Suppression of Staphylococcal Enterotoxin B-induced Toxicity by a Nuclear Import Inhibitor*

Danya Liu†, Xue Yan Liu‡, Daniel Robinson‡, Christie Burnett†, Charity Jackson‡, Louis SeelenRuth Ann Veach‡, Sheila Downs§, Robert D. Collins¶, Dean W. Ballard†, and Jacek Hawiger†

From the Departments of †Microbiology and Immunology and ‡Pathology, Vanderbilt University School of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee 37232

Staphylococcal enterotoxin B and related toxins that target T cells have the capacity to elicit systemic inflammation, tissue injury, and death. Genes that encode mediators of inflammation can be globally inhibited by blocking the nuclear import of stress-responsive transcription factors. Here we show that cell-permeant peptides targeting Rch1/importin α/karyopherin α 2, a nuclear import adaptor protein, are delivered to T cells where they inhibit the staphylococcal enterotoxin B-induced production of inflammatory cytokines ex vivo in cultured primary spleen cells and in vivo. The systemic production of tumor necrosis factor α, interferon γ, and interleukin-6 was attenuated in mice either by a cell-permeant cyclized form of SN50 peptide or by a transgene whose product suppresses the nuclear import of transcription factor nuclear factor of activated T cells in T cells. The extent of liver apoptosis and hemorrhagic necrosis was also reduced, which correlated with significantly decreased mortality rates. These findings highlight nuclear import inhibitors as a potentially useful countermeasure for staphylococcal enterotoxin B and other toxins that trigger harmful systemic inflammatory responses.

Staphylococcal enterotoxin B (SEB)1 causes a spectrum of human diseases, including food poisoning and non-menstrual toxic shock syndrome (NMTSS) (1, 2). SEB is one of the major virulence factors regulated by a quorum-sensing mechanism in the setting of staphylococcal infections caused by antibiotic-resistant strains. These high-risk community-acquired infections, which may lead to NMTSS, occur with increasing frequency as compared with the greater than 2 million hospital-acquired infections recorded annually in the United States (3, 4). Strikingly, SEB induces a fatal respiratory distress syndrome in non-human primates, suggesting its potential use as a bioweapon on the battlefield or in mass civilian settings (5, 6). Potential air-borne, water-borne, and food-borne use of SEB led to its designation by the United States Centers for Disease Control as a category B agent.

In terms of its mechanism of action, SEB is avidly bound by the T cell receptor Vβ chain and by major histocompatibility complex class II proteins on dendritic cells or macrophages (7–9). The resulting intercellular “synapse” generated by SEB engagement leads to excessive production of the inflammatory cytokines tumor necrosis factor α (TNFα), interferon γ (IFNγ), interleukin (IL)-1β, IL-2, and IL-6. T cell-produced inflammatory cytokines contribute to massive vascular injury, organ failure, and depending on the mode of exposure potentially lethal respiratory distress syndrome or toxic shock (1, 2, 5, 6). Active immunization prior to SEB exposure and passive immunization immediately after exposure are not readily available (6). We have designed an alternative approach to antibody-mediated neutralization of SEB and related toxins by targeting a common step in their intracellular signaling to the nucleus required for inflammatory cytokine gene expression.

The genes that encode inflammatory cytokines are under the control of stress-responsive transcription factors (SRTFs), including nuclear factor κB (NFκB), activator protein 1, nuclear factor of activated T cells, and signal transducer and activator of transcription 1 (STAT1) (10). For example, SRTFs are translocated to the nucleus in CD4+ T cells in response to staphylococcal enterotoxin A, which is structurally and functionally related to SEB (11). Following their mobilization to the T cell nuclear compartment, SRTFs act in concert to stimulate transcription of multiple genes encoding cytokines, chemokines, and other mediators of inflammation (12–14). We reasoned that simultaneous blockade of these four SRTFs at the level of cytoplasmic/nuclear shuttling may yield in vivo protection from T cell-mediated toxicosis induced by SEB.

