

Article

Equal Neutralization Potency of Antibodies Raised against Abrin Subunits

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Abstract: Abrin and ricin are potent AB toxins, which are considered biological threats. To date, there are no approved treatments against abrin or ricin intoxications. Previously, we showed that the administration of polyclonal anti-abrin antibodies to mice that were intranasally exposed to abrin, even very late post-exposure, conferred an exceedingly high-level of protection, while following ricin intoxication, similar treatment with anti-ricin antibodies resulted in negligible survival rates. To probe this unexpected difference in protection ability, we first examined whether the efficient anti-abrin-induced protection was due to neutralization of the A-subunit responsible for the catalytic effect, or of the B-subunit, which enables binding/internalization, by evaluating the protection conferred by antibodies directed against one of the two subunits. To this end, we generated and immunized rabbits with chimeric toxins containing a single abrin subunit, $A_{\text{abrin}}B_{\text{ricin}}$ in which abrin A-subunit was linked to ricin B-subunit, and $A_{\text{ricin}}B_{\text{abrin}}$ in which ricin A-subunit is linked to abrin B-subunit. Here, we show that antibodies raised against either $A_{\text{abrin}}B_{\text{ricin}}$ or $A_{\text{ricin}}B_{\text{abrin}}$ conferred exceptionally high protection levels to mice following intranasal exposure to a lethal dose of abrin, suggesting that the high level of protection conferred by anti-abrin antibodies is not related to the neutralization of a particular subunit.

Keywords: abrin; ricin; chimeric toxins; A-subunit; B-subunit; polyclonal antibodies

1. Introduction

Abrin and ricin, plant toxins derived from rosary peas (*Abrus precatorius*) and castor bean plant (*Ricinus communis*), respectively, belong to the type 2 ribosome inactivating (RIP-2) group of proteins. The heterodimeric glycoprotein of both toxins comprises subunit A, carrying the enzymatic activity of the toxin, and subunit B, which is a lectin that binds to eukaryotic cell surfaces via galactose residues and mediates toxin internalization [1]. Upon entry to the cytosol, the A-subunits of both ricin and abrin inactivate ribosomes irreversibly by site-specific depurination of a specific adenine nucleotide within the 28S rRNA, thereby precipitating cessation of cellular protein synthesis [2]. Due to their high availability, ease of preparation and high toxicity, ricin and abrin are considered potential bioterror agents and are classified as Category B agents by the US Center for Disease Control and Prevention (CDC). To date, there are no approved post-exposure therapeutic countermeasures for either toxin.

The toxicity of ricin and abrin depends on the route of exposure, inhalatory exposure being considered the most fatal [2]. The clinical manifestation following intranasal exposure of mice to these

toxins is the onset of a localized yet severe pulmonary edematous inflammation, which is accompanied by massive recruitment of neutrophils to the lungs and onset of a turbulent pro-inflammatory cytokine storm within this organ. Pulmonary (intranasal) ricin and abrin intoxications in mice, are similar with regard to pathological features and kinetics [3,4]. However, despite their resemblance in morbidity and mortality, the ability to protect mice against ricin and abrin intoxications by post-exposure antibody-mediated treatment differs radically. In the case of lethal ricin intoxication, rabbit-derived polyclonal anti-ricin antibody-based treatment up to three hours post exposure conferred protection to $\geq 90\%$ of the mice, nonetheless following treatment at 24 hours survival rates declined dramatically, to 34% [3]. When antibody treatment was administered at 48 hours post exposure to ricin, protection was no more than marginal [5]. In sharp contrast, the administration of polyclonal anti-abrin antibodies to mice intranasally exposed to a lethal dose of abrin, led to very high survival rates ($\sim 70\%$ – 80%), even when the antibodies were applied as late as 72 hours after intoxication [4].

The goal of the present work was to elucidate whether the efficient protection by polyclonal anti-abrin antibodies can be attributed to the neutralization of a single subunit, in other words, to define the critical neutralizing event that confers high-level of protection: prevention of toxin binding via the B subunit or abolishment of the A subunit-related catalytic activity. A specific conclusion could assist us, for example, to produce a subunit-directed abrin vaccine, which would be as efficient as holotoxin-based vaccine, but much safer. In addition, determining the subunit whose neutralization is critical for protection could assist in designing a more effective anti-ricin immunization protocol.

