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Human Monoclonal Antibodies Against Staphylococcal Enterotoxin B: Potential Therapeutics?

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ABSTRACT

Previous studies from our laboratory and others demonstrated the efficacy of antibodies towards different staphylococcal superantigens, such as the enterotoxins and toxic shock syndrome toxin-1, for therapy of disease associated with *Staphylococcus aureus*. Human monoclonal antibodies (Mabs) recognizing staphylococcal enterotoxin B (SEB) were generated by employing a human combinatorial antibody library and compared to mouse monoclonal, rabbit and human polyclonal antibodies. Select Mabs effectively inhibited T cell responses in cultures of human peripheral blood mononuclear cells (PBMCs) for up to 12 hours after toxin exposure. Cross-reactivity studies revealed that some Mabs recognized other superantigens, such as the SEC family, and streptococcal pyrogenic exotoxin C (SpeC) from *Streptococcus pyogenes* likely due to the high similarity in protein structure. Binding of the Mabs to SEB varied most in off-rates and KDs ranged from micro- to nanomolar. Our results suggest that Mabs may be useful for therapeutic treatment of diseases caused by SEB. To our knowledge, this is the first assessment of unique, human-based Mabs focused upon superantigens of *S. aureus* and *S. pyogenes*.

INTRODUCTION

Antibodies are invaluable immunoreagents for detection, prophylaxis, and therapy of various diseases. The known therapeutic value of antibodies spans a century with the pioneering efforts of Drs. Behring, Kitasato, and Roux on Corynebacterium diphtheriae and Clostridium tetani (1), as well as contemporary reagents that have been clinically used to neutralize infectious viruses or life threatening toxins of bacterial and snake origins. By harnessing current recombinant-based technologies, studies with more refined and characterized reagents seem logical for the near and distant future.

Staphylococcus aureus is a rather ubiquitous pathogen found throughout our biosphere that is involved in diverse diseases of particular interest to the medical and biodefense communities. This bacterium has become quite a daunting medical problem due to the spread of antibiotic resistance among nosocomial- and community-acquired isolates. The economic burden of S. aureus upon healthcare is remarkable and different means of controlling this pathogen are clearly needed for better patient care.

Enterotoxins and related virulence factors induced by S. aureus are referred to as superantigens because of their profound effect upon the immune system. To date, there are at least twenty different staphylococcal enterotoxins (SEs) described in the literature that fall within three major groups based upon amino acid sequences (11) while all are similar in protein structure. By definition, these molecules possess superantigenic properties that involve binding to major histocompatibility complex class II (MHC II) and specific T-cell receptors. The ultimate effect of SEs upon a host involves profound T-cell proliferation and elevated, pathological levels of the proinflammatory cytokines interferon gamma (IFNγ), interleukin 2 (IL-2), and tumor necrosis factor alpha (TNFα). This toxic shock syndrome can lead to multi-organ failure and death.

Previous studies with S. aureus toxic shock syndrome toxin-1 (TSST-1), a virulence factor similar in structure to SEs, reveal a predisposition towards toxic shock syndrome among patients lacking pre-existing antibodies (8). The same phenomenon is true for non-menstrual forms of toxic shock elicited by either TSST-1 or SEB (2). Intravenous immunoglobulin (IVIg), a human polyclonal antibody currently used, has proven effective against streptococcal- and staphylococcal-induced shock but the number of clinical trials is surprisingly limited in scope (5). The IVIG used clinically seems more efficacious towards streptococcal, versus staphylococcal, superantigens and there is batch to batch variation (4,7). It is possible that human monoclonal antibodies (Mabs) against toxins like SEB might afford a better characterized reagent than the human polyclonal antibodies commonly employed to date. The Mabs that we describe may be useful therapeutics against SEB intoxication.

RESULTS

Human Monoclonal Antibodies (hMabs)

The Mabs were isolated from a human combinatorial antibody library (HuCAL) developed by MorphoSys (Martinsreid, Germany). Each antibody contained a 6xHis and Myc tag to facilitate subsequent purification and detection of antibody-bearing phage. Bivalent, SEB-specific antibodies were expressed in E. coli and then detected by phage panning using a recombinantly-attenuated (L45R, Y89A, Y94A) SEB vaccine (SEBv) from previous murine and non-human primate studies (3,10). Panning was preformed either by adsorption of SEBv to plastic surface of microplates (Mabs 1-8) or by attachment of biotinylated SEBv to streptavidin-coated paramagnetic beads (Mabs 9-10).

