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Comparison of Immunoreactivity of Staphylococcal Enterotoxin B Mutants for Use as Toxin Surrogates

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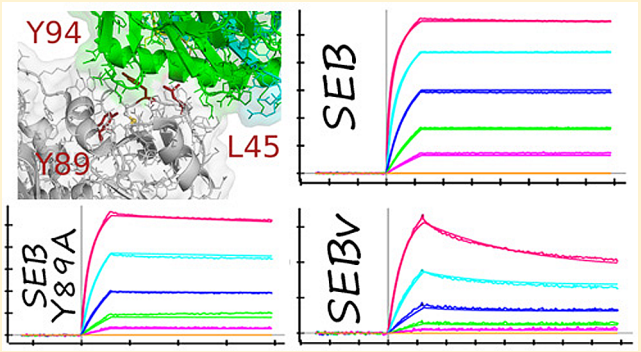
Supporting Information

ABSTRACT: The development and testing of detection methodologies for biothreat agents are by their very nature complicated by the necessity to handle hazardous materials. Toxoids prepared by thermal or chemical inactivation are often used in place of the native toxin; however, the process of detoxification can decrease the agent's ability to be detected at similar concentrations. One method to overcome this limitation is the use of toxin mutants which have altered amino acid sequences sufficient to abrogate or greatly reduce their toxic activity. While this method of toxoid preparation is much more controlled, there is still no guarantee that the resulting product will be equal in detectability to the native toxin. In this work, we have evaluated the utility of two recombinantly expressed Staphylococcal Enterotoxin B (SEB) mutants, a single point mutant (Y89A), and a mutant with three amino acids changed (L45R, Y89A, Y94A), to act as surrogates for SEB in immunoassays. We evaluated the affinity of a number of anti-SEB monoclonal antibodies (mAb) and an anti-SEB single domain antibody (sdAb) for SEB and its surrogates. One of the mAb's affinity was decreased by a factor of 3000 for the triple mutant, and another mAb's affinity for the triple mutant was decreased by 11-fold while the others bound the mutants nearly as well as they did the native toxin. MAGPIX sandwich immunoassays were used to evaluate the ability of all combinations of the recognition reagents to detect the SEB mutants in comparison to SEB and a chemically inactivated SEB. These results show that recombinant mutants of SEB can serve as much more useful surrogates for this hazardous material relative to the chemically inactivated toxin; however, even the point mutant impacted limits of detection, illustrating the need to evaluate the utility of toxin mutants on a case-by-case basis depending on the immunoreagents being employed.



The *Staphylococcus aureus* bacterium produces a number of potent toxins, of which Staphylococcal Enterotoxin B (SEB) is the most common cause of food borne poisoning. In addition to SEB's role in food poisoning, the toxin is considered a potential biological threat agent and is listed as a category B select agent by the Centers for Disease Control. SEB is toxic by both inhalation and ingestion and has the potential for significant morbidity after exposure, with 80% or more of the victims developing symptoms.[1] Symptoms depend on the route of exposure and include nonspecific flu-like symptoms such as fever and headache, gastrointestinal symptoms such as vomiting and diarrhea, and respiratory signs including chest pain and cough. SEB is a single-chain polypeptide of molecular mass 28.4 kDa[2,3] and is a superantigen, activating 5−30% of the T-cell population, resulting in cytokine release and inflammation.[2,4,5] Overstimulation of the immune system in response to superantigens can potentially lead to organ failure or toxic shock.

The development of methods to rapidly and reliably detect biothreat agents has gone from being an effort primarily located in Defense laboratories to a goal that includes numerous academic and commercial entities. While the additional efforts have enhanced our ability to meet our nation's biodefense requirements, the desire to test and validate sensor systems using the threat agents of concern can be problematic. Active toxins, bacteria, or virus can pose a risk to laboratory personnel and increases the concern that the proliferation of research efforts equates to a proliferation in the number of potential sources of the biothreats.[6] Additionally, once a detection system is developed and fielded, it is still necessary to provide a means to test and train on that system. The most obvious means to abrogate both of these concerns is to use inactivated material in lieu of the threat agent; in the case of toxins, toxoid material is used. Traditionally, toxoids have been prepared by either heat or chemical inactivation. While these methods can reliably lower the toxicity of the agent, they can also result in a material that is no longer recognizable by the immunoreagents being utilized in the detection instrumentation.[7] Moreover, many toxins are highly stable making them difficult to completely