To test this hypothesis, we applied cell-permeant peptides initially engineered by us to inhibit nuclear import of SRTFs in monocytes and macrophages (15, 16). This process is stimulated by lipopolysaccharide (LPS) through Toll-like receptor 4-generated signaling. Because LPS does not stimulate T cells, we thus aimed to inhibit nuclear import evoked by a distinct recognition- and signaling-based mechanism initiated by SEB interaction with T cells. The in vivo SEB toxicity model analyzed in this study is characterized by cytokine-dependent fulminant liver injury (17–19) not observed previously with LPS-induced lethal shock (16). The novel inhibitors of nuclear import employed by us in the SEB toxicity model contain a linear or cyclized form of the nuclear localization signal (NLS).
from the p50/NFkB1 subunit of NFkB (16). NLS was fused to the signal sequence hydrophobic segment from fibroblast growth factor 4. This hydrophobic segment serves as a membrane-translocating motif (MTM), which enables peptide or protein cargo to cross freely the plasma membrane of multiple cell types in various organs (16, 20–24) through a receptor/transporter-independent mechanism (25). Of equal importance, these cell-permeant peptides carrying NLS have been shown to simultaneously block the nuclear import of multiple SRTFs in the cultured Jurkat T cell line, a process that is mediated by the shutting molecule Rch1/importin α/karyopherin-α2 (26). Thus, we envisaged that global in vivo inhibition of SRTF-regulated genes encoding multiple mediators of inflammation in T lymphocytes, which are essential for SEB-induced toxicity (27, 28), would provide a new and useful platform to counteract noxious intracellular signaling evoked by SEB and related toxins.

**Experimental Procedures**

**Peptide Synthesis, Purification, and Labeling—**MTM-containing peptides (SN50, cSN50, and SM), and MTM-deficient peptide (N50c) were synthesized, purified, filter-sterilized, and analyzed as described elsewhere (16, 26). To monitor the delivery of peptides to T cells, the SN50 and SM peptides were coupled with fluorescein isothiocyanate (FITC) (Pierce) according to the manufacturer's protocol. After extensive dialysis against water to remove free FITC, labeled peptides were concentrated in a speedvac and used immediately. The N50c peptide was coupled with fluorescein-5-maleimide (FM) via an NH2-terminal cysteine.

**Delivery and Intracellular Detection of Cell-permeant Peptides ex Vivo and in Vivo—**For ex vivo detection of fluorescein-labeled peptides in primary T cells, spleens were harvested from wild type C57BL/6 mice and T cells were isolated by negative selection using magnetic beads coated with anti-major histocompatibility complex class II (1a) monoclonal antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Purified CD3+ T cells (>89% CD3+ as determined by fluorescence-activated cell sorter (FACS)) (BD Pharmingen) were resuspended in RPMI 1640 without supplements and incubated with 5 μM FITC-labeled SN50 or SM peptide or unconjugated FITC for 30 min at 25 °C in duplicate. Fluorescein-labeled N50c, which lacks an MTM, was used as a control. One of each was then treated with 5 μg/ml proteinase K (BD Biosciences, Clontech, Palo Alto, CA) for 10 min at 37 °C. To document susceptibility of peptides to proteolytic degradation, they were incubated with 5 μg/ml proteinase K for 10 min at 37 °C prior to the addition of T cells. After all treatments, cells were washed twice with phosphate-buffered saline. Cell fluorescence was measured in FACScalibur using forward versus side light scatter; green fluorescence was collected with a 530 ± 30-nm band pass filter. This protease accessibility test assures measurement of that pool of fluorescein-labeled peptide that was translocated across the plasma membrane to reach an intracellular compartment (cytoplasm), making it inaccessible to proteinase K action. For ex vivo detection of fluorescein-labeled peptides in T cells, blood and spleens were harvested (16) from wild type BALB/c mice 30 min after intraperitoneal (ip) injection of 500 μl of labeled peptide or FITC solutions with equivalent fluorescence units. T cells were isolated and analyzed by FACS as in ex vivo experiments.

