (3) SEB (2 μg/ml); (4) SEB130–160 (1 μg/ml); (5) SEB130–160 (2 μg/ml); (6) SEB 93–112 (2 μg/ml); (7) SEB Ab (1 μl/ml); (8) SEB (2 μg/ml) + SEB Ab (1 μl/ml); (9) SEB 130–160 (1 μg/ml) + SEB Ab (1 μl/ml); (10) SEB 130–160 Ab (1 μl/ml); (11) SEB (2 μl/ml) + SEB 130–160 Ab (2 μl/ml); (12) SEB 130–160 (2 μl/ml) + SEB 130–160 Ab (1 μl/ml). We noted that SEB and SEB peptide 130–160 dose-dependently induced apoptosis in PT cells. We also noted that antibody against SEB completely reversed SEB and/or SEB peptide 130–160–induced apoptosis, whereas antibody against SEB peptide 130–160 significantly mitigates SEB and SEB peptide130–160–induced apoptosis.

As shown in Figure 7A, incubation of PT cells with SEB and SEB peptide 130–160 stimulated the activity of neutral sphingomyelinase ~1.5-fold and ~1.8-fold, respectively. Metabolic labeling using [^{14}C]palmitic acid followed by quantitation revealed that SEB peptide 130–160 increased the cellular level of ceramide and decreased the cellular level of sphingomyelin compared with control.

Discussion

The present investigation employing cultured human kidney proximal tubular cells revealed that SEB peptide 191–220 binds to the digalactosylceramide receptor in PT cells with high affinity and specificity. Moreover, this SEB peptide imparts a modest effect on PT cell proliferation. SEB 191–220 bound to PT cells more than did SEB peptide 93–112. However, SEB 191–220 exerted a marked increase in PT cell proliferation compared with SEB 93–112. In sharp contrast, SEB 130–160 exhibited less binding but exerted a concentration-dependent and profound inhibition (4- to 6-fold) in cell proliferation. Furthermore, our mechanistic studies revealed that SEB 130–160 induces apoptosis *via* activation of the neutral sphingomyelinase-ceramide pathway. Moreover, SEB- and SEB peptide 130–160–mediated apoptosis was mitigated by preincubation of cells with either SEB antibody or antibody against SEB peptide 130–160. We recognize that these data are in part preliminary, and further controls will need to be employed in ongoing and future experiments (such as scambled sequence peptides and mutant variations of whole toxin).

Previously, a series of 13 synthetic SEB peptides (spanning the entire toxin sequence) has been used to evaluate the peptides' effects on T-cell proliferation in a culture system containing human peripheral blood lymphocytes incubated with a specific ratio of mononuclear cells (21). In this previous study, four peptide regions that inhibit SEB-induced peripheral blood mononuclear cell (PBMC) proliferation were identified. The regions included sequences 1–30, 51–92 (such sequences have been related to the T-cell receptor site), 93–112 (a linear sequence corresponding to the 16 loop), and 130–160 (containing highly conserved sequence, KKKVTAQEL; Fig. 1). Moreover, neutralizing antibodies against the latter domain were capable of abrogating SEB-induced proliferation. The peptide 130–160 also inhibited the binding of ^{125}I-SEB to lymphocytes.

In another study investigating the functionality of the SEB 130–160 region, peptide KKKVTAQELD and anti-peptide sera to this region were seen to impressively block SEB movement across cultured gut monolayers. Further peptide and sera were also shown to block the movement of SEA, SEE and, to a lesser extent, TSST-1 (7).

These studies reveal that the residues containing and surrounding the sequence KKKVTAQEL(D) may not only be critical in SEB-induced T-cell proliferation but also in lesions of nonimmunologic tissue. This region of the molecule may be of therapeutic interest.