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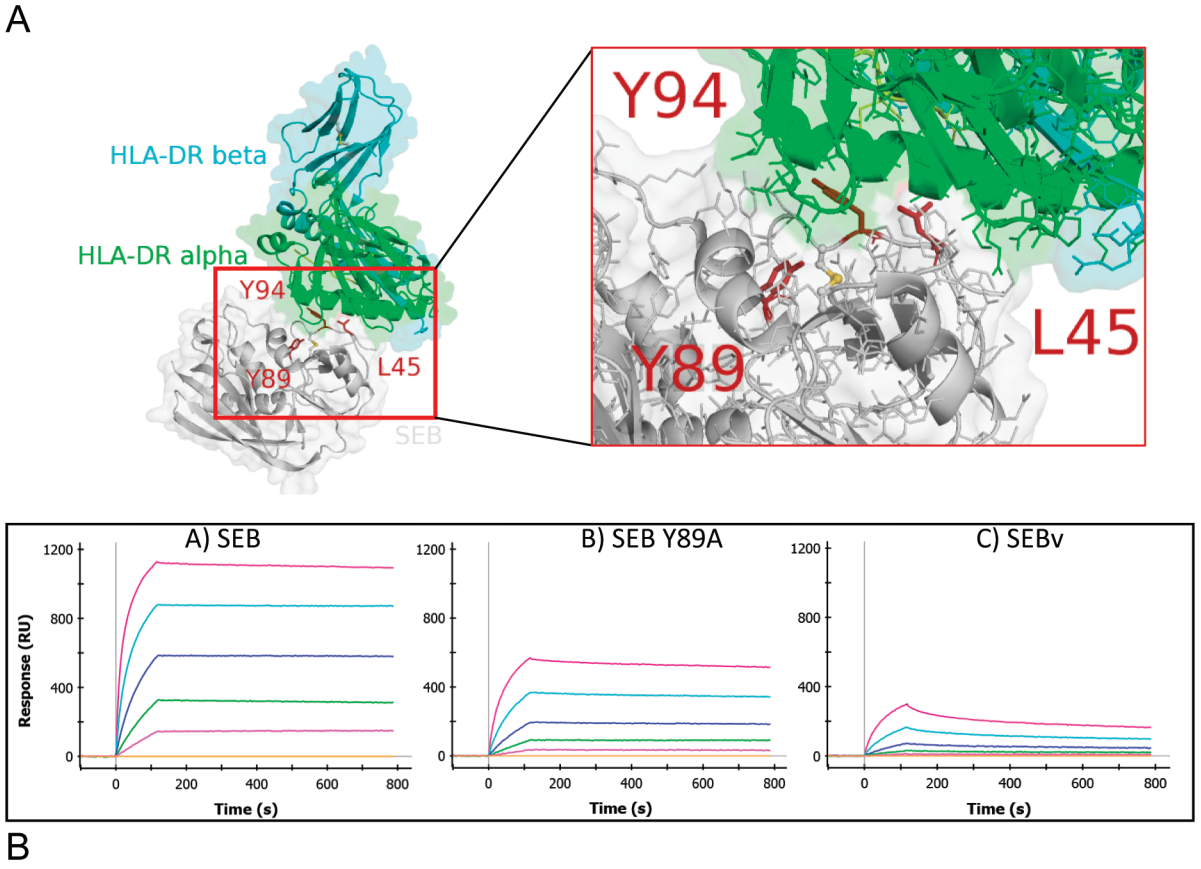

Figure 1. (A) Structure of SEB bound to HLA-DR4 (PDB 1D5M). The HLA-DR4 alpha subunit is shown in green, and the beta subunit is shown in cyan. SEB is shown in gray, and the three residues which were mutated to form SEBv are colored in red. The disulfide bond in the loop is shown in stick and sphere. All three residues are in the interfacial region between HLA-DR4 alpha and SEB. The interfacial region is enlarged on the right. (B) SPR binding traces of mAb 2F2 to SEB, SEB Y89A, and SEBv. Dilutions of the mAb 2F2 (indicated by the colored traces as follows: red 100 nM, light blue 33 nM, dark blue 11 nM, green 3.7 nM, pink 1.24 nM, and yellow 0 nM) were flowed over spots on which SEB, SEB Y89A, and SEBv had been immobilized, as described in the Materials and Methods.

denature, one of the properties that made them a viable threat agent to begin with; thus, a small but finite risk to the users may remain. Ideally, it would be preferable to utilize a surrogate material that behaves identically in most tests but is totally nontoxic; at the same time, one would still desire to be able to distinguish between the toxin and surrogate.