Production of effective polyclonal antibodies against a specific subunit of the abrin (or ricin) toxin is complicated by the tendency of isolated subunits to adopt an unstable conformation (in particular, B_{abrin} is insoluble in aqueous solutions when separated from the A_{abrin} , due to its hydrophobicity [6]). To overcome this difficulty, we incorporated each of the abrin subunits into a dimeric AB molecular fold by generating two chimeric heterologous toxins, $A_{\text{abrin}}B_{\text{ricin}}$ and $A_{\text{ricin}}B_{\text{abrin}}$, utilizing the monomeric subunit constituents of abrin and ricin. These chimera toxins served as antigens for rabbit vaccination, which resulted in elicitation of antibodies against each of the anti-abrin-subunits.

In the present study, we found that anti- A_{abrin} and anti- B_{abrin} antibodies were equally effective in recognizing and neutralizing abrin in-vitro. Highly efficient protection of mice against pulmonary abrin intoxication, comparable to that conferred by anti-abrin holotoxin antibodies, was obtained by post-exposure application of either one of the anti-subunit directed antisera. These findings strongly suggest that the neutralization of either A_{abrin} or B_{abrin} is sufficient for achieving enhanced protection.

2. Materials and Methods

2.1. Extraction and Purification of Abrin and Ricin

Crude abrin was prepared from *Abrus precatorius* seeds essentially as described previously [7,8]. Briefly, seed kernels were soaked in 5% acetic acid/phosphate buffer (Na_2HPO_4 , pH-7.4) overnight and homogenized in a Waring blender. Proteins obtained from 80% ammonium sulfate precipitation were centrifuged and dialyzed extensively against PBS.

Crude ricin was prepared from seeds of endemic *Ricinus communis*, essentially as described before [9]. Briefly, seeds were homogenized in a Waring blender in 5% acetic acid/phosphate buffer (Na_2HPO_4 , pH-7.4), the homogenate was centrifuged and the clarified supernatant containing the toxin was subjected to ammonium sulfate precipitation (60% saturation). The precipitate was dissolved in PBS and dialyzed extensively against the same buffer.

For the preparation of purified toxins, crude abrin and ricin were loaded consecutively on 2 columns, the first column containing activated Sepharose which binds and thereby depletes the *Abrus precatorius* Agglutinin (APA) and *ricinus communis* agglutinin (RCA), respectively, and the second column, containing α -lactose (lactamyl) agarose (Sigma-Aldrich, Rehovot, Israel), which binds the toxins. Proteins bound to the lactamyl agarose column were eluted with 0.5M Galactose in PBS.

2.2. Subunit Purification

Purified abrin was loaded on a lactamyl agarose column (1 mg pure abrin:2 mL lactamyl agarose) and the column was washed with 1M Trizma-HCl pH-8 (Sigma-Aldrich, Rehovot, Israel), which was diluted 1:10 in PBS (abrin washing buffer). For toxin reduction and collection of A_{abrin} , the column was rinsed with 1% v/v β -mercaptoethanol in abrin washing buffer. Each 1 mL A_{abrin} was neutralized with 25 μ L of 1M KH_2PO_4 . The column was washed with PBS (pH 7), and B_{abrin} was eluted with 0.5M galactose in the presence of 1% v/v β -mercaptoethanol. A_{abrin} and B_{abrin} were concentrated in 10 kDa cutoff Amicon-Ultra centrifugal filters.

Purified ricin was loaded on a lactamyl agarose column (1 mg pure ricin:1 mL lactamyl agarose) and the column was washed with 1M Trizma-HCl pH-9 (Sigma-Aldrich, Rehovot, Israel) diluted 1:10 in PBS (ricin washing buffer). For toxin reduction and the collection of A_{ricin} , the column was rinsed with 1% v/v β -mercaptoethanol (Sigma-Aldrich, Rehovot, Israel) in ricin washing buffer. Each 1 mL A_{ricin} was neutralized with 100 μ L of 1M KH_2PO_4 . The column was then washed with PBS (pH-7), and B_{ricin} was eluted with 0.5M galactose (Sigma-Aldrich, Rehovot, Israel), in the presence of 1% v/v β -mercaptoethanol. A_{ricin} and B_{ricin} were concentrated in 10 kDa cutoff Amicon-Ultra centrifugal filters (Mercury, Rosh Haayin, Israel).