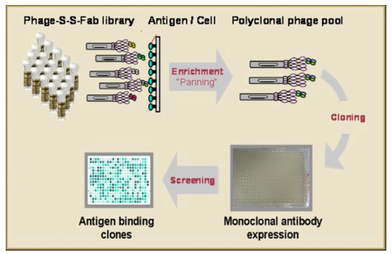
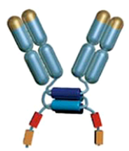


Figure 1. Antibody selection from HuCAL GOLD.



Fab-dHLXMH0
(mini-antibody used in our studies is devoid of Fc)

Figure 2. Bivalent antibody (~110kd).

ELISA Reactivity of SEB Mabs with Staphylococcal and Streptococcal Superantigens

COAT
5μg/ml SEA, SEB, SEC1, SEC2, SEC3, TSST-1, SpeA and SpeC, 2h/37°C

↓

BLOCK
0.2% casein, overnight/4°C

↓ *Wash PBST*

PRIMARY Ab
2μg/ml of each Mab, 2h/37°C

↓

SECONDARY Ab
1:2000 Goat anti-human (F)ab, '2 HRP conjugated, 1h/37°C

↓

DETECTION
TMB substrate, 40 min/RT, read @ 650nm

Table 1. SEB Mab Crossreactivity

	Antigen							
Antibody	SEA	SEB	SEC1	SEC2	SEC3	TSST-1	SpeA	SpeC
1	-	+++	+++	-	-	-	-	-
2	-	++++	-	-	-	-	-	-
3	-	++++	-	-	-	-	-	+
4	-	-	-	+	-	-	-	+
5	-	+++	-	-	-	-	-	-
6	-	++++	++++	+	+	-	-	++
7	-	+++	+++	+++	-	-	-	-
8	-	+++	+++	-	-	-	-	-
9	-	+++	-	-	++++	-	-	++
10	-	++++	-	++	++	-	-	++
anti SEB	-	-	+	++	++	++	+	-

-= <0.5 mean absorbance (650nm) of triplicate wells +/- standard deviation;
-+ = 0.5-1.0; ++ = 1.0-2.0; +++ = 2.0-3.0; ++++ = 3.0-4.0.

SUMMARY – All Mabs, except Mab 4, strongly reacted with wild-type SEB by ELISA. Since each clone was originally panned with SEBv as the target antigen, Mab4 may detect subtle differences between the vaccine with three amino acid changes and wild-type toxin. A few antibodies strongly reacted with closely related SECs as well as the more distant SpeC. These crossreactive Mabs are potentially useful therapeutic against other related superantigens. Staphylococcal and streptococcal toxins used for screening cross-reactivity share the following amino acid sequence homologies with SEB: SEA (33%); SEC1 (68%); TSST-1 (26%); SpeA (52%); SpeC (21%). Though sequence homology does not directly correlate with Mab reactivity (i.e. SpeA and SpeC), overall conformations of the staphylococcal and streptococcal superantigens are quite similar, as shown below:

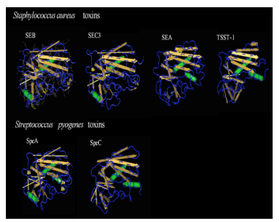


Figure 3. Staphylococcal and Streptococcal Superantigens share common three-dimensional structures.

Kinetic Analysis of Antibody – SEB Interactions

IMMOBILIZE
High density surface (8000 RUs) -his and goat anti-human F9ab) '2, CM5 sensor chip

↓

Mab CAPTURE
Mabs captured at various densities to reach optimal Rmax of analyte for kinetic analysis (50 -250 RUs)