Various SEB peptides within and around the 130–160 region were previously shown to abrogate SEB toxicity completely in mouse lymphocytes and partially in human lymphocytes (21). The data presented here show a 6-fold decrease in PT cell proliferation with SEB 130–160. Moreover, amino acid variation surrounding the sequence KKKVTAQEL (Table 1) compromised such an effect. Our studies implicate SEB 130–160 but not SEB peptide 93–112 to induce apoptosis. To understand the biochemical basis of SEB- or SEB peptide 130–160–induced apoptosis, we investigated the effect of these molecules on the neutral sphingomyelinase-ceramide pathway in PT cells.

Neutral sphingomyelinase is a cell membrane–associated phospholipase that cleaves sphingomyelin to ceramide and phosphocholine. *N*-SMase has been implicated in a variety of cell systems to mediate the effects of cytokines, such as TNF-α, IL-1, and interferon-γ. The basic mechanism may involve the binding of these cytokines to receptors. This, in turn, activates the *N*-SMase. *N*-SMase then cleaves the sphingomyelin to ceramide (22). Ceramide, in turn, stimulates programmed cell death (23), presumably by activating nuclear factors, such as nuclear factor κ-B, pBcl, and the integrating-conjugative element (ICE) family (24).

The data presented in this manuscript reveal that SEB and SEB peptide 130–160 rapidly activate *N*-SMase and generate ceramide. In turn, ceramide may well induce apoptosis *via* the signaling cascade above. Interestingly, our data suggest that preincubation of PT cells with antibodies against SEB or SEB peptide 130–160 can mitigate apoptosis induced either by SEB or SEB peptide 130–160.

Glycosphingolipids (GSLs) are integral components of cell membranes and serve as receptors for bacterial toxins (25). GSLs are composed of sphingosine, fatty acids, and sugars (26). Ceramide, composed of sphingosine and a fatty acid, is the backbone of all GSLs, to which monosaccharide units are attached. GSLs are synthesized in the Golgi apparatus *via* sequential addition of monosaccharide units from nucleotide sugars to ceramide *via* specific glycosyltransferases (26). The GSLs then are transported to various subcellular organelles. In mammalian cells, lactosylceramide has been shown to be located within cytoplasmic membranes (20). In PT cells, GSLs have been shown to be localized with the apical and basolateral membranes (27). Whether the topology of a GSL is a determinant of its functional role as a receptor for bacterial or viral proteins is not known.

Kidney PT cells have previously been reported to contain high-affinity saturable binding receptors for ^{125}I-SEB (15). Pretreatment of cells with endoglycoceramidase (which specifically cleaves the oligosaccharide backbone of GSL) or human kidney neutral GSLs completely compromised the binding of ^{125}I-SEB to PT cells (15). Furthermore, direct binding of ^{125}I-SEB to GSL separated on thin layer plates and solid-phase binding assays on microtiter plates has identified digalactosylceramide (diGalCer) as a receptor

