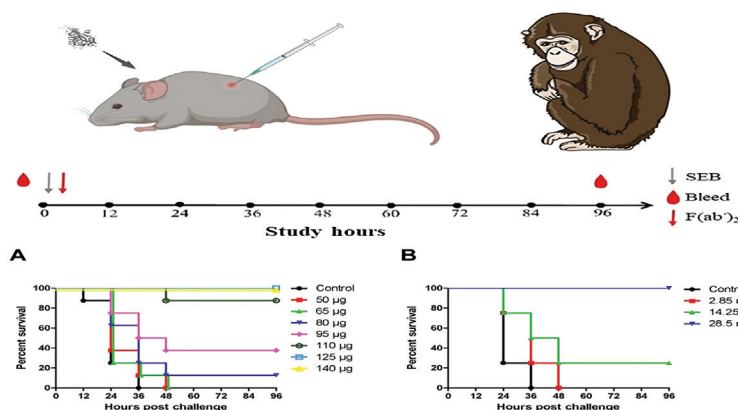




ORIGINAL ARTICLE

Purified Immunoglobulin F(ab')₂ Prevents Lethal Staphylococcal Enterotoxin B Intoxication in Mice and Rhesus Monkeys

Graphical abstract



Highlights

- An antibody that efficiently blocks lethal SEB-induced toxicity.

Authors

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Brief statement

Purified F(ab')₂ fragments are an effective antidote for a lethal dose of SEB in mice and rhesus monkeys, suggesting their potential of being a good candidate for treating patients suffering from severe SEB intoxication.

Purified Immunoglobulin F(ab')₂ Prevents Lethal Staphylococcal Enterotoxin B Intoxication in Mice and Rhesus Monkeys

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Abstract

Objective: Staphylococcal enterotoxin B (SEB), an exotoxin produced by single- or multi-drug-resistant *Staphylococcus aureus* (*S. aureus*), can induce food poisoning and toxic shock syndrome. Because no treatment is available for SEB-poisoned patients, development of a safe and effective SEB antidote is urgently needed.

Methods: First, SEB was prepared, and native SEB (nSEB) was used to construct lethal mouse and rhesus monkey models. Second, F(ab')₂ fragments of IgG antibodies were cleaved with pepsin from horses inoculated with Freund's adjuvant-purified nSEB. Finally, protective efficacy was evaluated in mouse and rhesus monkey models of lethal SEB intoxication.

Results: In mouse and monkey model studies, the purity of the prepared nSEB reached 90%, and that of the F(ab')₂ fragments reached 83.09%. In mice and rhesus monkeys, the median lethal dose (LD₅₀) of staphylococcal enterotoxin B (SEB) was 21.87 µg/kg and 23.77 µg/kg, respectively. Additionally, administration of 6.25 mg/kg and 7.125 mg/kg of F(ab')₂ fragments, respectively, effectively prevented SEB-induced lethality. Finally, single-cell sequencing of peripheral blood immune cells was used to detect the effects of the therapeutic antibody on peripheral blood immune cells. The underlying mechanism was found to involve inhibition of neutrophil activation, proliferation, and differentiation.

Conclusion: Purified F(ab')₂ fragments were an effective antidote to lethal SEB doses in mice and rhesus monkeys, and therefore might be a favorable candidate for treating patients with severe SEB intoxication.

Key words: SEB, Equine immunoglobulin F(ab')₂ fragments, Animal models

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INTRODUCTION

Staphylococcal enterotoxin B (SEB) is a protein exotoxin derived from *Staphylococcus aureus* (*S. aureus*) and a member of the superantigen family [1]. SEB poisoning in humans and livestock typically occurs accidentally, through ingestion of poisoned foods or inhalation of SEB aerosols. Clinical toxicological

studies have shown severe damage to the lungs after SEB inhalation or the small intestine after SEB ingestion [1]. Different exposure routes lead to varying clinical symptoms. SEB ingestion causes nausea, vomiting, abdominal cramps, and diarrhea; SEB inhalation results in fever, respiratory complaints, and gastrointestinal symptoms; and severe intoxication leads to pulmonary edema, adult

respiratory distress syndrome, or even death [2,3]. In addition, because of the relative ease of SEB production, the rise of multi-drug-resistant *S. aureus*, and lethality after exposure to minute amounts of aerosols, SEB is considered a high-risk toxin and a research focus of interest [1,4]. An effective countermeasure against SEB intoxication must critically be developed.

Despite SEB's substantial threat to public health, no therapies have been approved for the prevention and treatment of SEB poisoning. STEBVax, an *Escherichia coli*-expressed recombinant SEB vaccine, is under investigation in phase I clinical trials [4]. Furthermore, several nontoxic SEB mutant vaccines have achieved complete protection against lethal SEB challenge in mice and therefore might provide an effective recombinant SEB vaccine candidate [5]. To date, the treatment of patients with SEB poisoning has primarily been supportive, given the lack of specific antidotes. Many monoclonal antibodies (such as M0313 and 20B1) with potent SEB-neutralization activity, and inhibitors of SEB-mediated T cell activation or inflammatory cytokines, have shown therapeutic effects in SEB poisoned cells and/or animal models [6–10]. In cell culture, M0313 effectively inhibits SEB-induced proliferation and cytokine release by mouse spleen lymphocytes and human peripheral blood monocytes. However, much research remains necessary to develop an effective SEB-specific antidote.