**Cytokine Production by Cultured Spleen Cells—**Murine lymphocytes were isolated from the spleens of wild type C57BL/6 mice and transgenic C57BL/6 mice that express IxB.DN (inhibitor of NFkB nuclear translocation) as previously described (38) and cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum containing no detectable LPS (<0.006 ng/ml as determined by the manufacturer, Atlanta Biologicals, Norcross, GA), 2 mM l-glutamine, 55 μg 2-mercaptoethanol, streptomytin (100 μg/ml), and penicillin (100 units/ml). Splenocytes (2 × 10^7/ml) were treated with peptides SN50 and SM at 10, 20, and 30 μM for 30 min at 25 °C followed by the addition of 0.5 μM/ml of SEB (Toxin Technology, Sarasota, FL). Splenocytes were then distributed in 200-μl aliquots (6 × 10^5 cells/well) to a 96-well plate and cultured for 24 h at 37 °C in 5% CO2. Plates were spun at 800 × g for 2 min. The supernatant was removed and frozen at −80 °C for later assay.

**Animal Treatment Protocols—**Wild type C57BL/6 and BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All mice were female (8–12 weeks old) with an average weight of 20 grams. Wild type BALB/c mice were injected ip with 5 μg of SEB (25 μg/ml; Sigma) and 20 μg of α-galactosamin (α-Gal) (100 μg/ml; Sigma), both in pyrogen-free saline. SEB contained less than 1 endotoxin unit of LPS/mg of SEB as determined by the Limulus chromogenic assay (Associates of Cape Cod, Falmouth, MA). Peptides cSN50 (0.7 mg) and SN50 (0.7 mg) or 5% dimethyl sulfoxide in sterile H2O as diluent were injected in 200 μl volumes ip into mice before (30 min) and after (30, 90, 150, 210 min, and 6 and 12 h) SEB and α-Gal challenge. In some experiments, cSN50 peptide and diluent were injected only after the SEB and α-Gal challenge, with the first ip injection of cSN50 peptide 30 min after SEB and α-Gal followed by 5 ip injections at 90, 150, 210 min, and 6 and 12 h.

**Transgenic C57BL/6 mice expressing IxB.DN in the T cell line were engineered and bred as previously described (29). Wild type and transgenic C57BL/6 mice were injected ip with 150 μg of SEB (0.75 mg/ml; Toxin Technology) and 20 mg of α-Gal. Highly purified SEB contained less than 1 endotoxin unit of LPS/mg of SEB after its additional purification by the manufacturer. Animals were observed at hourly intervals for signs of toxic shock (piloerection, ataxia, and the lack of reaction to cage motion). Inactive animals were euthanized. Surviving animals were euthanized at 72 h, except for three survivors that were observed for 10 days before euthanasia. Animal handling and experimental procedures were performed in accordance with the American Association of Accreditation of Laboratory Animal Care guidelines and approved by the Institutional Animal Care and Use Committee.

**Cytokine Assays of Blood and Cultured Cell Supernatants—**Blood samples (40 μl) taken from the saphenous vein were collected in heparinized tubes before (30 min) and after SEB challenge at intervals shown in Figs. 2–4. Plasma levels of IL-6 were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. IL-2, -4, and -5, IFN-γ, and TNF-α proteins were utilized. Cytokine capture beads were mixed with the phycoerythrin-conjugated detection materials.
cytokine production by cell-permeant peptide inhibitor of nuclear import or by transgene inhibitor of NFκB nuclear translocation. T cells from the spleens of C57BL/6 mice were incubated at 25°C for 30 min with 5 μM FITC-SN50 peptide (A), 5 μM FITC-SM peptide (B), or 5 μM fluorescein-5-maleimide-N50c peptide (C) without proteinase K treatment (black) or subsequently treated with 5 μg/ml proteinase K for 10 min at 37°C (green). Alternatively, fluorescein-labeled peptides were pretreated with 5 μg/ml proteinase K for 10 min at 37°C before incubation with cells at 25°C for 30 min (pink). Control cells not exposed to any peptide or incubated with unconjugated FITC showed levels of fluorescence similar to cells incubated with fluorescein-labeled peptides pretreated with proteinase K (data not shown). D, spleen cells from wild type C57BL/6 mice were treated with diluent (culture medium, open bars) or SN50 peptide (30 μM, solid bars) or SM peptide (30 μM, gray bars) for 30 min and then exposed to SEB. E, spleen cells from wild type C57BL/6 mice (open bars) or from transgenic C57BL/6 mice bearing T cell-specific IkB-ΔN inhibitor of nuclear translocation of NFκB (solid bars) exposed to SEB. Cytokines were measured in culture supernatants 24 h following stimulation with 0.5 μg/ml SEB. Error bars indicate the ± S.E. from three-five independent experiments. Student’s t test was used to determine p values.