↓

ANALYTE CAPTURE
SEB injected at concentrations ranging from 11 -2700nM

↓

REGENERATION
Glycine buffer, pH 1.8

↓

ANALYSIS
BIA evaluation software, 1:1 Langmuir Model

Binding Affinities of SEB Mabs and Control Anitbodies

Table 2. Control Antibodies

Control Antibody	ka (1/Ms)	kd (1/s)	KD (M)
Polyclonal Rabbit anti SEB	$6.6x10^5$ ($+/-3.5x10^4$)	$2.6x10^{-4}$ ($+/-3.4x10^{-5}$)	$3.9x10^{-10}$ ($+/-4.0x10^{-11}$)
Mouse SEB Monoclonal Antibody 1	$2.2x10^5$ ($+/-4.7x10^3$)	$1.1x10^{-3}$ ($+/-5.0x10^{-5}$)	$4.9x10^{-9}$ ($+/-1.1x10^{-10}$)
Mouse SEB Monoclonal Antibody 2	$2.1x10^5$ ($+/-3.7x10^3$)	$7.1x10^{-4}$ ($+/-1.6x10^{-5}$)	$3.3x10^{-9}$ ($+/-1.0x10^{-10}$)
Mouse SEB Monoclonal Antibody 3	$1.2x10^5$ ($+/-4.7x10^3$)	$4.5x10^{-4}$ ($+/-2.4x10^{-5}$)	$3.6x10^{-9}$ ($+/-1.1x10^{-10}$)

Table 3. SEB Mab Results

	Mouse anti-6xHis Capture			Goat anti-human F(ab)'2 Capture		
Mab	ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)
1	$4.7x10^3$ ($+/-2.9x10^2$)	$6.9x10^{-3}$ ($+/-2.3x10^{-4}$)	$1.7x10^{-6}$ ($+/-1.7x10^{-7}$)	$5.4x10^3$ ($+/-1.3x10^2$)	$6.1x10^{-3}$ ($+/-7.3x10^{-4}$)	$1.1x10^{-6}$ ($+/-1.1x10^{-7}$)
3	$1.5x10^4$ ($+/-1.8x10^2$)	$3.3x10^{-4}$ ($+/-3.3x10^{-6}$)	$2.3x10^{-8}$ ($+/-0$)	$1.8x10^4$ ($+/-3.8x10^3$)	$2.3x10-4$ ($+/-1.5x10^{-5}$)	$1.4x10-8$ ($+/-2.2x10^{-9}$)
6	96.9 ($+/-2.5$)	$4.1x10^{-5}$ ($+/-4.2x10^{-6}$)	$4.2x10^{-7}$ ($+/-4.4x10^{-8}$)	*1.9x10^5	*3.5x10^-3	*1.9x10^-8
9	$7.3x10^3$ ($+/-9.4x10^2$)	$1.6x10^{-4}$ ($+/-2.0x10^{-5}$)	$2.2x10^{-8}$ ($+/-5.0x10^{-11}$)	$6.1x10^3$ ($+/-1.3x10^2$)	$2.0x10^{-4}$ ($+/-8.3x10^{-6}$)	$3.2x10^{-8}$ ($+/-2.1x10^{-9}$)
10	$2.7x10^4$ ($+/-2.1x10^3$)	$1.2x10^{-4}$ ($+/-1.6x10^{-5}$)	$4.2x10^{-9}$ ($+/-2.8x10^{-10}$)	$1.9x10^4$ ($+/-9.6x10^3$)	$9.1x10^{-5}$ ($+/-7.7x10^{-6}$)	$2.3x10^{-9}$ ($+/-2.2x10^{-10}$)

*Not determined, below instrument limitations

SUMMARY- Kinetic analysis was performed on all ten Mabs. Only five yielded reliable data (i.e. Rmax of analyte between 50 and 250 RUs). Of the five Mabs analyzed, only Mab 10 performed to a level equivalent to the native mouse monoclonals.

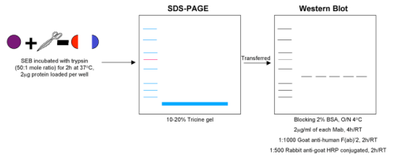


Figure 4. Epitope mapping using Mab recognition in western blots.

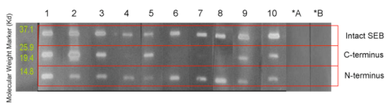


Figure 5. N- vs C- terminal epitope recognition. *Negative control Mabs A (TSST-1) and B (SEA) were also not cross-reactive with SEB by ELISA.

SUMMARY – All Mabs recognize SDS / beta-mercaptoethanol / heat denatured SEB. Trypsin digestion conditions generated two large fragments, as previously described by Spero et al. (9). Although there are distinct patterns of reactivity with SEB fragments, fragment epitope recognition and Mab performance (i.e. toxin crossreactivity and neutralization) were not consistent.