All subunit purification processes were conducted at 4 °C, and the subunits were stored under this temperature until further used.

2.3. Preparation of Chimeric Toxins

Chimera $A_{\text{abrin}}B_{\text{ricin}}$ preparation: the subunits were mixed (20% excess A_{abrin}) and extensively dialyzed (10 kDa cutoff) against 10mM phosphate buffer (1M phosphate buffer solution, pH-7.4, diluted 1:100 in DDW) containing 100 mM galactose, at 4 °C for 7 days, and additional 2 days against the same buffer without galactose. $A_{\text{abrin}}B_{\text{ricin}}$ was loaded on a lactamyl agarose column and the column was washed with PBS in order to remove residual monomeric A_{abrin} . $A_{\text{abrin}}B_{\text{ricin}}$ eluted with 0.5M galactose in PBS was dialyzed against PBS and kept frozen until used.

Chimera $A_{\text{ricin}}B_{\text{abrin}}$ preparation: the subunits were mixed (20% excess A_{ricin}) and extensively dialyzed (10 kDa cutoff) against PBS for 2 days at 4 °C. $A_{\text{ricin}}B_{\text{abrin}}$ was loaded on a lactamyl agarose column and the column was washed with PBS in order to remove residual monomeric A_{ricin} . $A_{\text{ricin}}B_{\text{abrin}}$ eluted with 0.5M galactose in PBS was dialyzed against PBS and kept frozen until used.

2.4. Gel Electrophoresis

Samples were visualized using Coomassie Blue stained non-reducing 10% polyacrylamide gel, which was subjected to sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or non-reducing conditions.

2.5. ELISA Titer Determination

Maxisorp 96-well plates (Nunc, Sigma-Aldrich, St. Louis, MO, USA) were coated overnight with 2 μ g/mL of abrin in 50 mM pH-9.6 Carbonate-Bicarbonate Buffer (Sigma, C3041), then washed and blocked with PBST buffer (0.05% Tween 20, 2% BSA in PBS) for one hour. Antisera were then added to the plates for a one-hour incubation. The plates were then washed with PBST and incubated with the detecting antibody, AP conjugated goat anti-rabbit (Jackson, 111-055-003) followed by detection with SIGMAFAST p-Nitrophenyl phosphate (Sigma, N1891).

2.6. In-Vitro Abrin Neutralization Assay

Ub-FL cells (HeLa cells stably expressing ubiquitin-luciferase [10]), a kind gift from Professor Piwnicka-Worms (University of Texas, MD Anderson Center, Austin, TX, USA), were seeded in a 96-well plate (1.5×10^4 cells/well) and incubated over night at 37 °C. Cell-culture medium was removed and serial dilutions of anti-holotoxin_{abrin}, anti- A_{abrin} , or anti- B_{abrin} antisera in the presence or absence of

abrin (7 ng/mL) were added to the plate. Twenty four hours later, cell-culture medium was removed, and the cell medium containing the proteasome inhibitor MG132 (Sigma, C2211 1 μ M) was added for another hour. After medium aspiration, cells were lysed by the addition of 50 μ L lysis buffer (Promega, E1941). Residual luciferase activity was determined by mixing equal volumes of the cell lysate and luciferase assay reagent (Promega, E1483) followed by immediate measurement of the luminescence levels. Residual luciferase activity was expressed as percent of luminescence levels obtained from untreated cells.

2.7. Epitope Recognition

Maxisorp 96-well microtiter plates were coated overnight with 5 μ g/mL Streptavidin (Sigma, S0677), then washed and blocked as described above. Biotin labeled 15aa length peptides (Table S1) (5 μ g/mL in PBST) were then added for 20 minutes. The plates were washed, anti-holotoxin_{abrin}/anti-A_{abrin}B_{ricin}/anti-A_{ricin}B_{abrin} antisera were diluted in PBST and added to the plates for one-hour incubation. The plates were washed and incubated with AP conjugated anti-rabbit antibodies (Jackson, 111-055-003) for 15 minutes followed by detection with SIGMAFAST p-Nitrophenyl phosphate (Sigma, N1891).