The ability to recombinantly produce both mutants and individual components of threat agents has permitted a very detailed understanding of structure/function relationships. In addition to enhancing our understanding of function and activity, these recombinant proteins provide a source of agent surrogates which are safe to handle yet may still be recognized by immunoreagents in a manner similar to the live threat. In the case of bacteria and virus, this can be an immune-dominant viral coat protein or bacterial cell wall/membrane protein. In the case of dichain protein toxins, such as ricin and botulinum toxin, an effective surrogate can be a recombinantly produced catalytic subunit lacking its receptor binding domain, which is nontoxic on its own. For toxins, like SEB, which consist of a single polypeptide, one cannot simply produce a nontoxic truncation variant to act as the toxoid. Thus, to recombinantly prepare toxoids for SEB and similar toxins, one is limited to amino acid mutants that possess reduced binding to HLA-DR, which are class II major histocompatibility complex (MHC) molecules that are expressed by antigen presenting cells (APC).[5]

Mutant forms of SEB have been developed and tested primarily for use as a vaccine.[8−10] The superantigens staphlococcal entertoxin A and B and toxic-shock syndrome toxin 1 (TSST-1) share a common fold and binding strategy (to HLA-DR). A hydrophobic binding loop, ^{43}QFL^{45}, conserved in all superantigens (with the exception of Streptococcal pyrogenic exotoxin C) is important for HLA-DR binding (Figure 1A). Mutation of L45 to arginine (L45R) in this loop significantly reduces HLA-DR binding. A second binding motif in superantigens (except TSST-1) is a polar pocket formed by Y89, Y115, and E67 which binds the HLA-DR α-subunit. Residues unique to the superantigen, such as Y89, which is within a disulfide-bonded loop, determine binding specificity for superantigen/HLA-DR complexes. For the development of a vaccine immunogen, a triple mutant of SEB, SEBv (L45R, Y89A, Y94A), was utilized primarily for added safety, as the likelihood that reversion to a toxic form is greatly reduced by formation of multiple mutations each of which was sufficient to abrogate toxicity by itself.

While the use of SEB mutants as a protective vaccine has been well studied, their use as surrogates for the toxin in immunoassays has not been previously reported. In this work, we tested four conventional monoclonal antibodies (mAbs) and a single domain antibody (sdAb) for their binding affinity for SEB and two mutants, SEB Y89A and SEBv. In addition, they were tested along with a polyclonal antibody for their

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limits of detection (LOD) as both capture and recognition reagents in bead-based multiplexed fluoroimmunoassays. The sdAb tested, A3, was recently evaluated for its binding to SEB.[11] SdAb are the recombinantly expressed heavy domains derived from camelid heavy chain only antibodies. Since their recognition site is composed of only three CDRs, their binding foot print is most likely smaller than most conventional IgGs, which are composed of six CDRs. Most sdAb, unlike conventional antibodies or their derived binding fragments, are able to refold and function after denaturation.[12−15] Our focus was on the use of monoclonal reagents, as they possess a high degree of specificity and are preferred especially for use in multiplexed analysis formats. We reasoned that monoclonal reagents would be more likely to have a measurably altered affinity toward the mutants, as the binding of polyclonal antibodies is an ensemble response, which is not easily interpretable. Thus, focusing on monoclonals permits us to obtain a clearer picture on the utility of SEB mutants to function as replacements for the SEB toxin during the testing and evaluation of biothreat detection systems.

