Humanized Staphylococcal Enterotoxin B (SEB)–Specific Monoclonal Antibodies Protect From SEB Intoxication and *Staphylococcus aureus* Infections Alone or as Adjunctive Therapy With Vancomycin

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**Background.** Staphylococcal enterotoxin B (SEB), a potential biological warfare agent, is a potent superantigen that contributes to the virulence of methicillin-resistant *Staphylococcus aureus* (MRSA), which is a major health threat in the United States. Efforts to develop toxin-neutralizing antibodies as adjunctive therapies are justified, given the high mortality and frequent failure of therapy despite available antibiotics.

**Methods.** Murine SEB-specific mAb 20B1 was humanized, and treatment benefits of Hu-1.6/1.1 and Hu-1.4/1.1 variants were investigated in mice in an SEB intoxication model, as well as in sepsis and deep-tissue infection models.

**Results.** Hu-1.6/1.1 and Hu-1.4/1.1 protected mice against SEB-induced lethal shock. Hu-1.6/1.1 also enhanced survival of mice that developed fatal sepsis after challenge with a SEB-producing MRSA strain. Combined treatment of Hu-1.6/1.1 with vancomycin further increased survival and altered cytokine responses, compared with monotherapy with either monoclonal antibody or vancomycin alone. Efficacy was also demonstrated in the deep-tissue infection model, where Hu-1.4/1.1 bound to SEB in vivo and decreased abscess formation, as well as proinflammatory cytokine levels.

**Conclusions.** SEB-neutralizing mAb 20B1 was successfully humanized. The mAb affects outcome by modulating the proinflammatory host response in both the sepsis and the intoxication models, which justifies further development.

**Keywords.** *Staphylococcus aureus*; virulence; adjuvant therapy; monoclonal antibody; superantigen.

Treatment failure rates and mortality remain high in patients with complicated *Staphylococcus aureus* infections despite the introduction of newer antistaphylococcal antibiotics [1]. These challenges and the development of antimicrobial drug resistance extending even to vancomycin necessitate investigations on new approaches to antibacterial prophylaxis and therapy. Most *S. aureus* strains secrete several different toxins, including a large number of enterotoxins with variable degrees of superantigenic activity [2]. Established successful active and passive immunotherapies against various toxins, including diphtheria, tetanus, anthrax, and botulinum toxin, have promoted work on targeting staphylococcal toxins, as well as [3–6]. Of the enterotoxins, staphylococcal enterotoxin B (SEB) is one of the most potent superantigens and thus is classified as a class B biological warfare agent. SEB forms a bridge between the major histocompatibility complex class II molecules on antigen-presenting cells and Vβ chains of T-cell
receptors, resulting in a massive release of cytokines, which can kill patients. Clinical studies and animal infection models support the concept that superantigens like SEB constitute significant virulence factors and contribute to bad outcome of *S. aureus* infections [7–9].

Previous work from our group demonstrated that the SEB-specific murine monoclonal antibody (mAb) 20B1 protects against SEB intoxication in vitro and in vivo [9] and effectively treats *S. aureus* infections in mice [10]. We generated a set of humanized variants of murine mAb 20B1 and investigated the neutralizing efficacy in vitro in proliferation and in vivo models. Two humanized mAbs (Hu-1.4/1.1 and Hu-1.6/1.1) were explored in an intoxication model, and later the therapeutic efficacy of Hu-1.6/1.1 was examined alone or as an additive therapy in combination with vancomycin in a murine *S. aureus* septicemia model, as well as a deep tissue thigh infection model. We found that treatment with Hu-1.6/1.1 alone was effective against SEB-induced toxic shock in mice and that treatment with Hu-1.6/1.1 either alone or in combination with vancomycin significantly improved survival among mice with *S. aureus* sepsis. Efforts are on the way to develop this humanized Ab further for human use.