2.8. Animal Studies

Animal experiments were performed in accordance with the Israeli law and were approved by the Ethics Committee for animal experiments at the Israel Institute for Biological Research. The treatment of animals was in accordance with regulations outlined in the USDA Animal Welfare Act and the conditions specified in the Guide for Care and Use of Laboratory Animals (National Institute of Health, 1996). New Zealand white rabbits (Charles River Laboratories Ltd., UK) weighing 2.5 to 3 kg were immunized in order to produce rabbit-derived polyclonal antibodies. Female CD-1 mice (Charles River Laboratories Ltd., UK) weighing 27–32 g were used for survival studies.

Prior to all studies in mice and rabbits, the animals were habituated to the experimental animal unit for 5 days. All mice were housed in filter-top cages in an environmentally controlled room and maintained at 21 \pm 2 $^{\circ}$ C and 55 \pm 10% humidity. Lighting was set to mimic a 12/12 hours dawn to dusk cycle. Animals had access to food and water *ad libitum*.

2.9. Polyclonal Anti-Chimera Antibody Production

Rabbits were immunized with A_{abrin}B_{ricin} or A_{ricin}B_{abrin} in a stepwise manner, injection 1 containing 1 μ g toxin in Freund's adjuvant, and injections 2–5 (with incomplete Freund's adjuvant) in increasing concentrations (4, 25, 50, and 100 μ g toxin/rabbit) with 4-week intervals between injections. Blood samples were collected (1 week after injection) to ascertain anti-chimera antibody titer build-up.

2.10. In-Vivo Protection Assay

Mice were anesthetized by an intraperitoneal (i.p.) injection of ketamine (1.9 mg/mouse, Vetoquinol, Lure, France) and xylazine (0.19 mg/mouse, Eurovet Animal Health, AD Bladel, The Netherlands), and intoxicated by intranasal instillation (2 \times 25 μ L) of 2LD₅₀ of abrin (8 μ g/kg). For antibody treatment 100 μ L of the relevant rabbit-derived polyclonal antibodies were applied intravenously at 48 hours following abrin intoxication. Mortality was monitored over 14 days and Kaplan-Meier analysis was performed for survival curves.

3. Results

3.1. Characterization of the Chimeric Toxins

Chimeric abrin/ricin toxins were generated from isolated A and B-subunits of abrin and ricin, based upon a method developed by Olsnes et al. [11], with slight modifications (for details see "Materials and Methods"). Under non-reducing conditions, the chimeric A_{abrin}B_{ricin} and A_{ricin}B_{abrin}

toxins, as well as reconstituted ricin and abrin toxin molecules generated by co-incubation of A and B-subunits of the same source, appear as a main band of approximately 60 kDa on SDS-PAGE (Figure 1A). The formation of $A_{\text{abrin}}B_{\text{ricin}}$ was incomplete, as indicated by detection of a weak ~30 kDa band, in addition to the major band. Interestingly, this observation was also reported previously [11]. Since excess A-subunits are removed prior to the final stage of the experimental protocol for chimeric toxin generation, this ~30 kDa band probably represents monomeric B_{ricin} . As seen in Figure 1B, reduction with β -mercaptoethanol of reconstituted ricin and abrin (lanes 1 and 2, respectively) resulted in the generation of two typical major bands, somewhat different in molecular weights (the molecular weight of A_{abrin} is less than that of A_{ricin} , the molecular weight of B_{ricin} is slightly less than that of B_{abrin}). Reduction of the chimera toxins (lanes 3 and 4) resulted in electrophoretic migrating bands, which confirm the composition of each toxin.