MATERIALS AND METHODS

Reagents and Buffers. Staphylococcal Enterotoxin B (SEB) was obtained from Toxin Technology, Inc. (Sarasota, FL). The following reagents were obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Staphylococcal Enterotoxin B Toxoid, recombinant from *Escherichia coli*, NR-10049 (referred to as SEBr), and Staphylococcal Enterotoxin B Toxoid, chemically inactivated from *Staphylococcus aureus* subsp. aureus, NR-4672 (referred to as SEB-CI). The mAb 03B2A was kindly provided by Dr. Jill Czarnecki, Naval Medical Research Center, Rockville, MD. The mAb 2F2 was the kind gift of Dr. Thomas Obrien, Tetracore, Rockville, MD. The mAbs S222 and S643 were the kind gifts of Dr. Peter Sveshnikov, Research Center for Molecular Diagnostics and Therapy, Moscow, Russia. Carboxy-functionalized standard MagPlex microspheres were obtained from Bio-Rad (Herules, CA). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide (sulfo-NHS), and NHS-LC-LC-biotin were purchased from Pierce (Rockford, IL). The streptavidin-R-phycoerythrin (SA-PE) was obtained from Columbia Biosciences (Columbia, MD). Enzymes used for cloning were from New England Biolabs (Ipswich, MA). Phosphate buffered saline (PBS), Tween 20, and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St. Louis, MO).

Biosafety. The amount of active SEB that the research group possessed did not at any time exceed 5 mg, in compliance with the department of health and human services (HHS) reporting requirements for select agents. All protocols for handling and disposal of toxin were approved by the NRL biosafety review process.

Production of SEB Mutants. The plasmid inserts for SEB Y89A and SEBv (Y89A/Y94A/L45R) were codon optimized for *E.coli* expression and were synthesized by Genscript Inc. (Piscataway, NJ). Inserts were subcloned into pet15b (Novagen, EMD Chemicals, USA) in frame with an N-terminal His-tag and thrombin cleavage site. The expressed protein sequences are shown in Figure S1, Supporting Information. Plasmid DNA of the SEB variants were transformed into *E.coli* BL-21(DE3) pLysS. Protein expression was induced overnight with 0.3 mM IPTG at 17 °C. Cells harvested from 0.75 to 1.5 L of media were lysed in 50 mL of lysis buffer (50 mM Tris pH 7.6, 500 mM NaCl, 2 mM β-mercaptoethanol (BME), 62.5% BugBuster (Novagen, EMD Chemicals) and 30 mg lysozyme) and sonicated for 1 min in an ice bath. Cell lysates were clarified by centrifugation (20 000*g* for 30 min, 4 °C) and were loaded onto a Chelating Sepharose column (G.E. Healthcare) charged with nickel and equilibrated with buffer A (50 mM Tris pH 7.6, 500 mM NaCl, and 2 mM BME). The column was washed with 20% elution buffer (buffer A containing 300 mM imidazole), and the protein was eluted with 100% elution buffer. Fractions containing protein were dialyzed against 50 mM Tris pH 7.6, 200 mM NaCl, 2 mM BME with 50U of thrombin overnight at room temperature. Thrombin was removed using a 1 mL benzamidine Sepharose column (G.E. Healthcare) and loaded onto an SP Sepharose column equilibrated with 50 mM Tris pH 7.6, and the flow through was collected. The protein was judged to be at least 95% pure based upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Proteins were buffer exchanged prior to use (1× PBS pH 7.4). Protein was flash frozen in liquid nitrogen and stored at −80 °C.

Production of SdAb A3. The plasmid insert for SdAb A3 was codon optimized and synthesized by Geneart, Inc. (Regensburg, Germany) and was subcloned into pet15b (Novagen, EMD Chemicals, USA) in frame with an N-terminal His-tag and thrombin cleavage site. Protein was expressed in the Shuffle T7 strain (New England Biolabs) grown at 30 °C in TB media and induced for 3 h with 1 mM IPTG (isopropyl-beta-D-thiogalactopyranoside). Cell lysis was accomplished with BugBuster HT (5 mL per gram of wet cell pellet) for 20 min at room temperature. Insoluble material was removed by centrifugation at 15 000 g for 20 min, and Ni Sepharose (GE Healthcare) in PBS was added directly to the BugBuster supernatant. After binding for 1 h at room temperature, the resin was packed in a column and eluted with 500 mM imidazole in PBS. Further purification and removal of imidazole was accomplished by fast protein liquid chromatography (FPLC) in PBS as described previously.[11]