To guide the development of a safe curative medicine, we evaluated the protective efficacy of equine immunoglobulin F(ab')₂ fragments, which were prepared as previously described in mouse and rhesus monkey models [11,12]. The pepsin-digested F(ab')₂ fragments of serum IgGs from horses inoculated with Freund's-adjuvanted purified SEB protected mice and rhesus monkeys from SEB lethality, and therefore might be a favorable candidate treatment for patients with severe SEB intoxication.

MATERIALS AND METHODS

S. aureus strains and animals

The *S. aureus* strain (WS-1) was purchased from the National Institutes for Food and Drug Control (Beijing, China). Healthy horses 4–6 years old were provided by Inner Mongolia Huaxi Bio-technology Co., Ltd. (Inner Mongolia, China). Eight-week-old female BALB/c mice were purchased from Beijing SPF Biotechnology Co., Ltd. (Beijing, China). Rhesus monkeys were purchased from the Beijing Laboratory Animal Center (Beijing, China). All animal experiments were approved by the Animal Experiment Committee of Preventive Medicine Research Project, Shandong University, China (assurance number LL20220603).

Preparation and identification of purified SEB

First, the *S. aureus* strain (WS-1) was rejuvenated in Dolman medium (Gibco, Grand Island, NY, USA) at 37°C overnight. Second, single WS-1 colonies were

collected on Dolman solid medium at 37°C for 48 hours and grown in Dolman medium at 37°C overnight. Third, to scale up the culture, we cultured WS-1 at 1% of the inoculation volume on Dolman solid medium at 37°C for 48 hours. Bacteria were collected with sterile saline (Shijiazhuang No.4 Pharmaceutical Co., Ltd., Hebei, China) and centrifuged at 20000 g for 10 min. The supernatant was concentrated with polyethylene glycol 8000 (Sigma-Aldrich Trading Co., Ltd., Shanghai, China) and dialyzed in a dialysis bag (Spectrumlabs, California, USA) with a 5 kD molecular weight cut-off at 4°C. Next, crude SEB was collected with cation exchange resin (CM-32). Finally, purified SEB was obtained through gel filtration chromatography (Sephadex G-75, Cytiva, USA) and stored at 4°C. For purity assessment, the purified SEB was assayed with SDS-PAGE to determine the intensity of the target protein bands, and with HPLC to determine the ratio of the target peak height to the total peak height.

To measure the median lethal dose (LD₅₀) of purified SEB, we intraperitoneally injected eight mice per group with 0.125, 0.25, 0.5, 1, 2, or 4 µg SEB. After 4 hours, 50 µg LPS [13] was intraperitoneally injected. Rhesus monkeys (four per group) were intraperitoneally injected with 60, 120, or 180 µg SEB. The mice and rhesus monkeys were monitored for 4 days. The LD₅₀ of the purified SEB was measured with the Reed-Muench method [14].

Preparation and identification of F(ab')₂ fragments of equine serum IgGs against SEB

We subcutaneously inoculated five healthy 4-to-6-year-old horses without detectable pathogens near the inguinal and submandibular lymph nodes with 2.0, 4.0, 8.0, or 16.0 mg purified SEB (with Freund's adjuvant) every 21 days. Two weeks after the final immunization, the activity of horse serum against SEB was detected according to inhibition of the colony-forming ability of SEB-sensitized spleen lymphocytes. When the serum titers exceeded 1:128, sera were collected and stored at 4°C.

The F(ab')₂ fragments of horse serum IgGs were prepared in the GMP facility of Shanghai Serum Biotechnology Co., Ltd., and stored at 4°C. The products were characterized by HPLC and sodium SDS-PAGE to ensure high quality and purity. SDS-PAGE was used to identify the F(ab')₂ fragments' purity according to the intensity of the target protein bands, and HPLC was used to identify the purity according to the ratio of the target peak height to the total peak height.

The neutralization titers of the F(ab')₂ fragments against SEB were determined by inhibition of colony formation by SEB-sensitized splenic lymphocytes [15]. The splenic lymphocytes of mice were aseptically isolated according to the Lymphocyte Separation Kit operation manual (Solarbio, Beijing, China). The splenic lymphocytes were transferred to a 96-well plate (Corning, New York, USA), at 1×10⁵ cells (100 µl) per well, and incubated at 37°C.

The F(ab')₂ fragments were two-fold serially diluted in RPMI 1640 (Gibco, Grand Island, NY, USA) containing 5% FBS (Minhai Bioengineering Co., Ltd., Lanzhou, China). FBS was inactivated at 56°C for 30 minutes. After the F(ab')₂ fragments were mixed with isopyknic suspension containing 0.5 µg purified SEB and maintained at 37°C for 1 hour in an incubator (Thermo Forma 311, Massachusetts, USA), the F(ab')₂ fragments and SEB mixtures were added to the splenic lymphocytes, which were subsequently maintained in an incubator at 37°C for 4 days. The control groups were a normal F(ab')₂ fragment control (with 0.5 µg SEB and F(ab')₂ fragments of equine serum IgGs without inoculation) and an SEB control (with only 0.5 µg SEB). The highest dilution of F(ab')₂ fragments inhibiting half the colony-forming cells observed with the normal F(ab')₂ fragment control was used as the neutralization antibody titer of the F(ab')₂ fragments against SEB.