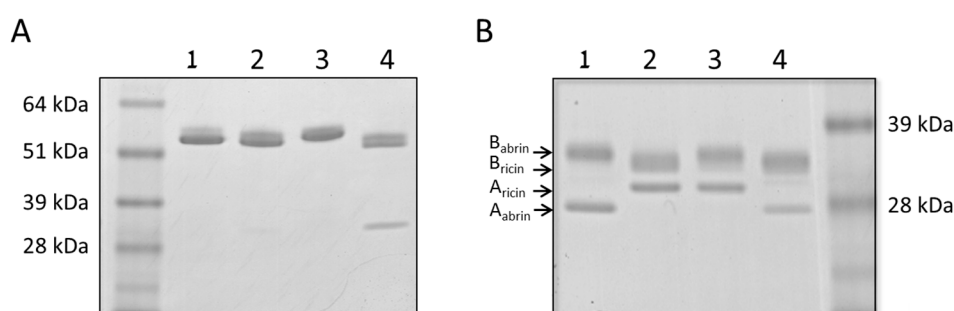


Figure 1. Gel electrophoresis of chimera abrin/ricin toxins. SDS-PAGE of: Reconstituted abrin (lane 1), Reconstituted ricin (lane 2), Chimera toxin $A_{\text{ricin}}B_{\text{abrin}}$ (lane 3), and Chimera toxin $A_{\text{abrin}}B_{\text{ricin}}$ (lane 4) was performed (A) in the absence of reducing agent, or (B) in the presence of β -mercaptoethanol.

3.2. Abrin Subunit Recognition and Neutralization

To produce polyclonal antibodies directed specifically against A_{abrin} or B_{abrin} , rabbits were immunized with the chimeric toxins, $A_{\text{abrin}}B_{\text{ricin}}$ and $A_{\text{ricin}}B_{\text{abrin}}$, respectively. The immunization protocol included repeated injection with increasing antigen doses, at four-week intervals, up to 100 μg antigen/rabbit. Hyperimmune sera directed against each of the chimeric toxins was collected and characterized for its ability to recognize A_{abrin} or B_{abrin} in an enzyme linked immunosorbent assay (ELISA). Accordingly, increasing concentrations of either the anti-chimera antisera were incubated on abrin-coated plates, after which abrin-bound antibodies were detected using secondary anti-rabbit antibodies. The two antisera displayed ELISA titers of similar magnitude against the corresponding abrin subunits, 4.5×10^5 and 8×10^5 ELISA units, against A_{abrin} and B_{abrin} , respectively (Table 1). Next, we asked whether these anti-subunit antibodies possess the capability to neutralize abrin in an in-vitro cell culture system that may potentially predict their efficacy in-vivo. In this assay, the neutralization potency of an antiserum is measured by monitoring its ability to block abrin-induced luciferase synthesis arrest in HeLa Ub-FL cells [12,13]. Abrin was incubated with increasing concentrations of the relevant antiserum, and added to the Ub-FL cells. Twenty four hours later, residual intracellular luciferase levels were measured and neutralizing antibody titers were determined for each antisera. As can be seen (Table 1), both subunit-directed anti-chimera antibodies possess the ability to effectively neutralize abrin in cell culture ($\sim 1 \times 10^3$ neutralizing antibody titers for both anti-chimera antitoxins).

Table 1. Anti-abrin titers of rabbit-derived anti- $A_{\text{abrin}}B_{\text{ricin}}$ and anti- $A_{\text{ricin}}B_{\text{abrin}}$ antisera.

Antiserum (Relevant Abrin Subunit)	ELISA-Antibody Titers ($\times 10^5$)	Neutralizing-Antibody Titers ($\times 10^3$)
Anti-AabrinBricin (A)	4.5 ± 0.1	1.1 ± 0.1
Anti-AricinBabrin (B)	$8.1 \pm 0.2^*$	0.9 ± 0.3

* $p < 0.05$ when compared to Anti-AabrinBricin ELISA-antibody titer.

3.3. Abrin Linear-Epitope Recognition by Anti- A_{abrin} - and Anti- B_{abrin} - Polyclonal Antibodies

We have recently determined the immunodominant epitopes of abrin in sera of rabbits that were immunized with abrin (manuscript in preparation). We now sought to characterize the antibody response elicited against A_{abrin} and B_{abrin} , when these abrin-derived subunits are embedded within the chimeric toxins. By allowing each of the anti-chimera antisera to react with overlapping 15-mer peptides spanning the protein sequence of either A_{abrin} or B_{abrin} , we could determine to which linear epitopes each sera is directed (Figure 2). It was found that the anti- $A_{\text{abrin}}B_{\text{ricin}}$ antiserum recognizes five epitopes of A_{abrin} (Table 2). While epitopes 1, 2, and 4 were previously mapped as immunodominant epitopes of anti-abrin antisera, epitopes 3 and 5 were discerned only by anti- $A_{\text{abrin}}B_{\text{ricin}}$ antisera. It should be noted however, that the signals of the sera reacting with the three known epitopes were high whereas the signal with the hitherto undetected two epitopes was relatively low (close to background level), suggesting that these two epitopes have minor contribution to the overall activity of the sera toward abrin. Mapping the epitopes of the anti- $A_{\text{ricin}}B_{\text{abrin}}$ reveals six epitopes (Table 2) all of which were previously mapped as immunodominant epitopes of B_{abrin} . It should be noted that epitope 9 is based on signal of the sera binding to a single peptide (No. 37). The sequence DGSI also appears as a part of a larger epitope sequence (epitope 10), suggesting that the binding of the antibodies to peptide 37 is due to possible cross-reactivity with this sequence. Taken together, these results indicate that the overall structure and immunogenicity of the chimeric subunits are similar to that of the native toxin.