Surface Plasmon Resonance Kinetics Analysis. The surface plasmon resonance (SPR) kinetic measurements were performed using the ProteON XPR36 (Bio-Rad). For testing the kinetics of the anti-SEB monoclonals, a GLC chip was coated with SEB, SEB Y89A, and SEBv, on two lanes each. For immobilization, proteins were diluted in 10 mM acetate buffer pH 5.0 and attached to the chip following the standard EDC coupling chemistry provided by the manufacturer. The RU obtained for the SEB, SEB Y89A, and SEBv was, respectively: 1890, 1924; 1287, 1414; 1646, 1910. Thus, similar amounts of the proteins were immobilized on the chip. All experiments were performed at 25 °C. The binding of the various mAb and the sdAb were tested by flowing six concentrations varying from 100 to 0 nM at 50 μL/min for 120 s over the antigen coated chip and then monitoring dissociation for 600 s. The chip was regenerated using 50 mM glycine-HCl (pH 2.5) for 36 s, prior to any additional testing. The data were analyzed with the ProteON Manager TM 2.1 software, corrected by subtraction of the zero antibody concentration column as well as interspot corrected; the binding constants were determined using the software's Bivalent analyte model (mAb) or Langmuir model (sdAb).

Preparation of MAGPIX Reagents and Assay Protocols. The carboxy-functionalized MagPlex microspheres were coated with antibodies using the manufacturer's suggested sulfo-NHS/EDC mediated coupling protocol. All steps

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Table 1. Binding Kinetics of Antibodies to SEB and Both Mutants

		ka (1/Ms)		kd (1/s)		KD (M)	
		average	error	average	error	average	error
mAb03B2A	SEBv	1.0×10^{6}	1.5×10^{5}	5.1×10^{-5}	1.8×10^{-5}	4.8×10^{-11}	1.1×10^{-11}
	SEB Y89A	8.2×10^{5}	9.0×10^{3}	1.7×10^{-5}	6.5×10^{-7}	1.9×10^{-11}	3.5×10^{-13}
	SEB	1.2×10^{6}	8.0×10^{4}	1.7×10^{-5}	3.1×10^{-6}	1.5×10^{-11}	3.8×10^{-12}
mAb S643	SEBv	3.9×10^{5}	6.6×10^{4}	9.5×10^{-3}	1.3×10^{-4}	2.5×10^{-8}	4.6×10^{-9}
	SEB Y89A	3.1×10^{5}	9.0×10^{3}	8.8×10^{-3}	7.4×10^{-4}	2.9×10^{-8}	1.6×10^{-9}
	SEB	3.3×10^{5}	1.7×10^{4}	4.9×10^{-3}	2.3×10^{-4}	1.5×10^{-8}	5.0×10^{-11}
mAb S222	SEBv	3.1×10^{5}	3.9×10^{4}	2.5×10^{-3}	2.6×10^{-4}	8.5×10^{-9}	1.5×10^{-9}
	SEB Y89A	2.6×10^{5}	1.3×10^{4}	1.9×10^{-3}	1.6×10^{-4}	7.2×10^{-9}	9.6×10^{-10}
	SEB	2.7×10^{5}	1.5×10^{4}	2.0×10^{-4}	4.0×10^{-6}	7.3×10^{-10}	2.4×10^{-11}
mAb 2F2	SEBv	6.2×10^{4}	2.2×10^{4}	1.1×10^{-2}	2.7×10^{-3}	2.2×10^{-7}	1.2×10^{-7}
	SEB Y89A	1.6×10^{5}	1.7×10^{4}	1.3×10^{-4}	1.7×10^{-5}	8.2×10^{-10}	2.5×10^{-11}
	SEB	3.0×10^{5}	9.0×10^{3}	2.2×10^{-5}	2.3×10^{-6}	7.1×10^{-11}	5.4×10^{-12}
sdAb A3	SEBv	2.6×10^{5}	1.2×10^{4}	3.2×10^{-5}	5.4×10^{-6}	1.3×10^{-10}	1.5×10^{-11}
	SEB Y89A	2.7×10^{5}	5.0×10^{2}	1.7×10^{-5}	1.0×10^{-5}	5.6×10^{-11}	3.0×10^{-11}
	SEB	2.1×10^{5}	2.0×10^{3}	3.0×10^{-5}	5.5×10^{-6}	1.4×10^{-10}	6×10^{-11}

requiring collection of microspheres and washing were performed with a permanent magnet for the MagPlex spheres. Briefly, 0.03 mL of the carboxylated microsphere stock suspension was diluted with 0.1 mL of 0.1 M sodium phosphate buffer (PB), pH 6.0, washed two times with the same buffer, and resuspended in 0.1 mL of PB, to which 0.01 mL of EDC (50 g/L in PB) and 0.01 mL of sulfo-NHS (50 g/L in PB) were added. Following 20 min of incubation, the activated microspheres were washed once with 0.1 mL of PB and once with 0.1 mL of PBS. Activated microspheres were then resuspended in 0.05 mL of PBS containing the desired antibody at ~1 mg/mL. After overnight coupling at 4 °C in the dark, the microspheres were washed twice with 0.2 mL of PBS and resuspended in 0.3 mL of PBS containing 0.05% Tween-20 (PBST).