antibodies and then incubated with recombinant standards or test samples to sandwich complexes (30). Following the acquisition of flow cytometric data, FACScalibur results were organized in graphical and tabular format using CBA analysis software.

**Histology Analyses—** Tissue samples (liver, spleen, kidney, lung, and heart) were collected from mice showing typical signs of toxicity shortly after death or in surviving mice that were euthanized after 72 h or 10 days of observation. Formalin-fixed, paraffin-embedded sections were stained with hematoxylin and eosin. Apoptosis among liver cells was evaluated by histology and by TUNEL (TdT-dependent dUTP-biotin nick end labeling) assay using the Apop Tag reagent (Chemicon) according to the manufacturer’s instructions.

**Statistical Analysis—** All in vivo experimental data were expressed as mean ± S.E. A two-way repeated measure analysis of variance and a log rank test were used to determine the significance of the difference in cytokine production and survival, respectively. Student’s t test was used to determine the significance of the difference in cytokine production in cultured splenocytes.

**RESULTS**

**Intracellular Delivery of Nuclear Import Inhibitors to T Cells—** Two cell-permeant peptides, SN50 and SM, were studied for their ability to enter primary T cells *ex vivo* and *in vivo*. The cell-permeant SN50 peptide ferries NLS as its cargo, whereas the cargo of the SM peptide was a mutated version of the same NLS (Table I). The NLS in the SN50 peptide is known to interact with the nuclear import adaptor protein Rch1/importin α/karyopherin α 2 (26). Conversely, the mutated sequence in the SM peptide is inactive in terms of inhibition of SRTFs nuclear import and served as a control. A third peptide, called N50c, is a truncated form of SN50 that lacks the MTM and was used as a plasma membrane translocation-negative control (Table I) (26).

The SN50, SM, and N50c peptides, labeled with fluorescein, were added to *ex vivo* cultured murine spleen-derived primary T cells. Although the SN50 and SM peptides were detected in primary T cells, the N50c peptide lacking a membrane-translocating motif was not, thus indicating the MTM dependence of *ex vivo* delivery of NLS peptide cargo to these cells (Fig. 1, A–C). To verify further the intracellular delivery of cell-permeant SN50 and SM peptides, we employed a protease-accessibility test that is based on the incubation of T cells with cell-permeant peptides, before and after treatment with a broad-range protease (proteinase K), followed by FACS. Treatment of fluorescein-conjugated peptides with proteinase K prior to their addition to T cells, degraded all peptides tested and prevented SN50 and SM delivery into T cells as compared with proteinase K-unreated peptides and cells (Fig. 1, A and B). On the other hand, treatment with protease following 30 min of incubation of T cells with FITC-labeled peptides did not ablate T cell-associated fluorescence. The observed reduction in T cell-associated fluorescence, as compared with the protease-unreated cells, was due to proteolytic removal of an extracellular pool of fluorescein-labeled peptides absorbed on the surface of T cells. Thus, the protease-accessibility test indicates that both cell-permeant peptides were similarly delivered to T cells, the principal targets of SEB (27, 28).