Effective dosages of F(ab')₂ fragments against SEB in mice and rhesus monkeys

To investigate the effective dosages of F(ab')₂ fragments against SEB, we weighed and intraperitoneally injected eight mice per group with 10 LD₅₀ of purified SEB. The mice were then intravenously injected with 50, 65, 80, 95, 110, 125, or 140 µg F(ab')₂ fragments against SEB at 3 hours post inoculation. Subsequently, the mice were intraperitoneally injected with 50 µg LPS (Sigma-Aldrich, USA) at 4 hours post inoculation, then monitored for mortality daily for 4 days. The mice injected with isopyknic normal equine F(ab')₂ fragments served as a control group.

To further investigate the effective dosages of F(ab')₂ fragments against SEB, we intraperitoneally injected four rhesus monkeys per group with 2 LD₅₀ of purified SEB. After 4 hours, the rhesus monkeys were intramuscularly injected with 2.85, 14.25, or 28.5 mg F(ab')₂ fragments against SEB. The monkeys' physical condition and mortality were monitored daily for 4 days. Four monkeys injected with isopyknic normal equine F(ab')₂ fragments served as a control group.

Histopathological analysis

Dying monkeys in the control group and those injected with 28.5 mg F(ab')₂ fragments 36 hours after inoculation were euthanized. Tissues from the small intestine and other main organs (such as the lungs, liver, gastrointestinal tract, and kidneys) were collected, fixed with 4% formalin (Sinopharm, Shanghai, China), paraffin-embedded, sectioned to 5 µm, stained with hematoxylin and eosin, and observed under a microscope (IX51, Olympus, Tokyo, Japan) [16].

Isolation of PBMCs

Two sets of mice, each containing ten mice, were abdominally injected with 2 LD₅₀ SEB in 200 µl. At 4 hours after SEB injection, a needle-free injection device was used to inject 0.5 ml containing 125 µg anti-SEB IgG or PBS

(Sinopharm, Shanghai, China) into mice in the treatment or control groups, respectively. At 24 hours after the injection, 1 ml peripheral blood was collected from the tail vein of each mouse. Subsequently, 3 ml red blood cell lysis buffer was added to the collected blood, which was incubated on ice for 5 min. PBS (5 ml) with 10% fetal bovine serum was added and mixed, and the samples were centrifuged at 500 g for 5 min. The supernatant was discarded, and the sediment was washed again with PBS containing 10% fetal bovine serum; this step was repeated twice. After a final centrifugation step, the supernatant was discarded, and the sediment was suspended in PBS and mixed to prepare a cell suspension. Five replicate samples of cells in the treatment group that met the sequencing requirements from the processing group, and a sample containing a mixture of five replicate samples of control group cells were prepared for single-cell sequencing analysis.

Single-cell sequencing library construction

Sequencing and subsequent data analysis were conducted by Shanghai Ouyi Biomedical Technology Co., Ltd. In the data analysis stage, we used Cellranger software from 10x Genomics to conduct preliminary quality control and determine gene expression statistics for the original high-throughput sequencing data. Subsequently, with the Seurat software package, we conducted deeper quality control and data processing. To effectively identify the dominant variation patterns in the data, we performed principal component analysis (PCA), a linear dimension reduction method. To intuitively present the analysis results, we further used the T-distributed stochastic neighbor embedding (t-SNE) method to transform the PCA results into two-dimensional space for visualization. For cell type identification, we used the SinglerR package and a public single cell reference data set. Through calculation of the correlation between the cell expression spectrum and the reference data set, the most correlated cell types were allocated to the cells to be identified, thereby avoiding interference from human factors to the greatest possible extent. On the basis of cell type identification, we used the FindAllMarkers function of the Seurat software package to further identify genes expressed in each cell classification relative to other cell groups. These differentially expressed genes (DEGs) were considered potential markers. To intuitively demonstrate the expression of these DEGs, we used functions such as FeaturePlot and HeatMap for visualization. Finally, we used the FindMarkers function to identify genes with statistically significant differential expression, using a threshold of $P < 0.05$ and a fold change > 1.5 .

Statistical analysis

Statistical analyses of the LD₅₀ of SEB, the effective dose of F(ab')₂ fragments against SEB in mice and rhesus monkeys, and the effects of anti-SEB antibodies in the peripheral blood, revealed by single cell sequencing, were performed in Prism software (GraphPad 5.0).

RESULTS

Production of SEB with high purity

Antibody preparation requires high quality antigen, we generated high-purity SEB with ion-exchange resin and gel filtration chromatography methods. We used SDS-PAGE and HPLC to measure the purity of the prepared SEB (28.3 kDa). The SEB preparation had >90% purity according to SDS-PAGE (Fig. 1A) and 96.1% purity according to HPLC (Fig. 1B).

The LD₅₀ of the SEB was then determined in mice and rhesus monkeys. The LD₅₀ of purified SEB was 21.87 µg/kg in mice (Fig. 2A) and 23.77 µg/kg in rhesus monkeys (Fig. 2B).