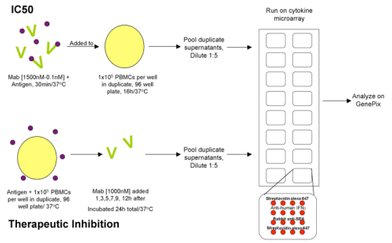


Figure 5. Mab inhibition of response to superantigens by human Peripheral Blood Mononuclear Cells (PBMCs).

Table 4. Mab Inhibition of Human T-cell Responses

	IC_{50} (nM)		
Mab	SEB	SEC1	SpeC
1	205	1487	nt[a]
2	1255	nt[a]	nt[a]
3	199	nt[a]	>1500
4	558	nt[a]	>1500
5	772	nt[a]	nt[a]
6	875	480	
7	>1500	>1500	>1500
8	>1500	>1500	>1500
9	444	nt	769
10	77	nt	>1500
Anti-SEB[b]	92	570	59
Anti-SEA Mab[c]	>1500	nt	nt[a]

a) nt = "not tested" because of weak cross-reactivity by ELISA
b) Affinity purified antibody from human sera
c) Does not cross-react with SEB by ELISA

SUMMARY – Human T-cell response (IFNγ secretion) was most effectively inhibited by Mab 10, which was slightly better than the purified polyclonal anti-SEB (human). Mabs 7 and 8 were least effective. This assay also revealed inhibition of the heterologous superantigens, SEC1 and SpeC by Mabs 6 and 9 respectively. Interestingly, Mab 4 (which was negative by ELISA for SEB) prevented SEB induction of IFNγ. These data suggest critical alteration of the SEB epitope in an ELISA well versus being in solution. Overall, these results reveal that most Mabs inhibit SEB-induced IFNγ from PBMCs and some are relatively effective against heterologous antigens like SEC1 and SpeC. The next series of experiments determined whether the Mabs could be used therapeutically in vitro with human PBMCs.

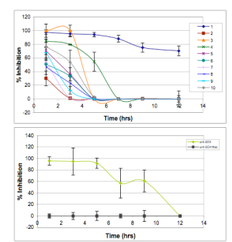


Figure 6. Therapeutic Inhibition of Human T-cell response to SEB.

SUMMARY – Preliminary studies with human PBMCs suggested that purified human anti-SEB (polyclonal) effectively prevented SEB-induced IFNγ release up to 7 – 9 h after toxin exposure. Certain Mabs possess a strong therapeutic effect in vitro that is evident 1 - 3 h after SEB exposure. Mab 1 was particularly effective against SEB up to 12 h after toxin exposure. Overall, such findings are very promising for future work using these Mabs as therapeutic reagents in vivo.

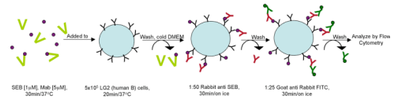


Figure 7. Mabs 9 and 10 effectively inhibit SEB binding to MHC II.

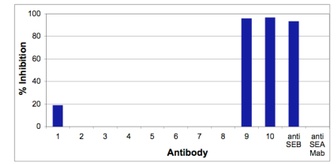


Figure 8. Mab effects upon SEB binding to MHC class II. % inhibition calculated from samples with antibody verses no antibody.

SUMMARY – These results suggest that only Mabs 9 and 10 inhibit SEB binding to MHC II. These unique Mabs are similar in epitope mapping studies (SEB peptide recognition) but markedly differ in heterologous toxin recognition by ELISA. Since Mabs 1-8 don't inhibit SEB binding to MHC II but still inhibit T-cell responses, one could deduce that they are involved in T-cell receptor inhibition.

CONCLUSIONS/FUTURE EFFORTS

1. Ten human Mabs screened against SEB represent potentially unique reagents for therapies targeting S. aureus and S. pyogenes toxins.
2. The biotinylated antigen solution panning method produced antibodies with better KDs than those antibodies screened by ELISA.
3. Select Mabs neutralize SEB-induced release of IFNγ from human PBMCs when premixed with toxin or used in a "therapeutic" fashion in vitro hours after SEB exposure.
4. Epitope mapping studies (trypsin digestion and site-directed mutants) of SEB reveal distinct patterns of recognition by Mabs; however, there is no obvious trend between Western Blot results and protective effects (decrement of IFNγ release by SEB-stimulated human PBMCs) in vitro.
5. Future studies will involve mixing of unique, toxin-neutralizing Mabs to ascertain additive / synergistic effects of therapeutic cocktails towards homologous (SEB) and heterologous (SEC and SpeC) antigens.

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