**Nuclear Import Inhibitors Suppress Inflammatory Cytokine Gene Expression in Cultured Primary T Cells—** To validate the T cell delivery of cell-permeant peptides by demonstrating their intracellular function, we evaluated the ability of SN50 and SM peptides to interfere with SEB-induced production of inflammatory cytokines in *ex vivo* cultured splenocytes. For these studies, splenocytes were isolated from wild type C57BL/6 mice and then treated with an SN50 or SM peptide prior to exposure...
to the T cell agonist SEB. The SN50 peptide at a concentration of 30 μM significantly inhibited the expression of inflammatory cytokines TNFα (p < 0.02), IFNγ (p < 0.05), and IL-2 (p < 0.05) in SEB-stimulated splenocytes derived from wild type animals (Fig. 1D). For SN50, the effective concentration leading to 50% inhibition (EC50) of cytokine production was 20 ± 4 μM (not shown). In contrast, cytokine expression was unaffected following treatment with the SM peptide (30 μM) containing the MTM fused to a mutated version of the NLS (26) (Fig. 1D). Thus, SN50 interferes significantly ex vivo in primary cells with SEB-induced inflammatory cytokine production by blocking the Rch1-dependent mechanism responsible for the nuclear import of NFκB and other SRTFs.

This SN50 peptide-directed ex vivo inhibition of inflammatory cytokine production was compared with the effect of the transgene that encodes an inhibitor of NFκB nuclear translo-
cation in T cells (29). IxB.DN is a truncated form of the cytoplasmic protein IxB with an NH2-terminal deletion that prevents its phosphorylation and degradation, thereby retaining NFkB in the cytoplasm of stimulated T cells. Splenocytes derived from transgenic C57BL/6 mice expressing the inhibitor IxB.DN produced significantly lower levels of inflammatory cytokines TNF-α (p < 0.02), IFN-γ (p < 0.05), and IL-2 (p < 0.02) in response to SEB as compared with splenocytes from wild type C57BL/6 mice (Fig. 1E). These results indicate that SN50 peptide produces a similar suppression of inflammatory cytokine expression as transgene-directed inhibition of NFkB nuclear translocation in SEB-stimulated T cells.

In Vivo Suppression of Inflammatory Cytokine Production by Nuclear Import Inhibitor—To track in vivo targeting of T cells by a peptide inhibitor of nuclear import, fluorescein-labeled peptides SN50, SM, and N50c were injected intraperitoneally into separate groups of BALB/c mice. T cells were isolated from the blood and the spleen 30 min after injection. T cells from mice injected with SN50 and SM stained positive for the presence of fluorescein-labeled peptides as compared with controls (Fig. 2, A and B). In contrast, injection with fluorescein-labeled N50c, which lacks an MTM, failed to produce any gain in fluorescence as compared with controls. The control mice received phosphate-buffered saline (not shown) or unconjugated FITC. These results establish the MTM dependence of a rapid in vivo delivery of nuclear import inhibitory peptides to T cells.

We next explored the in vivo toxicity of SEB. Although mice display heightened resistance to SEB toxicity as compared with humans (31), BALB/c mice expressing both I-A and I-E major histocompatibility complex class II isotypes of the H-2d haplotype are 50 times more susceptible to SEB than the C57BL/6 strain (32). To sensitize BALB/c mice to the deleterious effects of SEB-induced cytokines such as TNF-α, we used d-Gal. In this murine model, simultaneous administration of SEB and d-Gal via an ip route evokes acute liver injury followed by rapid death (17–19). Importantly, animals deficient for TNF-α and IFN-γ receptors are refractory to the lethal effects of SEB and d-Gal, a finding that further validates the physiologic relevance of this particular animal model (17–19). A related experimental model of SEB-induced toxicity employs LPS in lieu of d-Gal with a similar pattern of systemic inflammatory cytokine response (33, 34).