**MATERIALS AND METHODS**

**SEB Toxin and *S. aureus* Strain**

SEB toxin was purchased from Toxin technology. SEB toxoid, a nontoxic variant of SEB that has a mutation at positions L45R, Y36L, and Y94A [11], was supplied by Pfizer and used in surface plasmon resonance (SPR) assays, to comply with mandated laboratory safety regulations. A previously described clinical methicillin-resistant *S. aureus* (MRSA) strain (strain 38) was used for this study, and inocula were prepared and verified by dilution back plating, as described elsewhere [12].

**Sequencing of Variable Heavy (VH) and Variable Light (VL) Chains of mAb 20B1 and Humanization of Mouse 20B1 mAb**

VL and VH chains of mAb 20B1 were sequenced using standard methods. The complementary DNA was amplified with universal 5′ (sense) variable region and specific 3′ (antisense) constant region primers that were designed on the basis of an amino acid sequence derived by mass spectrometry (unpublished data). Sequence data obtained for VL and VH regions were further analyzed for homologous germ-line variable region genes in the database, using International ImMunoGeneTics (IMGT) Information System software, as described elsewhere [13]. The complementarity-determining regions (CDRs) of mouse antibody 20B1 VH were identified using the AbM definition, which is based on sequence variability and the location of the structural loop regions. Kabat definition was used for VL CDRs. For humanization of the heavy chain variable region, its CDRs were first grafted onto human germ-line acceptor framework IGHV3-7 (DP-54; GenBank accession no. CAA78224.1). For the J-segment, human IGHJ4 sequence was used. For humanization of the VL chain region, human germ-line acceptor framework IGKV1-39 (DPK9; GenBank accession X93627.1) and human IGKJ4 were used. The CDR-grafted versions of VH and VL were designated “VH1.0” and “VL1.0,” respectively. Back mutations were then introduced at the framework positions that were predicted to contribute to antigen recognition and/or variable domain stability. Specifically, back mutations A49G, I69F, R71L, and L78A, in various combinations, were introduced in VH, and Y36L, P44I, L46R, and G66R were introduced in VL (Table 1). The humanized versions of the heavy and the light chain were expressed in combinations to identify the antibody variants with best retention of antigen binding in vitro. Later, chimeric and humanized 20B1 antibodies were produced in human HEK293F cells (Invitrogen) and purified using affinity chromatography on protein A and size-exclusion chromatography. Antibody preparations were all tested for endotoxins and were below 1 EU/mg. An SEB-induced T-cell proliferation assay using Hu-mAbs was performed in murine splenocytes. The median inhibitory concentration (IC50) of the Hu-mAbs was calculated by XLfit software (IDBS, United Kingdom).

**Kinetic Rate and Binding Constants Analysis of Humanized mAbs**

SPR was performed in a T200 instrument (GE Healthcare Bio-Sciences) to determine the binding affinity and kinetics of SEB toxoid and chimeric or humanized Ab variants. On a carboxymethylated dextran sensor chip (CMS), 50–100 resonance units of the chimeric or the humanized Ab variants were captured with an antihuman immunoglobulin G (IgG) Ab capture kit following manufacturer’s guidelines (GE Healthcare, BR-1008-39). The SEB toxoid was serially diluted 3-fold, encompassing a range from 300 nM to 8 nM in HBS-EP+ buffer (0.01 M HEPES pH = 7.4, 0.15 M NaCl, 3 mM ethylenediaminetetraacetic acid, and 0.005% v/v surfactant P20), then injected at a flow rate of 50 μL/minute for a 50-second association period and allowed to dissociate for 300–2700 seconds, followed by sequential 15–30 second injections of 3 mM MgCl2.