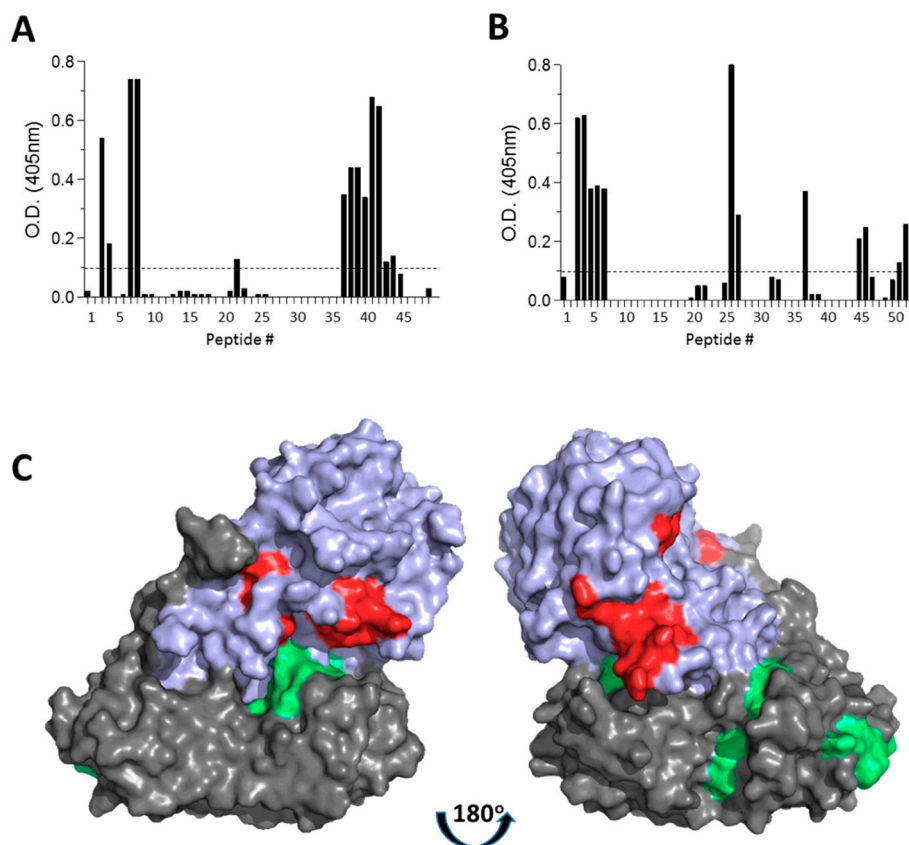


Figure 2. Linear epitopes recognized by anti-abrin-subunit antisera. Linear 15-mer peptides spanning (A) A_{abrin} or (B) B_{abrin} were reacted with either anti- $A_{\text{abrin}}B_{\text{ricin}}$ antisera or anti- $A_{\text{ricin}}B_{\text{abrin}}$ antisera, respectively. Dash line represents background binding. (C) Schematic representation of the linear epitopic regions recognized by anti- A_{abrin} antibodies (red) and anti- B_{abrin} antibodies (green) are depicted on the crystal structure of abrin (PDB 1abr) using PyMol. A_{abrin} and B_{abrin} are represented by cyan and gray colors, respectively.

Table 2. anti-A_{abrin}B_{ricin} and anti-A_{ricin}B_{abrin} antisera immunodominant epitopes of abrin.

