Toxicon 113 (2016) 55–59

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Preliminary investigation of human serum albumin-Vβ inhibition on toxic shock syndrome induced by *staphylococcus* enterotoxin B *in vitro* and *in vivo*

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ARTICLE INFO

Article history:
Received 19 December 2015
Received in revised form
6 January 2016
Accepted 10 January 2016
Available online 12 January 2016

Keywords:
Staphylococcus enterotoxin B
Human serum albumin
Toxic shock syndrome

ABSTRACT

Staphylococcus enterotoxin B (SEB) is a superantigen that can induce massive activation of T cells with specific Vβ and inflammatory cytokine cascades, which mediate shock. To date, no SEB vaccine has been developed for preventing toxic shock syndrome (TSS). Here, we evaluated the therapeutic effect of a fusion protein human serum albumin-Vβ (HSA-Vβ) on TSS induced by SEB. Compared with Vβ, the preparation of HSA-Vβ was much easier to handle owing to its solubility. Affinity testing showed that HSA-Vβ had high affinity for SEB. *In vitro* results showed that HSA-Vβ could effectively inhibit interferon (IFN)-γ and tumor necrosis factor (TNF)-α secretion by human peripheral blood mononuclear cells. Moreover, *in vivo*, HSA-Vβ reduced IFN-γ and TNF-α levels in the serum and protected mice from SEB lethal challenge when administered simultaneously with SEB or 30 min after SEB. In summary, we simplified the preparation of Vβ by fusion with HSA, creating the HSA-Vβ protein, which effectively inhibited cytokine production and protected mice from lethal challenge with SEB. These data indicated that HSA-Vβ may represent a novel therapeutic strategy for the treatment of SEB-induced TSS.

1. Instruction

SEB is produced by *Staphylococcus aureus* and has been implicated in many human diseases, such as food poisoning and toxic shock syndrome (TSS) (Torres et al., 2001). Moreover, SEB may be a potential biological warfare agent because it is simple and inexpensive to produce, stable in aerosol form, and easy to disperse (Henghold, 2004). Accordingly, SEB is listed as a category B priority agent by the Centers for Disease Control and Prevention (CDC). As a type of superantigen, SEB stimulates robust activation of T cells bearing certain T-cell receptor (TCR) Vβ elements, generating massive amounts of inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ, even when administered at picomolar concentrations (Gu et al., 2013; Jie et al., 2007; Wang et al., 2010). Such a cytokine storm in turn damages the tissues and may cause food poisoning or TSS.

Many researchers have been focused on the development of novel vaccines. For example, some groups have worked to develop anti-inflammatory agents that can inhibit staphylococcal exotoxin-induced T-cell proliferation and cytokine release, such as Pirfenidone (Hale et al., 2002) and dexamethasone (Krakauer and Buckley, 2006). Alternatively, some groups have focused on protein antagonists that inhibit the activation of T cells at the beginning of the toxicity cascade. Arad and colleagues designed a structurally conserved peptide antagonist that could inhibit cytokine expression and protect mice against lethal challenge (Arad et al., 2000). Additionally, Kranz and colleagues developed some effective receptor antagonists (Buonpane et al., 2007). Although many efforts had been made in the field of SEB vaccine research and development, no approved vaccines have yet been developed to prevention

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http://dx.doi.org/10.1016/j.toxicon.2016.01.050
0041-0101/

of SEB-related diseases.

Therefore, in the current study, we aimed to evaluate the therapeutic efficacy of the fusion protein human serum albumin (HSA)-Vβ for the treatment of SEB-induced TSS.

2. Materials and methods

2.1. HSA-Vβ preparation

HSA and Vβ were fused by overlap polymerase chain reaction (PCR). The linker between HSA and Vβ was GGGGSGGGGSGGGGS. The fusion protein was expressed in GS115 cells cultured in BMGY medium at 30 °C with shaking at 250 rpm. Every 24 h, 0.5% methanol was added to the culture. The supernatant was precipitated with 1 M $(NH_4)_2SO_4$ and then purified using Phenyl Sepharose HP and Source 30Q (GE Healthcare Life Science, Pittsburgh, PA, USA). The purity was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2.2. Affinity measurement

The affinities of HSA-Vβ and HSA for SEB were determined using biolayer interferometry (BLI) on a BLItz instrument (ForteBio; Pall Life Sciences, NY, USA). SEB was first biotinylated using a Thermo EZ-Link Sulfo–NHS–LC-Biotinylation kit according to the manufacturer's instructions. Next, the biotinylated SEB (0.391 mg/mL) was immobilized via a standard streptavidin-biotin-coupling procedure using an SA-sensor (ForteBio; Pall Life Sciences, NY, USA). The tip of the sensor was immersed in HSA-Vβ or HSA solutions having different concentrations. The association and dissociation phases were recorded for 120 s. The dissociation constant (KD) was calculated using the software associated with the instrument.