### Table 1. Humanized Variants of Murine Monoclonal Antibody 20B1 Heavy (VH) and Light (VL) Chains Generated With Back Mutations

<table>
<thead>
<tr>
<th>Humanized 20B1 VH or VL Variant</th>
<th>Back Mutations in Human Frameworks (Kabat Numbering)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hu-20B1 VH 1.1</td>
<td>A49G, R71L</td>
</tr>
<tr>
<td>Hu-20B1 VH 1.4</td>
<td>A49G, R71L, L78A</td>
</tr>
<tr>
<td>Hu-20B1 VH 1.6</td>
<td>A49G, I69F, R71L, L78A</td>
</tr>
<tr>
<td>Hu-20B1 VH 1.10</td>
<td>A49G, I69F, L78A</td>
</tr>
<tr>
<td>Hu-20B1 VL 1.1</td>
<td>Y36L, P44I, L46R, G66R</td>
</tr>
<tr>
<td>Hu-20B1 VL 1.4</td>
<td>Y36L, P44I, L46R</td>
</tr>
</tbody>
</table>
ionic (0.46 M KSCN, 1.83 M MgCl₂, 0.92 M urea, 1.83 M guanidine-HCl, [14]), and glycine pH = 1.5. All injections were performed at 25°C, and the resulting sensorgrams were double referenced with an anti-human IgG Ab flow cell and buffer injections. The data were fit with a 1:1 binding model, using Biacore T200 Evaluation Software, version 1.0, to determine the kd/ka rate constants and corresponding KD values.

Animal Experiments

Protective efficacy of Hu-1.4/1.1 and Hu-1.6/1.1 were tested in vivo in the SEB-induced lethal shock model, as described earlier [15]. Briefly, a small incision was made into the lateral aspect of the quadriceps muscle. MRSA strain 38 (10⁵ colony-forming units [CFU]/5 μL) was injected deep into the muscle tissue via the incision. The skin was closed with clips, and mice were observed for 5 days. Mice were treated 24 hours before infection with 500 μg of Hu-1.4/1.1, Hu-1.6/1.1, vancomycin alone or in combination with humanized mAbs, or phosphate-buffered saline (PBS). Vancomycin (Hospira) was given at a dose of 15 mg/kg intraperitoneally once daily for 3 days, starting 24 hours before infection with *S. aureus*. CFUs and SEB levels in abscess fluid, as well as neutrophil counts in blood, were analyzed as previously described [10]. The efficacies of Hu-1.4/1.1 and Hu-1.6/1.1 were also tested in the murine sepsis model [10]. Humanized mAbs were given intravenously 2 hours before infection, and control mice were treated either with PBS or with a human-isotype-specific mAb (anti-tetanus IgG1; clone 9F12, ATCC HB-8177) [16]. For combination therapy in the sepsis model, Hu-1.6/1.1 (250 μg) and vancomycin (15 mg/kg) was given intraperitoneally once daily for 3 days, starting 24 hours before *S. aureus* infection. Blood specimens for cytokine analysis by multiplex cytokine assay (ELISA; MSD System) were collected in the sepsis model on days 0 and 3.

Ethics Statement

Animal experiments were performed with the approval of the Albert Einstein College of Medicine Animal Institute Committee, in accordance with their rules and regulations.

Statistical Analysis

GraphPad Prism 6 software was used to generate log-rank survival curves and to perform t tests to compare CFUs. Cirasoft PROarray Analyst Software was used for multiplex cytokine data. To observe the effect of combination in the sepsis model, the data sets for cytokines were processed to remove the outliers, using the 1.5 interquartile range criterion. The normality and homocedasticity of the samples, two required conditions for analysis of variance (ANOVA), were assessed by the Shapiro-Wilk test and the Fligner & Bartlett tests, respectively. A two-way ANOVA with interaction was then applied to each data set, and switched to an additive model if the interaction was found to be not significant. If interactions were found, the post hoc comparisons were explored previously performing a 1-way ANOVA, otherwise, the main factor effects were properly investigated. The post hoc comparisons were performed using the Tukey honestly significant differences test and the Tukey-Kramer test. Analyses of this data set were performed using R, version 2.14.1.