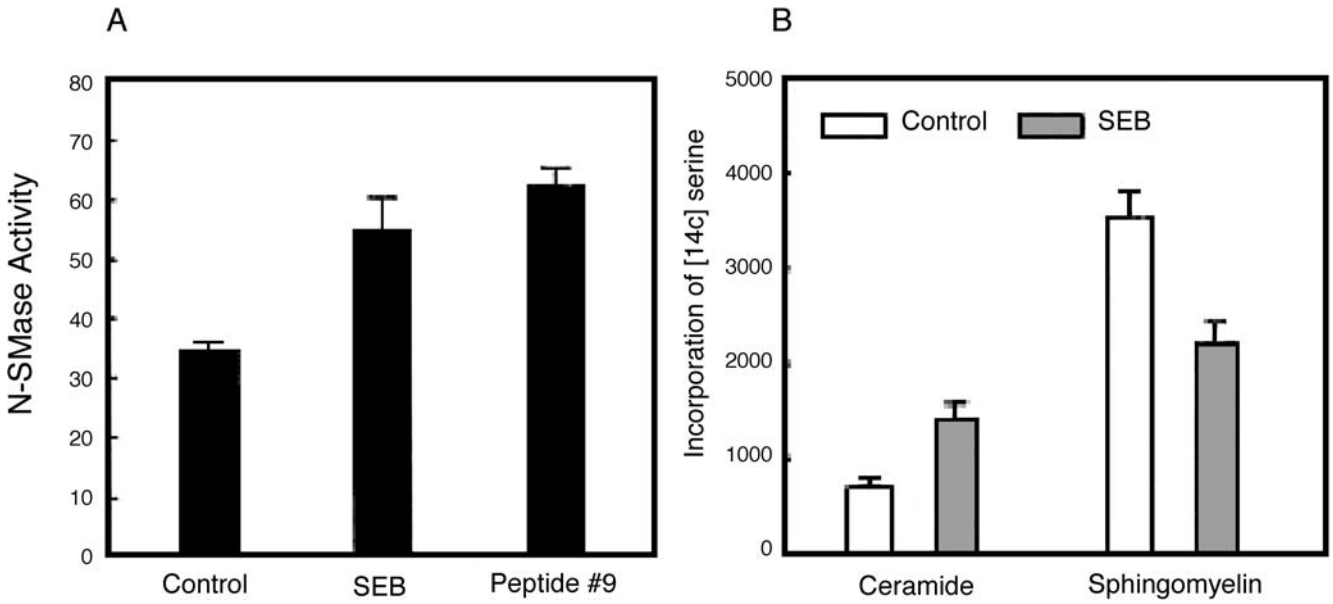


Figure 7. Effect of SEB and SEB peptide on the activity of neutral phingomyelinase (A) and the level of ceramide and sphingomyelin (B). Cells grown in six-well trays were incubated with SEB and SEB peptide 130–160 (1 μg/ml) for 15 mins at 37°C. Cells were harvested, and the activity of *N*-SMase was measured using [^{14}C]sphingomyelin as substrate (A). Cells were also metabolically labeled for 24 hrs with [^{14}C]serine (5 μCi/ml), washed, and then incubated with fresh medium containing SEB (1 μg/ml) for 15 mins at 37°C. Next, medium was removed, and cells were extracted with hexane-isopropanol (3:2 v/v) for 10 mins. The total lipid extract was dried in nitrogen and fractionated by high-performance thin layer chromatography. Gel area corresponding to ceramide and sphingomyelin was scraped, and radioactivity was measured.

for SEB in human kidney and PT cells. This GSL was not found in rat kidney cells; however, feeding these cells diGalCer resulted in saturable binding of ^{125}I-SEB (16). This finding may suggest a possible biochemical basis for the discrepancy seen in lower mammals challenged with SEB.

Most interestingly, SEB 191–220 was bound to PT cells with the highest affinity that was abrogated by preincubation of cells with endoglycoceramidase, antibody against SEB, and exogenous supply of human kidney neutral glycosphingolipids. Solid-phase binding assays suggest that SEB 191–220 may be involved in binding SEB to the diGalCer receptor in PT cells.

Similarly, SEB 93–112 was also bound to PT cells with lesser affinity, and this was strongly displaceable by the use of SEB, digalactosylceramide itself, or removal of the oligosaccharide backbone of glycosphingolipids by the use of endoglycoceramidase. What was intriguing is that unlike SEB peptide 191–220, peptide SEB 93–112 markedly induced PT cell proliferation. Moreover, the biochemical mechanisms by which SEB peptide 93–112 imparts a stimulation of cell proliferation is not known and warrants further studies on this line of investigation.

In sum, we have shown that specific peptide domains within the SEB molecule (SEB 191–220) bind to the digalactosylceramide receptor present in PT cells. SEB 93–112 binding results in the activation of downstream signaling events that ultimately lead to cell proliferation. In contrast, SEB peptide 130–160, which is bound to PT cells, activates the *N*-SMase–ceramide pathway to induce apoptosis and that, in turn, may contribute to a marked decrease in cell proliferation. Most important, the toxic/apoptotic effect of SEB peptide 130–160 was completely reversed by the use of corresponding antibodies.

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