For use as detection reagents in the MAGPIX assays, the antibodies were labeled using biotin (Bt)-LC-LC-NHS from Pierce. The Bt-LC-LC-NHS was dissolved in DMSO and then added to the antibody in PBS at a molar ratio of 10 to 1 for the mAbs and 3 to 1 for the sdAb. After ~30 min, the biotinylated antibodies were separated from the free biotin by gel filtration (Biogel P10, Bio-Rad, Hercules, CA).

The assays were performed by first mixing the various antibody coated MagPlex microspheres to be utilized (0.5 μL of each set/sample tested). The buffer was removed and replaced with PBSTB (PBS with 0.05% Tween 20 and 1 mg/mL BSA) sufficient to add 10 μL/sample. The samples were prepared by adding 10 μL of SEB (10 μg/mL) or mutant SEB to 90 μL of PBSTB in row A of a polypropylene round-bottom microtiter plate and making serial dilutions to yield concentrations of 1000, 100, 10, 1, 0.2, 0.04, 0.008, and 0 ng/mL. To each sample, 10 μL of MagPlex bead mixture was added and the microtiter plate was covered and incubated at room temperature in the dark for 30 min. The plate was then washed three times with PBST using the microtiter plate with a permanent magnet. To measure the amount of antigen captured, the appropriate biotinylated detection antibody was added to each sample to yield a final concentration of 1 μg/mL sdAb, 5 μg/mL mAb, or 10 μg/mL polyclonal IgG in PBSTB. After a 30 min incubation, the plate was washed 2 times with PBS and then 50 μL of SA-PE (2.5 μg/mL) was added to each well. After a final 30 min incubation, the plate was washed, and the microspheres were resuspended with 75 μL of PBST prior to evaluating using the MAGPIX instrument. The data reported is the median of an average of 125 ± 25 SD of microspheres per bead set. The median value of fluorescence intensity is most commonly utilized to evaluate MAGPIX data as it minimizes the influence of any outliers; however, the median and trimmed mean provide similar results. To provide a measure of data error, the standard deviation of the trimmed mean for the various data sets was less than 5% for all the data presented.

RESULTS AND DISCUSSION

The generation of SEB with mutations in the amino acid primary sequence with the goal of understanding toxin function and producing material with a reduced toxicity relative to native SEB for use as a vaccine has been well described.[8,16,17] Our work evaluated how well two of these mutants were recognized by five monoclonal immunoreagents. We determined binding affinities and then compared the LOD obtained in a sandwich immunoassay format, utilizing SEB mutants and wild type toxin, as well as a chemically inactivated toxin.

The affinity for four different standard mAb as well as a sdAb specific for SEB was determined for SEB as well as both mutants. Table 1 lists the on and off rates, while the SPR traces are shown in Figure S2, Supporting Information. Two of the mAb and the sdAb showed no significant change in binding between SEB and the two mutants, suggesting that SEBv could be utilized as a relatively safe positive control in combination with these antibodies. mAb S222 showed a decrease in affinity (as determined by the dissociation constant, KD = ka/kd) between SEB and SEB Y89A of ~10-fold and ~11-fold between SEB and SEBv. Interestingly, mAb 2F2 showed decreasing KD with increasing number of SEB mutations; it bound SEB Y89A 11-fold more weakly and SEBv 3000-fold more weakly (Figure 1). The affinity calculated for the binding of sdAb A3 to SEB and both mutants was very high indicating it bound well to all three; although the binding appeared to vary, the ability to accurately measure affinities becomes more problematic at high affinities (<100 pM), as one is evaluating very small differences in off rates that can be easily skewed by signal drift. Using long off rate binding intervals and doing repetitive measurement, one can get a better assessment of KD, but for this work, it was sufficient to establish whether the binding of the antibodies to the mutants stayed as strong as it was for SEB or whether it was abrogated by the mutations.