RESULTS

Sequencing of Murine mAb 20B1 and Development of Humanized mAbs

Sequence data of mAb 20B1 by means of new primers modified on the basis of an amino acid sequence derived by mass spectrometry (unpublished data) resulted in a variable sequence and V class assignment that differed from those previously published by us [9]. Immunoglobulin gene family of mAb 20B1 was identified using IMGT software and is shown in Supplementary Figure 1. VDJ analysis revealed that the VH chain belonged to the IGHV9-4*02 family and shared a IGHJ4*01 VH segment and IGHD2-1*01-D segment. The VL chain was identified as belonging to the IGKV9-124*01 family and shared the J segment of IGKJ1*01. All the mutations in mAb 20B1 were located in the AID- and Pol-η–associated hotspots in the VH and VL regions (Supplementary Table 1). On the basis of these sequence data, murine mAb 20B1 was humanized by CDR grafting onto human germ-line framework IGHV3-7 for the VH chain and IGKV1-39 for the VL chain. Several back mutations were introduced into the frameworks to retain binding affinity for SEB (Table 1). Five human mAb candidates—Hu-1.4/1.1, Hu-1.6/1.1, Hu-1.6/1.4, Hu-1.10/1.1, and 1.10/1.4—were successfully generated. These humanized 20B1 variants were generated as hlgG1/kappa mAbs that also contained mutations Leu234Ala, Leu235Ala, and Gly237Ala to eliminate FcyR interactions and the effector functions of wild-type IgG1.

Humanized mAbs Bind to SEB and Exhibit IC₅₀ Values in the Subnanomolar Range

SPR analyses determined that mean KD values (±SD) for Ch-20B1, Hu-1.4/1.1, Hu-1.6/1.1, Hu-1.6/1.4, and Hu-1.10/1.4 were 0.17 ± 0.03, 3 ± 1.83, 0.83 ± 0.05, 0.49 ± 0.04, and 15.9 ± 3.08 nM, respectively (Figure 1A–F). All 4 humanized mAbs had comparable IC₅₀ values (Hu-1.4/1.1, 0.64 nM; Hu-1.6/1.1, 0.37 nM; Hu-1.6/1.4, 0.28 nM, and Hu-1.10/1.4, 1.5 nM) for SEB-induced proliferation of murine splenocyte in vitro (Figure 2A–D). IC₅₀ values were in the low nanomolar range and comparable to that of the parent murine mAb 20B1 [10].

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SEB-Specific mAbs Protect Mice Against SEB-Induced Lethal Shock

The protective efficacy of Hu-1.4/1.1 and Hu-1.6/1.1 were further explored in vivo in BALB/c mice (5 mice/group) with SEB-induced lethal shock. Potent in vitro neutralizing efficacy with human T cells (data not shown) had been confirmed before these in vivo experiments. We demonstrated 80% survival among mice treated with Hu-1.4/1.1 or Hu-1.6/1.1, compared with 10% survival among untreated mice. This was comparable to the efficacy of murine mAb 20B1 (Figure 3A). In accordance with this finding, 2 hours and 8 hours after SEB injection, mean interferon γ (IFN-γ) blood levels (±SD) were significantly lower in mice that were treated with Hu-1.4/1.1, Hu-1.6/1.1, or mAb 20B1, compared with those in PBS-treated mice (272.86 ± 125.33 and 552.94 ± 79.72 pg/mL; P < .05; Figure 3B).

Treatment With Hu-1.4/1.1 and Hu-1.6/1.1 Reduces Abscess Size in the Murine Model of Thigh Infection