Injection of SEB and d-Gal into BALB/c mice caused a rapid rise in plasma TNF-α levels that peaked at 90 min (Fig. 2C), followed by a more progressive increase in IFN-γ and IL-6 (Fig. 2, D and E). In contrast, injection of d-Gal alone was without effect, thus confirming the SEB-dependent nature of this inflammatory cytokine response (data not shown). Administration of cSN50 (the cyclized form of SN50), before and after SEB exposure, suppressed the induction of inflammatory cytokines (Fig. 2, C–E). Plasma levels of inflammatory cytokines significantly differed for TNF-α (p < 0.0001), IFN-γ (p < 0.0001), and
IL-6 \( (p < 0.0001) \) using a two-way repeated measure analysis of variance. Administration of cell-permeant mutant SM peptide did not significantly suppress inflammatory cytokine production \textit{in vivo}, consistent with its lack of an inhibitory effect in cultured spleen cells (Fig. 1D).

Protection from SEB-induced Tissue Injury and Death by Nuclear Import Inhibitor—We also explored the effects of cSN50 on survival and tissue injury in mice treated with SEB and \( \beta \)-Gal. In control BALB/c mice that received ip injections of diluent before and after SEB and \( \beta \)-Gal, we observed a characteristic progression of morbid signs resulting in the death of 14 of 15 mice within 40 h (Fig. 2F). No systemic toxicity was detected upon the administration of SEB or \( \beta \)-Gal alone (data not shown). At death, all mice exhibited severe liver injury characterized by extensive apoptosis and hemorrhagic necrosis (Fig. 5, A and B). In contrast, the administration of cSN50 before exposure to SEB, and thereafter in six doses over 12 h, produced a pronounced protective effect. Fourteen of 15 mice recovered fully from SEB challenge and survived at least 72 h.

Thus, the cell-permeant cSN50 peptide reduced SEB-induced lethality by 87%. Based on the log rank test, the difference in the survival rate between cSN50 peptide-treated and control mice was statistically significant (\( p < 0.0001 \)), whereas the SM peptide, containing a mutated p50 NLS, had no \textit{in vivo} protecting activity (\( p > 0.2 \)) (Fig. 2F). Histologic examination of cSN50-treated mice surviving 72 h showed normal tissue architecture with no apoptotic and/or hemorrhagic liver injury in contrast to untreated controls (Fig. 5, C and D). Thus, the cytoprotective effect of the cSN50 peptide correlated with the survival of mice challenged with SEB and \( \beta \)-Gal.

Delayed Treatment with a Nuclear Import Inhibitor Is Effective in Suppressing Inflammatory Cytokine Production and Preventing Death—The protective effect was maintained when treatment with the cSN50 peptide was delayed for 1 h as compared with the protocol employed above. The mice received the first dose of cSN50 30 min after SEB and \( \beta \)-Gal. Despite omitting the first dose of cSN50, given previously 30 min before SEB and \( \beta \)-gal challenge, we observed significant suppression of inflammatory cytokines TNF-\( \alpha \) (\( p < 0.0001 \)), IFN-\( \gamma \) (\( p < 0.0001 \)), and IL-6 (\( p < 0.001 \)) using a two-way repeated measure analysis of variance concomitant with 60% survival (\( p < 0.02 \)) (Fig. 3, A–D). These findings indicate that time-delayed and dose-reduced administration of the cSN50 peptide during the early rise in TNF-\( \alpha \) production still attenuates SEB-induced lethal shock. Because of the fulminant nature of tissue injury in this experimental model (50% of the untreated animals died within 10 h), further delay in treatment results in less protection from death (not shown).

Transgenic Inhibitor of NF-kB Nuclear Import in T Cells Recapitulates the Cell-permeant Effects of Peptides—To explore the relative contribution of NF-kB to the set of SRTFs targeted by a cell-permeant peptide inhibitor of nuclear import, we next...
introduced SEB and d-Gal into C57BL/6 mice expressing an IxB.DN transgene under the control of a T-cell-specific promoter (29). Inflammatory cytokine responses were significantly attenuated in transgenic mice as compared with wild type controls (29). Inflammatory cytokine responses were significantly attenuated in transgenic mice as compared with wild type controls (29). Inflammatory cytokine responses were significantly attenuated in transgenic mice as compared with wild type controls (29).