The F(ab')₂ fragments of equine serum IgGs protect mice against SEB intoxication

Five healthy horses 4–6 years old were immunized with purified SEB with Freund's adjuvant. Inhibition of colony-forming splenic lymphocytes was assessed to detect

the SEB specific antibody titer in horse sera 2 weeks after the last immunization. The serum titers were above 1:128, indicating a strong immune response. The GMP plant at Shanghai Serum Bio-technology Co., Ltd. prepared the F(ab')₂ fragments from equine serum IgGs. The purity of the F(ab')₂ fragments, as determined with two methods, was >80% according to SDS-PAGE (Fig. 3A) and 83.09% according to HPLC (Fig. 3B). The neutralization antibody titer of the F(ab')₂ fragments against SEB was 1:228.

To confirm the effective dosages of F(ab')₂ fragments against SEB, we constructed a lethal SEB intoxication mouse model. At 3 hours after inoculation, the mice were injected with 50, 65, 80, 95, 110, 125, or 140 µg F(ab')₂ fragments. The control group mice died within 36 h. All mice injected with 50 or 65 µg F(ab')₂ fragments died within 2 days. One mouse injected with 80 µg F(ab')₂ fragments survived and recovered by the fourth day, whereas the other mice died within 2 days. Three mice injected with 95 µg F(ab')₂ fragments survived and recovered by the fourth day, whereas the other mice died within 2 days. One mouse injected with

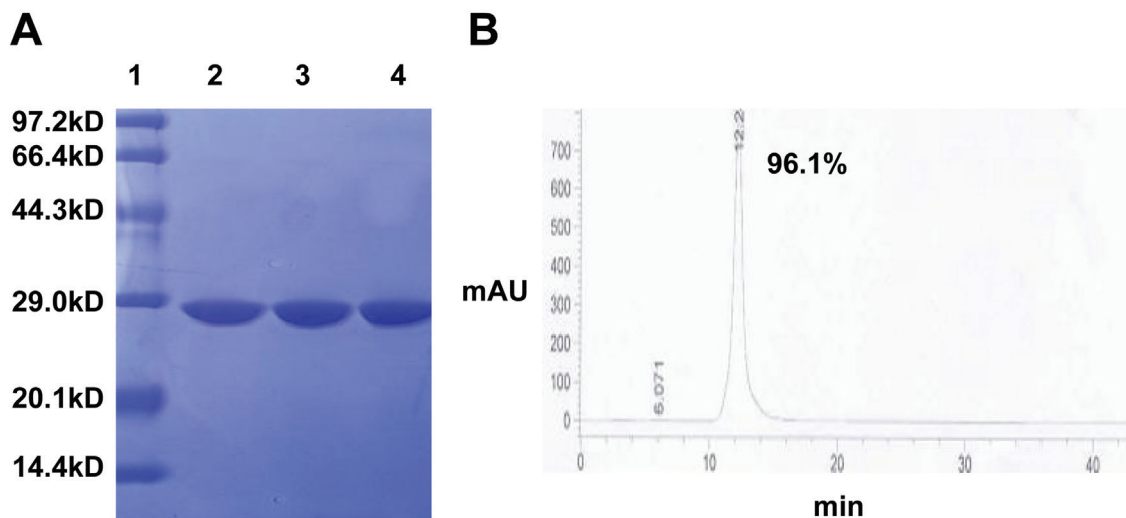


FIGURE 1 | Characterization of purified native SEB. (A) SDS-PAGE of purified native SEB. Lane 1, protein molecular weight ladder with the MW of each band indicated. Lanes 2–4, purified native SEB. (B) Purified native SEB, assayed with HPLC.

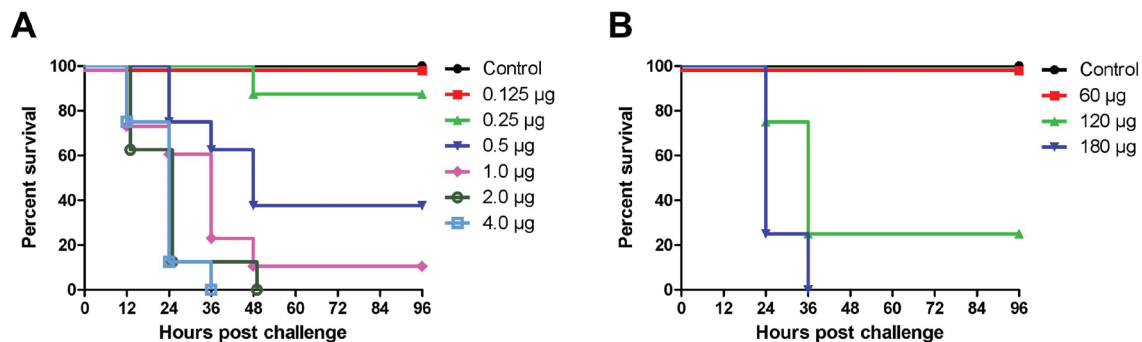


FIGURE 2 | The median lethal dose (LD₅₀) of SEB. (A) In the mouse model, mice (n=8/group) were intraperitoneally injected with six concentrations of purified native SEB and monitored for survival daily for 4 days. (B) In the rhesus monkey model, rhesus monkeys (n=4/group) were intraperitoneally injected with three concentrations of purified native SEB, and survival was monitored for 4 days. The LD₅₀ of SEB was measured with the Reed-Muench method.