Next, the efficacy of Hu-1.4/1.1 and Hu-1.6/1.1 was explored in a murine model of thigh infection. Abscess size reduction was observed by day 5 in mice treated with Hu-1.4/1.1 and Hu-1.6/1.1. The number of CFUs was lower in abscesses of mice treated with Hu-1.4/1.1 and Hu-1.6/1.1, compared with mice that received PBS (Figure 4A). As previously observed, the infection remained localized. Analysis of the peripheral complete white blood cell count revealed a trend toward lower leukocyte and neutrophil counts in the mAb treatment group, compared with the PBS group (data not shown). Multiplex cytokine analysis of abscess fluid yielded significantly lower levels of mIL-1ra (Figure 4B) in abscesses of mice treated with Hu-1.4/1.1, Hu-1.6/1.1, and murine mAb 20B1, compared with mice treated with PBS (P < .01), whereas interleukin 2 (IL-2), interleukin 4, interleukin 5, interleukin 10 (IL-10), interleukin 12 (IL-12), IFN-γ, and tumor necrosis factor α (TNF-α) levels in abscesses of mice were comparable (data not shown). ELISA confirmed the presence of SEB in the abscesses of all mice. Consistent with reduced abscess size and CFU count, SEB levels in mAb-treated mice were lower than those in PBS recipients and vancomycin recipients (Figure 4C). Additional ELISAs showed significantly lower levels of unbound SEB in the abscesses of mice treated with Hu-1.4/1.1, Hu-1.6/1.1, and mAb 20B1, compared with those treated with PBS (P < .05), when detection was performed with IgG2a isotype switch variants of mAb 20B1 (Figure 4D). In contrast, SEB remained detectable in mice that received PBS and those that received vancomycin. This finding was consistent with the conclusion that Hu-1.4/1.1 and Hu-1.6/1.1 bound their target SEB in vivo.
Treatment With Hu-1.6/1.1 Enhances Survival of Mice With S. aureus Sepsis

The protective efficacy of Hu-1.6/1.1 and Hu-1.4/1.1 was further explored using a murine S. aureus sepsis model (10 mice/group). These experiments demonstrated that intravenous treatment with Hu-1.6/1.1 (500 µg) in mice with S. aureus sepsis resulted in 90% survival ($P = .003$), compared with 10% survival among mice treated with isotype control mAb. The survival benefit was demonstrated in a dose-dependent manner (Figure 5A) and was still apparent at lower doses. The efficacy of treatment with Hu-1.6/1.1 (500 µg) in mice with S. aureus sepsis resulted in 90% survival ($P = .003$), compared with 10% survival among mice treated with isotype control mAb. The survival benefit was demonstrated in a dose-dependent manner (Figure 5A) and was still apparent at lower doses. The efficacy of

Figure 2. Humanized antibodies (Ab) inhibit staphylococcal enterotoxin B (SEB)–induced proliferation in murine splenocytes. A, Humanized (Hu) 20B1 variant VH1.4/VL1.1 with a median inhibitory concentration ($IC_{50}$) of 0.64 nM. B, VH1.6/VL 1.1 with an $IC_{50}$ of 0.37 nM. C, VH1.6/VL 1.4 with an $IC_{50}$ of 0.28 nM. D, Humanized 20B1 variant VH1.10/VL1.4 with an $IC_{50}$ of 1.5 nM. The circles represent an average of triplicates of the relative luminescence units (RLUs). Bars represent the standard error derived from measurements in triplicate wells in the same experiment.

Figure 3. BALB/c mice (5 mice/group) were treated with humanized (Hu) monoclonal antibodies (mAbs) 1.4/1.1 and 1.6/1.1, mAb 20B1, and phosphate-buffered saline (PBS) intraperitoneally and challenged with 20 µg of staphylococcal enterotoxin B (SEB) and 25 mg of galactosamine intraperitoneally. Mice treated with Hu-1.4/1.1 and Hu-1.6/1.1 showed significantly higher survival, similar to that for those that received mAb 20B1, compared with mice that received PBS ($P = .01$). Analysis of survival data were performed using the log-rank (Mantel-Cox) test. Inflammatory cytokine interferon γ (IFN-γ) levels 2 and 8 hours after SEB intoxication were significantly lower ($P<.01$) in blood of mice treated with either mAb 20B1, Hu-1.4/1.1, or Hu-1.6/1.1, compared with mice that received PBS.
Hu-1.4/1.1 was found to be less potent in this model (40% survival at a dose of 500 \( \mu \)g; data not shown). Next, we investigated whether the efficacy of a lower dose of Hu-1.6/1.1, which by itself resulted in 40% survival, would be more effective if combined with vancomycin, which also has a similar efficacy when used alone. These experiments demonstrated that combination treatment with Hu-1.6/1.1 (250 \( \mu \)g) and vancomycin significantly enhance survival (90%) in mice with MRSA sepsis. In mice with MRSA sepsis that were treated with combination therapy (Figure 5B), a survival benefit was observed when compared to mice that received PBS (\( P = .002 \)), vancomycin alone (\( P = .08 \)), and Hu-1.6/1.1 alone (\( P = .09 \)).