Histologic analysis of the liver sections obtained from non-surviving wild type C57BL/6 mice challenged with SEB and D-Gal received 5% dimethyl sulfoxide as a diluent (A, C, E, G) or with Apop Tag (B, D, F, H). Control BALB/c mice challenged with SEB and d-Gal received 5% dimethyl sulfoxide in H₂O as a diluent (A, B); treated mice received the cSN50 peptide (C, D).

Wild type C57BL/6 mice (E, F) and IxB.DN transgenic mice (G, H) were challenged with SEB and d-Gal. Note the hallmarks of acute liver injury (apoptosis, hepatocyte necrosis, and erythrocyte extravasation) in control BALB/c and C57BL/6 wild type mice (A, B, E, F) and preserved liver architecture without apoptosis in cSN50 peptide-treated BALB/c mice (C, D) and IxB.DN-transgenic C57BL/6 mice (G, H). Histologic examination of survivors observed for 10 d showed no lesions (not shown). Mice receiving either d-Gal alone (n = 5) or SEB alone (n = 5) survived and after 10 days of observation showed no evidence of tissue injury (not shown).

Three separate lines of evidence establish that nuclear import inhibitors SN50 and cSN50 were delivered to T cells ex vivo and in vivo where they substantially inhibited SEB-induced toxicity: (i) SN50 and cSN50 significantly reduced ex vivo production of inflammatory cytokines in cultured primary splenocytes and in vivo in blood, (ii) apoptotic and hemorrhagic injury in mouse liver was suppressed in vivo by cSN50, and (iii) the number of mice dying after SEB challenge was significantly reduced. The strikingly cytoprotective effect of the cSN50 peptide in the mouse liver indicates its capacity to counteract the expression and florid action of two inflammatory cytokines, TNFα and IFNγ, that are essential for SEB-induced hepatic toxicity in this experimental model (18, 19). Other organs with evidence of apoptosis (33, 34) are likely to be protected because cSN50 delivered intraperitoneally is able to reach T lymphocytes in the blood and the spleen within 30 min (Fig. 2). The protective in vivo effect of cSN50, administered during the first 12 h of SEB-induced systemic inflammation, lasts at least 72 h and does not seem to cause undesirable side effects. Further studies will be required to determine the pharmacokinetics, long-term toxicity, and therapeutic efficacy of this new class of peptide inhibitors.

Our comparative analysis of cell-permeant peptides versus a T cell-specific transgene that inhibits NFκB indicates a key role for this inducible transcription factor in the progression of SEB-induced disease (27, 28). The role of other SRTFs (activator protein 1, nuclear factor of activated T cells, and signal transducer and activator of transcription 1), whose nuclear translocation is blocked by the SN50 peptide, should be taken into account as well (26). Given their coordinated involvement in regulation of genes encoding the key inflammatory mediators of systemic inflammation, broad inhibition of SRTFs nuclear import becomes preferable as a treatment strategy over ablation of a single SRTF signaling pathway to the nucleus.

Taken together, our experiments highlight the in vivo efficacy of cell-permeant peptides as nuclear import inhibitors of SRTFs involved in SEB-induced and T cell-mediated toxicosis. Given their rapid but transient inhibitory activity (16, 20, 21), cell-permeant nuclear import inhibitors may provide a better therapeutic platform than previously reported gene transfer approaches (35). Nuclear import inhibitors may also have the capacity to suppress other inflammation-based systemic diseases induced by a much wider spectrum of potential biological warfare agents (www.niaid.nih.gov/dmid/biodefense/bankpriority.htm), including tularemia, smallpox, and Ebola virus (36–38).

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Danya Liu, Xue Yan Liu, Daniel Robinson, Christie Burnett, Charity Jackson, Louis Seele, Ruth Ann Veach, Sheila Downs, Robert D. Collins, Dean W. Ballard and Jacek Hawiger

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