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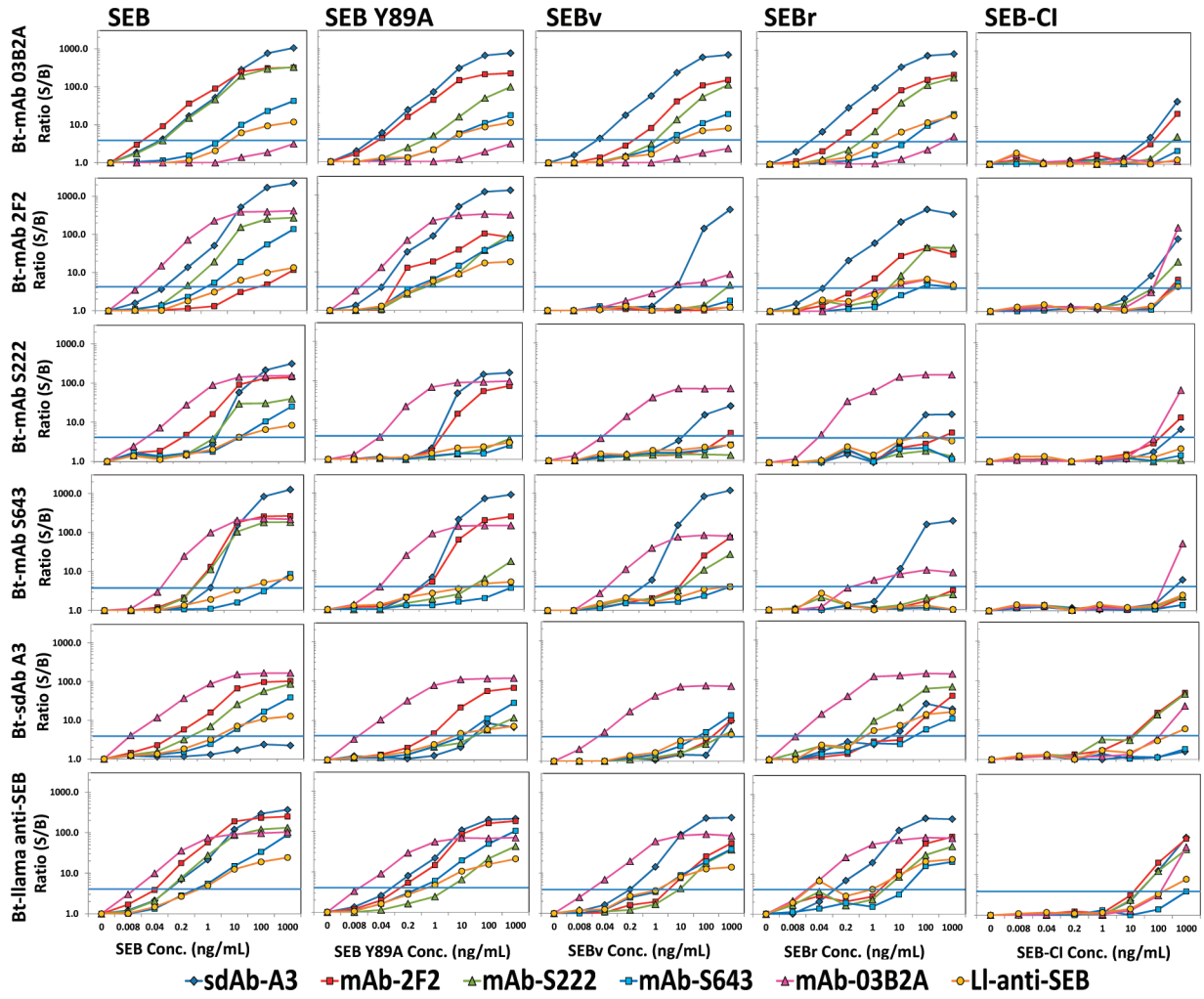

Figure 2. MAGPIX sandwich fluoroimmunoassay results for the detection of SEB and each of the possible surrogates using all six different capture antibodies each immobilized on a different Magplex bead set and then each as the biotinylated (bt) detection antibody. Each graph shows all six captures for the indicated tracer and target combination. Captures are coded by symbol and color as follows: sdAb A3 blue diamond; mAb 2F2 red square; mAb S222 green triangle; mAb S643 blue square; mAb 03B2A pink triangle; llama anti-SEB (Ll-anti-SEB) yellow circle. The *Y*-axis, signal divided by background, is shown on a log scale; the blue line represents the LOD (4-fold the background response). The *X*-axis shows 10-fold dilutions of target (SEB and surrogates) from 1000 ng/mL down to 1 ng/mL and 5-fold dilutions from 1 to 0.008 ng/mL. See Materials and Methods for details.