Cytokine levels were measured in serum of mice on days 0 and 3 after infection. Two-way ANOVA of the data set for IFN-\( \gamma \) showed a significant interaction between vancomycin and Hu-1.6/1.1, suggesting a synergistic effect (Table 2). The 1-way ANOVA showed a significant effect for both. The post hoc comparisons for IFN-\( \gamma \) showed the following groups to be significantly different (\( P < .05 \)): PBS–Hu-1.6/1.1; PBS–Hu-1.6/1.1 plus vancomycin; vancomycin–Hu-1.6/1.1 plus vancomycin; and vancomycin–PBS. No significant interaction was observed between vancomycin and antibody for interleukin 1\( \beta \) (IL-1\( \beta \)), interleukin 6 (IL-6), IL-10, IL-12, KC/GRO, and TNF-\( \alpha \), which confirmed that use of an additive model is appropriate. The 2-way ANOVA with an additive model revealed a significant effect of antibody on IL-10 and of vancomycin on IL-1\( \beta \), IL-10, interleukin 12p70 (IL-12p70), and TNF-\( \alpha \) (\( P < .05 \)). The post hoc comparisons to investigate the vancomycin or Hu-1.6/1.1 effect showed a significant difference for IL-1\( \beta \) (between the PBS–Hu-1.6/1.1 plus vancomycin group and the vancomycin–PBS group), IL-10 (between the PBS–Hu-1.6/1.1 group, the PBS–Hu-1.6/1.1 plus vancomycin group, the vancomycin–Hu-1.6/1.1 plus vancomycin group, and the vancomycin–PBS group), IL-12p70, and TNF-\( \alpha \) (the groups containing vancomycin and the ones in which it was absent).

**DISCUSSION**

At present, anti-SEB therapeutic or prophylactic measures are not licensed for human use, and there are no licensed

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**Figure 4.** In vivo thigh infection model (10 mice/group). A, The colony-forming unit (CFU) count was statistically lower in abscesses of mice treated with monoclonal antibody (mAb) 20B1, compared with those that received phosphate-buffered saline (PBS) or isotype control (IC). Trends of lower CFU counts were observed in mice treated with humanized (Hu) mAb 1.4/1.1 and, to a lesser extent, with Hu-1.6/1.1. B, Hu mAb treatment significantly lowered the level of interleukin 1ra (IL-1ra) in the abscesses of mice, compared with PBS. Each point represents the analysis of an individual mouse. Statistically significant differences were calculated using the 2-tailed heteroscedastic \( t \) test. C, The presence of staphylococcal enterotoxin B (SEB) was significantly lower in mAb-treated mice, compared with PBS recipients, when detection of mAb 6D3-IgG2a was used. D, Unbound SEB was significantly higher only in the abscesses of the vancomycin or PBS group, compared with the mAb20B1 and Hu mAb groups (\( P < .01 \)). Abbreviation: SI, sham incision.
immunotherapeutic agents for superantigens as potential adjuvant therapy in *S. aureus* sepsis. Almost all Food and Drug Administration (FDA)-licensed mAbs are human or humanized Abs, to avoid the potential risk of suboptimal pharmacokinetics of murine mAbs and the potential risk for allergic reactions in humans. Inhalational exposure to SEB in humans rapidly leads to symptoms, including fever, within a few hours of exposure [17]. This supports the notion that SEB-specific mAbs for human use will be most effective only if they have high affinity. Both Hu-1.6/1.1 and Hu-1.4/1.1 exhibited low nanomolar affinity to SEB, and accordingly they effectively blocked superantigen action both in vitro and in vivo. In previous studies, the murine parent mAb 20B1 was shown to neutralize the proliferation of T cells and inhibit the IFN-γ and IL-2 secretion in vitro, as well as prevent death in 2 murine models for SEB toxin–induced lethal shock [9]. In the clinical setting, however, the majority of SEB-associated diseases can be expected to occur in the setting of *S. aureus* infection. It is therefore reasonable to investigate the potential use of these mAbs in *S. aureus* infection models and to study whether treatment with humanized mAbs can also affect outcomes. Other reported human SEB-specific mAbs with nanomolar affinities have been tested only in vitro [18] or in SEB-induced lethal shock models that use lower doses of SEB and are potentiated by lipopolysaccharide if given 3–4 hours after exposure [18, 19]. One other lead humanized mAb [20] demonstrated good post exposure efficacy in systemic intoxication model, but like the others it has not been tested in sepsis or *S. aureus* infection models. In addition, soluble, high-affinity Vβ proteins have also been shown to be effective in the neutralization of SEB in vitro, as well as for protecting rabbits from pneumonia [21, 22]. There is, however, less human trial experience with these novel reagents, whereas >30 mAbs are now licensed by the FDA, and therefore more is known about the formulation, dosing, and pharmacokinetics of mAbs.