Epitope No.	Subunit	Epitope Sequence	Epitope Location
1	Aabrin	KQFIEALR	18–25
2		IPVLPDP	36–42
3		YGTYGDL	110–116
4		QPDAAMISLE	186–195
5		LTIRN	219–223
6	Babrin	VRIGGRDG	16–23
7		NGYHNG	31–36
8		QGWRTGN	134–140
9		DGSI	183–186
10		WVKFNDGSI	221–229
11		QIWTLTF	261–267

3.4. Post-Exposure Treatment of Abrin Intoxicated Mice with Anti-Abrin Subunit-Directed Antisera

As the goal of this study was to elucidate which abrin subunit needs to be neutralized in-vivo to promote the observed high level of protection following pulmonary abrin-intoxication [4], mice were intranasally exposed to 2LD₅₀ of abrin (8 µg/kg), and anti-abrin or anti-abrin-subunit (anti-chimera) antibodies (100 µL intravenously) were administered at 48 hours post exposure. Control intoxicated mice were not subjected to antibody treatment. The data in Table 3 establish that, as in the case of anti-abrin holotoxin antibody treatment, late administrations of either anti-A_{abrin} or anti-B_{abrin} antisera conferred very high protection levels (≥90%) against a lethal abrin intoxication.

Table 3. Survival rates of mice intoxicated with abrin and subjected to antibody treatment.

Antibody treatment	Survival	
	%	Survivors/Total
Control	0	0/10
Anti-Abrin	90	9/10
Anti-AabrinBricin	90	9/10
Anti-AricinBabrin	95	19/20

4. Discussion

Previous studies carried out in our laboratory, demonstrated that application of anti-abrin antibodies conferred extremely high survival rates to intranasally abrin-intoxicated mice, even when treatment was performed at very late time after exposure to a lethal dose of the toxin [4]. In contrast, late anti-ricin antibody treatment following lethal ricin intoxication resulted in insignificant protection [3]. This unexpected sharp dissimilarity in antibody-induced protection could stem from a difference in the quality of the anti-abrin and anti-ricin antibody preparations, or from differences in disease progression following exposure to the two toxins.

We have previously shown that anti-ricin and anti-abrin ELISA- and neutralizing- antibody titers, as well as the apparent affinities of the specific fraction of either anti-ricin or anti-abrin antibodies, were very similar [4]. However, this data could not rule out the possibility that neutralization of an essential epitope within a specific subunit, or perhaps neutralization of the functional activity of one of the two subunits, toxin catalytic activity or lectin binding of subunit A and B, respectively, is obligatory for obtaining high-level protection. To evaluate such subunit-related neutralization effects, we sought to produce anti-abrin polyclonal antibodies directed specifically against A_{abrin} or B_{abrin}. An obvious option for generating such antibodies, would be to immunize rabbits with isolated monomeric A_{abrin} or B_{abrin}, however isolated individual subunits, originating from an AB dimeric context, are unstable and their conformational structure is drastically impaired [14]. Indeed, export of the abrin (or ricin)

monomeric A-subunit from the endoplasmic reticulum (ER) into the cytosol relies on the fact that following toxin reduction in the ER, the isolated A-chain is identified as a misfolded protein, thereby allowing its retrieval to the cytosol via the ER degradation pathway [15,16]. In this regard, it has been suggested that ribosome depurination within the cytosol by monomeric A-subunit, requires its refolding, with the aid of the Hsc70 chaperone, to a catalytic conformation [17]. With respect to the B-subunit, it has been documented that B_{abrin} is insoluble in aqueous solutions when separated from the A_{abrin}, due to its hydrophobicity [6]. Accordingly, isolated subunit vaccination may lead to the production of antibodies that could not recognize and neutralize the holotoxin, resulting in inability to protect against in-vivo intoxications. Indeed, when recombinant A_{abrin} was used as the immunizing antigen for production of anti-A_{abrin} mAbs, in-vivo neutralization of abrin could be demonstrated only when these mAbs were applied prophylactically, while no post-exposure effects were reported [18,19]. MAbs directed against A_{abrin}-*abrus preicatorius agglutinin* (APA) chimeric toxin (1–123 amino acids of A_{abrin} linked to 124–175 amino acids of APA), in which A_{abrin} is not in an AB holotoxin context, were also found to be protective prophylactically, without any reports regarding post exposure effects [20]. Consequently, to produce an abrin-subunit specific antigen possessing proper abrin-subunit conformational structure for rabbit immunization, we generated chimeric abrin-ricin toxins composed of A_{abrin} or B_{abrin} linked via disulfide bond to their non-homologous counterparts, subunit B_{ricin} or A_{ricin}, respectively. Characterization of the two antisera raised against the two chimeric abrin/ricin toxins demonstrated that these recognized their cognate subunits to a similar extent and that both anti-chimeric toxin antibodies possessed potent in-vitro anti-cytotoxicity abilities. Most importantly, we found that following intranasal exposure of mice to abrin, both subunit-directed antisera, anti-A_{abrin}B_{ricin} (anti-A_{abrin}) and anti-A_{ricin}B_{abrin} (anti-B_{abrin}), were highly protective ($\geq 90\%$ survival) even when treatments were applied as late as 48 hours following intoxication. This high level of protection is due to the antibodies elicited against the abrin subunit of the chimeric toxins and not to those formed against the ricin subunit, since anti-ricin antibodies were completely ineffective when given to mice even at early hours following intranasal exposure to a lethal dose (2LD₅₀) of abrin (data not shown).