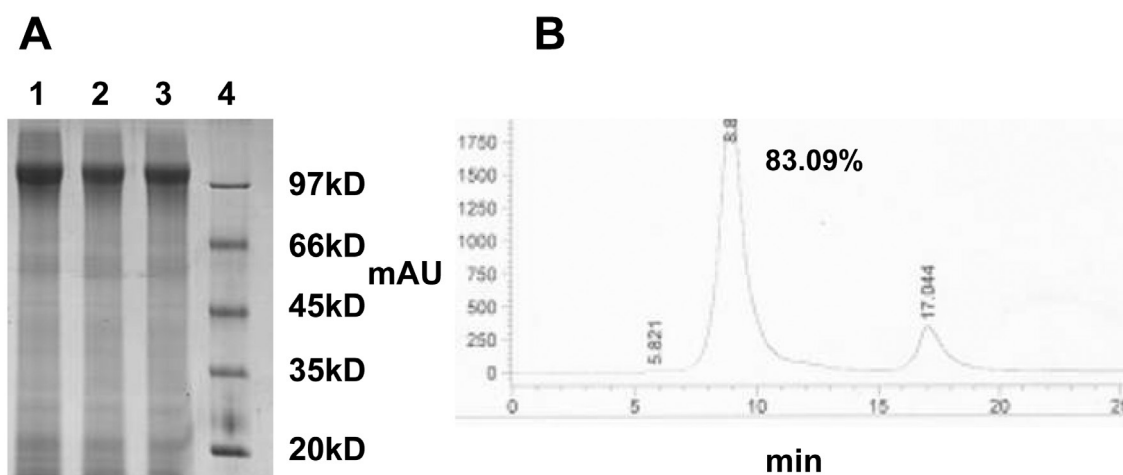


FIGURE 3 | Characterization of the $F(ab')_2$ fragments of equine serum IgGs against SEB. (A) SDS-PAGE of the $F(ab')_2$ fragments. Lanes 1–3, purified $F(ab')_2$ fragments. Lane 4, protein molecular weight ladder, with the MW of each band indicated. (B) Purified $F(ab')_2$ fragments, assayed with HPLC.

110 μg $F(ab')_2$ fragments died on the second day, whereas the other mice survived and recovered by the fourth day. All mice injected with 125 or 140 μg anti-SEB $F(ab')_2$ fragments against SEB survived and recovered by the fourth day. The survival rates of the mice are shown in Fig. 4A.

The $F(ab')_2$ fragments protect rhesus monkeys against SEB intoxication

To further confirm the effective dosages of the $F(ab')_2$ fragments against SEB, we constructed a rhesus monkey model of SEB intoxication through injection with 2 LD_{50} of purified SEB. Four hours later, the rhesus monkeys were injected with 2.85, 14.25, or 28.5 mg $F(ab')_2$ fragments, and their survival rates were assessed (Fig. 4B). All rhesus monkeys in the control group developed symptoms such as diarrhea, anorexia, and agitation, and died within 2 days. The histopathological results (Fig. 5) indicated exfoliation of the small intestinal mucosa. The rhesus monkeys were injected with 2.85 mg of $F(ab')_2$ fragments and monitored for 6 hours. All the monkeys exhibited symptoms of anorexia and agitation, and all succumbed to the effects by day 1 or day 2. After being injected with 14.25 mg of $F(ab')_2$ fragments, three monkeys exhibited symptoms of anorexia, while one monkey returned to normal condition after 48 hours of observation. At 0 hours after $F(ab')_2$ fragment injection with 28.5 mg, only one rhesus monkey showed anorexia and subsequently returned to normal after 24 hours, whereas the other rhesus monkeys showed no abnormalities. The histological morphology of all tissues from the rhesus monkeys injected with 28.5 mg $F(ab')_2$ fragments was normal.

$F(ab')_2$ fragments protect against lethal neutrophil toxicity in mice, according to single cell sequencing

The number of cells in the antibody therapy group and model group samples after quality control was 11209 and

7077, respectively. After clustering and analysis of DEGs, B cells, dendritic cells, monocytes, neutrophils, basophils, natural killer cells, and T cells were found to participate in the SEB poisoning process (Fig. 6A, B). Subsequently, we conducted statistical analysis of the percentages of eight peripheral blood mononuclear cell (PBMC) types (Fig. 6C).

PBMCs, mononuclear cells in the peripheral blood, include lymphocytes, monocytes, and dendritic cells. The lymphocytes comprise CD3+T cells (accounting for approximately 45%–70%), B cells (accounting for approximately 15%), and NK cells (accounting for approximately 15%). Monocytes account for approximately 10%–30%, whereas dendritic cells account for approximately 1%–2%. CD3+T cells comprise CD4+T (approximately 25%–60%) and CD8+T (approximately 5%–30%) cells, in an approximately 2:1 ratio. CD4+T cells and CD8+T cells can be further divided into naive T cells, antigen-exposed central memory T cells, effector memory T cells, and effector T cells.

CD4+T cells, also known as helper T cells, can be divided into functional subsets according to cytokine expression. CD8+ cells, also known as toxic T cells, can be divided into approximately 200 functional phenotypes.