Similar to our previous study, which demonstrated mAb 20B1–mediated protection in *S. aureus* sepsis, a significant survival benefit was observed after treatment with Hu-1.6/1.1. Reasons for the lack of efficacy of Hu-1.6/1.1 in the *S. aureus* thigh infection model, despite its superiority in the sepsis model, are not clear. The 2 humanized mAbs differ by only 1 amino acid; however, a high variability in the pathogen burden may have contributed to the lack of significance. Other investigators have observed such variability, as well [23]. Cytokine analysis was more consistent. In this study, we also established a clear correlation between efficacy and dose. Previously published

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**Table 2. Multiplex Cytokine Analysis of Serum From 10 Mice Treated With Hu-1.6/1.1, Hu-1.6/1.1 Plus Vancomycin (Van), Van Alone, or Phosphate-Buffered Saline (PBS) and Infected Intravenously With 5 × 10⁷ Colony-Forming Units of *Staphylococcus aureus* Strain 38**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IFN-γ¹</th>
<th>IL-10²,³</th>
<th>IL-12p70²,³</th>
<th>IL-1β⁴</th>
<th>IL-6</th>
<th>KC/GRO</th>
<th>TNF-α⁵</th>
</tr>
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<tbody>
<tr>
<td>Hu-1.6/1.1</td>
<td>2.26 ± 0.77²⁺</td>
<td>12.42 ± 1.61²⁺</td>
<td>46.77 ± 9.96²⁺</td>
<td>5.03 ± 1.74</td>
<td>433.50 ± 314.41</td>
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<tr>
<td>Hu-1.6/1.1_Van</td>
<td>1.66 ± 0.89²⁺</td>
<td>9.04 ± 2.76²⁺</td>
<td>42.88 ± 11.29²⁺</td>
<td>4.58 ± 3.97</td>
<td>384.27 ± 363.03</td>
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<td></td>
</tr>
<tr>
<td>Van</td>
<td>2.98 ± 1.64</td>
<td>16.75 ± 6.67</td>
<td>61.55 ± 23.71²⁺</td>
<td>3.51 ± 1.08²⁺</td>
<td>397.72 ± 335.46</td>
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<tr>
<td>PBS</td>
<td>5.03 ± 1.33</td>
<td>17.26 ± 4.12</td>
<td>69.33 ± 28.54</td>
<td>5.87 ± 1.69</td>
<td>465.80 ± 255.05</td>
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</tbody>
</table>