Once we had determined that each of the antibodies not only could bind SEB but also could recognize both the SEB mutants, we set out to evaluate how the changes in affinity would impact our ability to detect the mutant SEBs relative to the native SEB. We elected to perform multiplexed fluorimmunoassays on the MAGPIX platform. To complete these assays, each of the monoclonal antibodies along with a polyclonal llama anti-SEB were immobilized on MagPlex microspheres to provide capture reagents; they were also biotinylated to allow them to be used as reporters as well. Mixtures of all the capture antibody coated microspheres were incubated with a range of concentrations of SEB, SEB Y89A, SEBv, SEBr, and SEB-CI. The amount of antigen captured by each bead set was detected by addition of one of the biotinylated antibodies. Results are shown in Figures 2 and S3, Supporting Information. This assay format is ideal for this application, as one can incubate all the capture sets with the exact same concentration of antigen for exactly the same amount of time, making the comparison of antigen captured highly robust.

An important observation was that none of the capture–detector antibody pairs performed well for the detection of the SEB-CI (LOD $\geq$ 100 ng/mL), this confirmed the need for improved surrogates for testing and evaluation of detection platforms, as a LOD for SEB of 40 pg/mL was obtained for a number of antibody pairs. This represents a 2500-fold decrease in detectability upon chemical inactivation. The next observation is that the two antibodies, mAb 03B2A and sdAb A3, which each bound the surrogates toxins well, also functioned well as a pair. The mAb 2F2 also bound SEB well (LOD of 40 pg/mL in combination with mAb 03B2A regardless of orientation), but similar to the SPR data, mAb 2F2 generated less signal for SEB Y89A and even less for SEBv and SEBr with an LOD of 200 pg/mL as a capture molecule, but as the detector molecule, the LOD increased to 10 ng/mL. This 50-fold difference in sensitivity simply due to swapping the antibodies from capture to reporter is of interest. One possible explanation is that when the 2F2 is immobilized on the surface of the microsphere it behaves as though it is at a high concentration; thus, when the SEB mutants dissociate, they

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would diffuse faster along the surface of the microsphere and rebind to another 2F2 antibody instead of diffusing away, thereby enhancing the LOD relative to its use as a detector antibody. Examining the results from the other antibody combinations reinforces the conclusion that the point mutant SEB Y89A has the least impact on LOD and would by that virtue be the best surrogate for the native SEB toxin; however, it may have some residual toxicity, as well as a greater risk of reversion to the fully toxic form with a single point mutation. The SEBv/SEBr mutants are clearly detected much more poorly than SEB by most of the antibody pairs; however, they are still much better surrogates than SEB-CI. The two triple mutants behaved nearly identical in the immunoassays as expected since their differences are relatively minor, being at the toxin's amino or carboxyl terminal end. However, 2F2 did bind differently, implying preparation of the surrogate in combination with the antibodies being employed can yield differences; i.e., the presence of aggregates in the antigen sample can allow a monoclonal to act as both capture and detector and may explain some of the differences observed. A final observation is that use of multiple detection antibodies would permit one to easily discriminate between the active toxin and the use of nontoxic surrogates, an important advantage provided by platforms with high multiplexing capabilities.

CONCLUSION

We have demonstrated that the use of SEB mutants are an effective alternative to chemically inactivated SEB for the testing and evaluation of detection platforms. However, some high affinity immunoreagents did show significant loss in sensitivity upon challenge with the mutants as compared to the native toxin, suggesting that they bind to surfaces involved in HLA-DR alpha binding,[18,19] as the mutations in SEBv were selected on the basis of their inability to bind HLA-DR, stimulate T-cells, and induce cytokines.[8,20] This highlights the necessity to evaluate the immunoreagent being employed prior to replacing the native toxin with more safely handled surrogates.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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