Circulating B cells include transitional cells, unsensitized cells, memory cells, and plasmablasts, in differing proportions. Circulating dendritic cells include plasmacytoid dendritic cells and myeloid derived dendritic cells. Circulating monocytes can be divided into classical monocytes and non-classical CD16 pro-inflammatory monocytes (approximately 10% of monocytes).

The percentages of eight cell types in PBMCs are shown in Table 1. The results indicated a clear decline in the neutrophil proportion and a marked increase in B cells after antibody treatment in mice, which might have been associated with avoidance of the lethal consequences of SEB. Furthermore, B-Rag1, B-Lipg, B-Igcl1, B-Ighd, B-Hist1

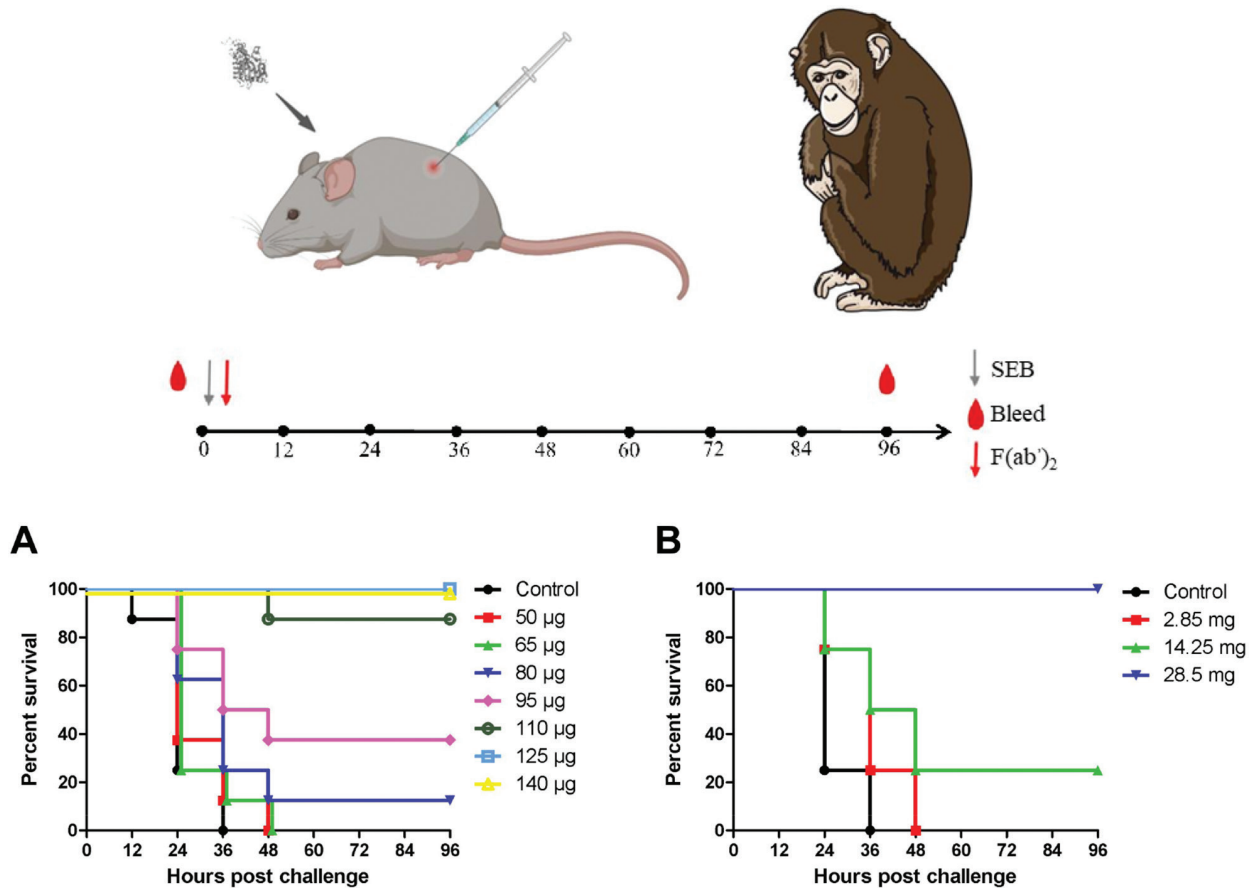


FIGURE 4 | Effective dose of F(ab')₂ fragments against SEB in mice and rhesus monkeys. (A) After injection with 0.2 ml of 10 LD₅₀ SEB for 3 hours, the mice (n=8/group) were injected with 0.2 ml of 50, 65, 80, 95, 110, 125, or 150 µg F(ab')₂ fragments. One hour later, the mice were intraperitoneally injected with 50 µg LPS. The mice injected with PBS served as a control group. The mice were monitored for mortality daily for 4 days. (B) After injection with 2 LD₅₀ SEB for 4 hours, the groups of rhesus monkeys (n=4/group) were injected with 2.85, 14.25, or 28.5 mg F(ab')₂ fragments. Rhesus monkeys injected with PBS served as a control group. The rhesus monkeys were monitored for physical condition and mortality daily for 4 days.