Data are mean ± standard errors.  
Abbreviations: IFN-γ, interferon-γ; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-12, interleukin 12; IL-12p70, interleukin 12p70; TNF-α, tumor necrosis factor α.  
¹ Synergistic effect of Van and monoclonal antibody.  
² Additive effect of monoclonal antibody.  
³ Additive effect of vancomycin.  
⁴ P < .05, by the t test, showing significantly lower levels of IFN-γ, IL-10, IL-12p70, and TNF-α, compared with PBS-treated mice.
experiments demonstrated efficacy even if Abs were given 2 hours after infection. Those experiments could not demonstrate a significant effect on serum levels of IL-6, IL-1β, IFN-γ, or IL-10, which are associated with poor outcomes in human S. aureus sepsis [10]. Therefore, cytokine levels were measured in this study at 72 hours, as the most-pronounced changes were seen in the prior study between 48 and 96 hours. Significantly lower IFN-γ, IL-10, and IL-12p70 levels are now seen in treated mice.

Complicated MRSA infections are inherently difficult to treat, and despite the availability of effective antibiotics high rates of treatment failure and mortality are commonly encountered in deep complicated tissue infection, as well as in sepsis [24]. This study demonstrates that standard therapy with vancomycin can be made more efficacious if combined with Hu-1.6/1.1. So far, the FDA has approved 2 anti-infective mAbs [3, 25] for use in humans; other anti-infective mAbs are currently being tested in human clinical trials [26, 27]. In a randomized, double-blind, placebo-controlled study that tested human mAbs against Clostridium difficile toxins A (CDTA) and B (CDB1), the Abs were also administered as adjunctive therapy in patients with symptomatic C. difficile infection who were receiving either metronidazole or vancomycin [26]. Given the high mortality and morbidity of many infections, anti-infective agents, in most cases, will be administered as an adjunct to appropriate antibiotic therapy, rather than as monotherapy.

Of note, murine mAb 20B1 appeared to be slightly more effective than the humanized variant in the thigh model. Treatment with Hu-1.4/1.1 and Hu-1.6/1.1 resulted in lower CFU counts and lower SEB levels in abscesses. It also modulated the immune response, but inhibition of neutrophil recruitment was only marginal. Although Hu-1.4/1.1 bound SEB in vivo, it is conceivable that the mAb must also bind to FcyR on inflammatory cells to optimize its effect on the host response. In the sepsis model, this may be achieved only by neutralizing the toxin efficiently, but in the thigh model it may require engagement of FcyR to neutralize the effects of SEB toxin on host cells [28]. A future study with chimeric Abs and genetically modified mice will have to be designed to conclusively investigate the requirement of FcyR binding for optimal efficacy. Of importance, our data highlight that Hu-1.6/1.1 is an excellent lead candidate to be developed further for treatment of human diseases. We also demonstrated the feasibility of generating initial murine mAbs with subsequent humanization of potent lead candidates. This approach may also be important in generating cross-reactive mAbs that recognize >1 enterotoxin. Before moving forward with the development of passive immunotherapy for S. aureus, the challenge of S. aureus strain diversity in the empirical treatment of infections due to S. aureus clones [12, 29] has to be overcome. Pretreatment typing would not be feasible in septic patients, who require immediate empirical therapy. We concur with other investigators that mAb cocktails targeting various virulence factors [30, 31] may ultimately constitute the best treatment option. Opposed to Clostridium botulinum and C. difficile toxin, for which combinations of mAbs or polyclonal Abs were required for efficient toxin neutralization [32, 33], humanized mAb 1.6/1.1 alone is sufficient to neutralize SEB toxin, although previous data from our laboratory also indicated that combination of mAbs may even enhance neutralization efficacy [9]. For S. aureus infections, several polyclonal or mAbs directed against surface determinants such as IsaA, IsdB, ClfA, and ABC transporter have showed promising preclinical results, and even though many have failed as monotherapy in clinical trials [30, 34–36] they could still be efficacious in treating S. aureus sepsis when combined. Although therapeutic mAbs are expensive, given the medical costs of MRSA infection [37, 38] continued preclinical evaluation of these mAbs against complicated staphylococcal infections caused by SEB-excreting S. aureus isolates is warranted.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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