Taken together, it seems unlikely that the difference in protection conferred by anti-abrin and anti-ricin antibodies against abrin and ricin intoxications, respectively, is related to variances in the quality of the two antibody preparations. Rather, it seems that the striking difference in the ability to protect against ricin- and abrin- challenges stems from dissimilarities in disease progression. Supporting this argument, we have previously shown that following intranasal intoxication, the in-vivo catalytic performance of abrin, i.e., ribosomal depurination of pulmonary tissue, is significantly lower than that observed following ricin intoxication. In particular, the depurination level of pulmonary epithelial cells was markedly reduced following abrin intoxication, approximately five times lower than after ricin intoxication (16% and 81% depurination following exposure to abrin and ricin, respectively [21]). We further determined by FACS analysis that the epithelial cell damage following intoxications with abrin (unpublished data) and ricin [22] was mostly confined to the type II epithelial cells, responsible for lung tissue regeneration [23]. The injury to the epithelial cell sub-population was correlative to the trend described above for the whole-epithelial population, namely, the specific insult to the type II cells following abrin intoxication was less prominent than the damage observed following ricin exposure (unpublished data). Thus, anti-abrin and anti-ricin antibodies may halt further toxin-induced damage in an equally efficient manner, yet the anti-abrin antibodies will provide greater protection to abrin-intoxicated subjects due to the reduced levels of irreversible cellular damage inflicted by this particular toxin at a given post-exposure time-point. Furthermore, the notion that antibody-related protection ability is mostly due to toxin pathology progression and not to antibody quality, is in line with experiments recently carried out in our laboratory, in which mice were intoxicated with lethal doses of the chimeric toxins, and then treated with their corresponding anti-chimeric antibodies. Preliminary results suggest that significantly higher survival rates are achieved following late antibody treatment of mice that were intranasally intoxicated with the chimera toxin A_{ricin}B_{abrin}, even though both

anti-A_{abrin}B_{ricin} and anti-A_{ricin}B_{abrin} conferred equally high levels of protection to abrin-intoxicated mice. Future studies will be required for characterization of the different damage patterns in the pulmonary cell subpopulations following intranasal intoxications with the two chimeric toxins.

In parallel to the results described above, recent studies carried out in our laboratory established that the low-density lipoprotein Receptor Related-protein 1 (LRP-1) is the major receptor for ricin in murine lung cells (Falach et al, manuscript in preparation). A different receptor for abrin (and A_{ricin}B_{abrin}), if indeed detected, may further explain the dissimilarity between the disease progressions following pulmonary abrin and ricin intoxications.

In summary, both anti-A_{abrin}- and anti-B_{abrin} directed rabbit-derived polyclonal antibodies confer extremely effective protection against abrin lethality when administered lately following intranasal abrin intoxication in mice, allowing us to conclude that the significant difference in protection levels following antibody treatment after intranasal abrin and ricin intoxications stems from a dissimilarity in disease progression inflicted by these two toxins.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4468/9/1/4/s1>, Table S1: List of 15-mer peptides of Aabrin and Babrin.

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