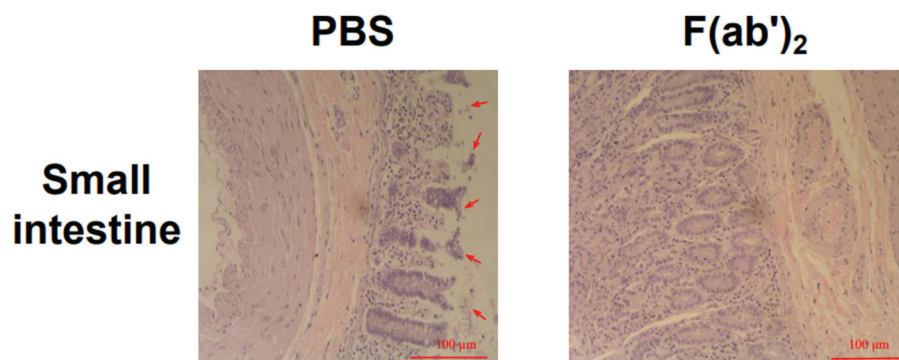
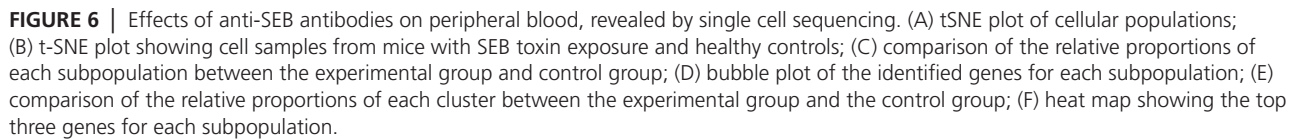


FIGURE 5 | Histopathological examinations of small intestinal tissues from rhesus monkeys injected with PBS or 28.5 mg F(ab')₂ fragments from Fig. 4. After the moribund monkeys from the control group and those injected with 28.5 mg F(ab')₂ fragments at 36 hours after inoculation were euthanized, small intestinal tissues were collected and subjected to pathological analysis.

h2ae, B-Hbb-bt, B-H2-Aa, B-Crip1, B-Ciita, B-Cecr2, B-Cd83, neutrophils-Wfdc17, neutrophils-Trbc2, neutrophils-Retnlg, neutrophils-Pf4, neutrophils-Ngp, and

neutrophils-Ill1b (Fig. 6D) were identified through transcriptomic characterization. The proportions of subtypes of neutrophils significantly changed after antibody treatment



Cells	Basophils	DC	Mnocytes	Monocytes	NK	B	T	Neutrophils
SEB+PBS	0.35	0.10	3.25	1.81	6.03	23.15	18.09	47.22
SEB+antibody	0.07	0.26	0.05	0.38	4.25	45.75	23.37	25.87

(Fig. 6E). B-Ciita and neutrophils-Pf4 were dominant in the control group, whereas B-Cecr2, B-Cd83, B-Hist1h2ae, B-Ragl, B-Lipg, and neutrophils-Ngp were dominant in the antibody-treated group. In addition, after antibody treatment, the B cells and neutrophil transcriptomic patterns in major subsets were significantly altered (Fig. 6F). The immune cell population markers are shown in Table 2.

DISCUSSION

To develop a safe and effective SEB detoxification agent, we successfully prepared F(ab')₂ fragments and evaluated their anti-SEB effects in mice and rhesus monkeys

receiving a lethal dose of SEB. The preparations met Chinese Pharmacopoeia standards of purity and biological activity. Previous studies [17] have confirmed that the use of full-dose purification F(ab')₂ fragments effectively protects animal models against SEB poisoning. At present, these anti-SEB horse F(ab')₂ fragments are undergoing clinical trials to further verify their efficacy and safety.

Although crudely purified SEB and equine F(ab')₂ fragments can be made easily, high purity of SEB antigens and the F(ab')₂ fragments is necessary for immunization and treatment, to improve the bio-specific activity and avoid adverse effects. Recent studies have reported purification of recombinant SEB by cation exchange chromatography [18] to meet bio-product requirements. Equine F(ab')₂ fragments have also been purified by size exclusion chromatography in addition to common methods, to meet the requirements [19,20]. In this study, the purification methods were screened and coordinated to further improve the bio-specific activity of our bio-product, which satisfied the technical guidance principles for clinical pharmacological studies of antibody-based drugs.

Because bioactivity is critical, optimization of purified antigens, immunization regimens, and blood collection benchmarks is required to improve the quality of F(ab')₂ preparations. In this study, we used purified nSEB as immunization antigens, because of their greater bioactivity than that of recombinant SEB under safe dosages [21]. Healthy adult and horses were inoculated with the elevated adjuvanted, purified nSEB at sites near the lymph nodes many times until the serum titers exceeded 1:128, thus providing further quality assurance. The F(ab')₂ preparations had more satisfactory protective efficacy than other reported antibody types, the F(ab')₂ preparations had satisfactory protective efficacy [22].

SEB can induce a fatal inflammatory response, primarily through extensive activation and proliferation of neutrophils. However, the application of F(ab')₂ preparations significantly decreased the proportions of peripheral blood neutrophils in mice, thereby attenuating the systemic inflammatory immune response. This attenuation was attributed to the potent neutralizing effect of F(ab')₂ and the absence of Fc fragments. The Fc portion of antibodies is well known to have various biological functions in activating the immune response, including antibody-dependent cellular cytotoxicity, complement activation, and induction of apoptosis. The rational design of human IgG1 antibodies lacking antibody-dependent cellular cytotoxicity or other Fc functions is a particularly promising approach enabling the creation of therapeutic antibodies without unwanted Fc activity.