2.3. Human cytokine detection

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood of healthy donors using Ficoll–Paque PLUS density gradient centrifugation (GE Healthcare; endotoxin tested < 0.12 EU/mL), as previously described (Gu et al., 2013). Freshly isolated PBMCs were suspended in RPMI 1640 culture medium containing 10% fetal bovine serum (FBS) and then seeded (1×10^6 cells/well) into 24-well plates. Different stimuli were added to the wells. After incubating for 16 h at 37 °C in an atmosphere containing 5% CO_2, 100 μL of supernatant was used to test the concentrations of cytokines. TNF-α and IFN-γ levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (cat. nos. EHC103A for TNF-α and EHC102g for IFN-γ; NeoBioscience, Shenzhen, China). Absorbance values at 490 nm were recorded using a SpectraMax Plus spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

2.4. Animal experiment

To evaluate the therapeutic effects of HSA-Vβ, we used a lipopolysaccharide (LPS)-potentiated murine model for SEB-induced shock (Hale et al., 2002). Two treatment models were evaluated. For the first model, 1 μg SEB (about 0.03 nmol) in 0.1 ml was first mixed with 25 ug (about 0.32 nmol) or 125 ug (about 1.6 nmol) of HSA-Vβ in 0.1 ml or corresponding equimolar amounts of HSA in 0.1 ml at 4 °C overnight. Female BALB/C mice (18–22 g/mouse, 7–10 mice/group) then received an intraperitoneal (i.p.) inoculation of SEB plus HSA-Vβ, SEB plus HSA, SEB alone or PBS. Four hours later, mice receiving SEB, SEB plus HSA-Vβ or HSA, PBS alone were administered 80 μg LPS purified from *Escherichia coli* 055:B5 (Sigma Aldrich, St. Louis, MO, USA). The mice were checked every 12 h. The serum was collected 6 h after SEB was administered, and cytokine levels in the serum were measured using ELISAs (EMC101g for IFN-γ and EMC102a for TNF-α; NeoBioscience, Shenzhen, China). For the second model, SEB was administered i.p. at 1 μg/mouse (about 0.03nmol/0.2 ml·mouse). Thirty minutes later, mice were treated with 25ug/mouse (about 0.32nmol/0.2 ml·mouse , i.e. 1.6 μM) or 125ug/mouse (about 1.6 nmol/0.2 ml·mouse , i.e. 8 μM) HSA-Vβ or HSA i.p. Each group contained 10–11 mice. The remaining experimental procedures were the same as those for the first model.

All animals were purchased from the Animal Center of the Academy of Military Medical Science (AMMS) and housed under specific pathogen-free (SPF) conditions. All experimental procedures were approved by and carried out in accordance with the guidelines of the Animal Experiment Committee of Beijing Institute of Microbiology and Epidemiology.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) and Kaplan–Meier survival analysis were performed using SPSS software, version 19. The results are presented as the mean ± standard deviation (SD). The significance level was set at 0.05.

3. Results

3.1. HSA-Vβ was expressed in yeast and exhibited high affinity for SEB

In order to be expressed stably in yeast, the gene sequence of Vβ was optimized and fused with HSA. For optimization, yeast biased codons were chosen to improve the stability of mRNA. Moreover, by fusion with HSA, HSA-Vβ was expressed in soluble form in GS115 yeast cells. The production yield reached about 25%, and after purification, the fusion protein was more than 95% pure (Fig. 1). Affinity testing using BLItz showed that the KD for the interaction between HSA-Vβ and SEB was 7.272×10^{-9} M (Fig. 2A). In contrast,

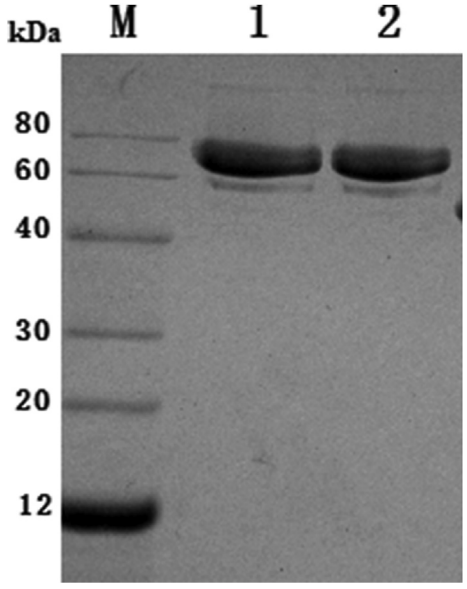

Fig. 1. SDS-PAGE analysis of HSA-Vβ. HSA-Vβ was purified by affinity purification. Twenty microliters of the elution fraction was added per lane to test the purity of the protein. Lane M: low-molecular-weight protein marker; Lanes 1 and 2: different elution fractions of HSA-Vβ.