The Ngp gene encodes a neutrophil granule protein involved in neutrophil development and function [23], which has critical roles in antimicrobial defense. During the immune response, the Ngp protein contributes to neutrophil chemotaxis, adhesion, and degranulation [23], thereby enhancing the response to pathogens. In this study, antibody treatment significantly increased the proportion

TABLE 2 | List of immune cell population markers.

Identity	Features
B-Cd83	Cd19, Cd79b, Ebf1, Ccr5, Il1b, S100a9, Ccl5
B-Cecr2	Cd19, Cd79b, Ebf1, Il1b, S100a9, Ccl5
B-Ciita	Cd19, Cd79b, Ebf1, Csf1r, Il1b, Csf3r, S100a9, Ccl5, Cd3d
B-Crip1	Cd19, Cd79b, Ebf1, Il1b, Csf3r, S100a9, Ccl5
B-H2-Aa	Cd19, Cd79b, Ebf1, Il1b, Csf3r, S100a9, Ccl5
B-Hbb-bt	Il1b, Csf3r, S100a9, Ccl5
B-Hist1h2ae	Cd19, Cd79b, Ebf1, Il1b, S100a9, Ccl5, Cd3d
B-Ighd	Cd19, Cd79b, Ebf1, Cd4, Il1b, S100a9, Prf1, Ccl5, Cd3d, Cd3e, Cd3g
B-Iglc1	Cd19, Cd79b, Ebf1, Il1b, S100a9, Ccl5
B-Lipg	Cd19, Cd79b, Ebf1, Il1b, Csf3r, S100a9, Ccl5, Cd3d
B-Rag1	Cd19, Cd79b, Ebf1, Il1b, S100a9, Ccl5
Basophils	Mcpt8, Il6, Ccl9, Cdh1, Ccr2, Il1b, S100a9, Ccl5
DC	Siglech, Cd4, Cdh1, Ccr2, Ccr5, Il1b, S100a9
Mnocytes	Ccl9, Ccr2, Csf1r, Il1b, Csf3r, S100a9, Ccl5
Monocytes	Il6, Ccl9, Ccr2, Ccr5, Csf1r, Il1b, Csf3r, S100a9, Ccl5
Neutrophils-Il1b	Csf1r, Il1b, Csf3r, S100a9, Ccl5
Neutrophils-Ngp	Il1b, Csf3r, S100a9, Ccl5, Cd3d
Neutrophils-Pf4	Csf1r, Il1b, Csf3r, S100a9, Ccl5, Cd3d
Neutrophils-Retnlg	Il1b, Csf3r, S100a9, Ccl5, Cd3d
Neutrophils-Trbc2	Cd4, Csf1r, Il1b, Csf3r, S100a9, Ccl5, Cd3d, Cd3e, Cd3g
Neutrophils-Wfdc17	Csf1r, Il1b, Csf3r, S100a9, Cd3d
NK	Ccr2, Ccr5, Il1b, Csf3r, S100a9, Prf1, Gzma, Ccl5
T-Cd8b1	Ccr2, Ccr5, Il1b, Csf3r, Prf1, Cd3d, Cd3e, Cd3g
T-Ctla4	Cd4, Ccr2, Ccr5, Il1b, Csf3r, Ccl5, Cd3d, Cd3e, Cd3g
T-Ms4a4b	Cd4, Il1b, Csf3r, Cd3d, Cd3e, Cd3g

of neutrophils–Ngp+; therefore, Ngp-associated pathways might serve as therapeutic antibody targets.

Furthermore, in contrast to findings from our previous study on ricin [24], monoclonal antibody treatment had a more pronounced effect on B cells in experimental mice. The quantities of B–Ccr2+, B–Cd83+, B–Hist1h2ae+, B–Ragl+, and B–Lipg+ were enhanced by antibody treatment. The biological functions of the associated genes have clinical implications as diverse as gene recombination and the immune response (RAG1 [25], CD83 [26]); gene expression regulation (CECR2 [27], HIST1H2AE [28]); and lipid metabolism (LIPG [29]). However, the effects of these genes on B-cell function require further exploration in subsequent research.

This study has several limitations. Non-human antibodies elicit an immune response from the body, and the neutralization reaction occurring with the use of non-human antibodies can limit the use of antibody drugs in the treatment of human diseases. Immunogenicity issues may also occur; for example, polyclonal antibodies of equine origin can cause immunogenic human anti-equine antibody reactions. These reactions can not only decrease the therapeutic effect of antibodies but also cause serious adverse reactions, such as allergic reactions. The underlying cause of immunogenicity is immune rejection reaction between equine-derived antibodies and the human immune system, which can result in rapid antibody clearance in the human body or an antibody neutralization effect. Because of genetic differences between horses and humans, equine polyclonal antibodies may cross-react with non-target proteins. This cross-reactivity might potentially lead to false-positive or false-negative results, thus affecting disease diagnosis and treatment. In addition, cross-reactivity might lead to unpredictable adverse effects and increased therapeutic risk.

CONCLUSION

In summary, bio-products against SEB were successfully produced and might potentially be used to treat patients and animals with SEB intoxication in the near future.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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