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Title: A Tripartite Cocktail of Chimeric Monoclonal Antibodies Passively Protects Mice against Ricin, Staphylococcal Enterotoxin B and Clostridium perfringens Epsilon Toxin

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Abstract: Due to the fast-acting nature of ricin, staphylococcal enterotoxin (SEB), and Clostridium perfringens epsilon toxin (ETX), it is necessary that therapeutic interventions following a bioterrorism incident by one of these toxins occur as soon as possible after intoxication. Moreover, because the clinical manifestations of intoxication by these toxins are likely to be indistinguishable from each other, especially following aerosol exposure, we have developed a cocktail of chimeric monoclonal antibodies that is capable of neutralizing all three toxins. The efficacy of this cocktail was demonstrated in mouse models of lethal dose toxin challenge.

Suggested Reviewers:

Opposed Reviewers:

Wadsworth Center
New York State Department of Health

BMS | Department of Biomedical Sciences

August 28, 2014

Dr. Alan Harvey
Editor in Chief, *Toxicon*

Dear Dr. Harvey:

I am pleased to submit to you a revised version of manuscript Toxcon-D-13-0312 entitled “A Tripartite Cocktail of Chimeric Monoclonal Antibodies Passively Protects Mice against Ricin, Staphylococcal Enterotoxin B and Clostridium perfringens Epsilon Toxin” for consideration as an **Short Communication** in *Toxicon*.

The manuscript has been *re-revised* in response to the Reviewer’s comments and a point-by-point rebuttal letter is provided. Specifically, we have made a concerted effort to highlight the fact that we have not investigated the potential of the tripartite cocktail as a therapeutic except in the case of ricin toxin.

As noted previously, this study represents an advance within the field of antibody-based therapeutics for biothreat toxins. In this study we describe a cocktail of chimeric monoclonal antibodies that is capable of neutralizing three toxins: staphylococcal enterotoxin (SEB), *Clostridium perfringens* epsilon toxin (ETX) and ricin toxin. We demonstrate the efficacy of this cocktail in mouse models of lethal dose toxin challenge. This cocktail may prove useful as a therapeutic following a bioterrorism incident by one of these toxins.

Thank you in advance for re-considering this manuscript for publication in *Toxicon*.

Sincerely,

Nicholas Mantis

Nicholas Mantis, Ph.D.

Response to Reviewer (Toxcon-D-14-00312)

Reviewer #1: My poorly -articulated objection resulted from the extrapolation of the therapeutic potential to all three toxins. Data was shown that there is therapeutic potential against ricin. No data was shown for the other toxins. Yet, the authors seem to generalize these results to all three toxins when they talk about using "these mAbs" in "...military personnel as a post exposure therapeutic". This is where I think there needs to be editorial adjustment. The authors should clearly state the limitations of their data. They are free to state their hypothesis/belief that the cocktail will indeed be effective against all three, but should point out that the studies confirming this are either upcoming or in progress. With this modification, I believe this manuscript is worthy of publication in Toxicon.

We thank the Reviewer for taking time to clarify his/her critique and to point out what is clearly a notable limitation of the study that warrants further discussion. We have made the following three modifications to the manuscript in response to the Reviewer's comments:

modification 1: We have removed the sentence (formerly lines 145-146) stating "Nonetheless, these data underscore the potential utility of a cocktail as a therapeutic against three disparate toxins" so as not to overplay the therapeutic side of the cocktail

modification 2: We have inserted the sentence (line 152) "We therefore envision that the chimeric mAbs (or fully humanized derivatives) could be used as a means of providing passive immunity to first responders, laboratory staff or military personnel in the event that they may be at risk of toxin exposure."

modification 3: The following paragraph has been inserted into the manuscript: "We also envision the possibility that the cocktail could be used as a post-exposure therapeutic, although it is important to underscore that in this study we have only examined the potential of the tripartite cocktail to rescue mice following ricin challenge. We did not investigate whether the combination of cPB10, c19F1 and c4D7 had any therapeutic activity against SEB or ETX. Indeed, rescuing an individual following *C. perfringens* ETX exposure may be a particularly formidable challenge considering that the toxin exerts its effects on host cells virtually instantaneously. In the ETX-challenge model employed in this study, control mice succumbed to intoxication with 12 h, indicating that the therapeutic window (in rodents, at least) is likely to be very narrow. However, as is the case for ricin and SEB, the actual therapeutic potential of any antibody against ETX is going to be dependent on the amount and route (i.e., systemic versus mucosal) of toxin exposure. From the results of our limited *in vitro* and *in vivo* analysis of the combination of cPB10, c19F1 and c4D7 antibodies, we can only speculate that the tripartite cocktail has therapeutic utility in humans in an actual clinical setting."

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Ethical Statement

With this letter I attest that the manuscript entitled "A Tripartite Cocktail of Chimeric Monoclonal Antibodies Passively Protects Mice against Ricin, Staphylococcal Enterotoxin B and Clostridium perfringens Epsilon Toxin" to be submitted to *Toxicon* is an original study that has not previously been submitted to this (or any other) journal. In submitting this article we (the authors) have adhered to the ethical guidelines as described by the publisher: http://www.elsevier.com/publishingethics and http://www.elsevier.com/ethicalguidelines.

HIGHLIGHTS

- Ricin, staphylococcal enterotoxin (SEB), and *Clostridium perfringens* epsilon toxin (ETX) are biothreat toxins
- We developed a cocktail of chimeric monoclonal antibodies (mAbs) that neutralizes all three toxins
- Chimeric mAbs were expressed using a robust plant-based platform
- The tripartite cocktail also passively protected mice against ricin, SEB, and ETX in relevant challenge models
- These studies represent a major advancement towards a broad antitoxin antibody-based therapeutic

A Tripartite Cocktail of Chimeric Monoclonal Antibodies Passively Protects Mice against Ricin, Staphylococcal Enterotoxin B and *Clostridium perfringens* Epsilon Toxin

Erin K. Sully[1], Kevin Whaley[2], Natasha Bohorova[2], Ognian Bohorov[2], Charles Goodman[2], Do Kim[2], Michael Pauly[2], Jesus Velasco[2], Frederick W. Holtsberg[3], Eric Stavale[3], M. Javad Aman[3], Chandra Tangudu[4], Francisco A. Uzal[5], Nicholas J. Mantis[1,6,*] and Larry Zeitlin[2,*]

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Running title: Antibody Cocktail against Category B Toxins

Keywords: antibody, toxin, biodefense, therapeutic

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HIGHLIGHTS

- Ricin, staphylococcal enterotoxin (SEB), and *Clostridium perfringens* epsilon toxin (ETX) are biothreat toxins
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ABSTRACT

Due to the fast-acting nature of ricin, staphylococcal enterotoxin (SEB), and *Clostridium perfringens* epsilon toxin (ETX), it is necessary that therapeutic interventions following a bioterrorism incident by one of these toxins occur as soon as possible after intoxication. Moreover, because the clinical manifestations of intoxication by these toxins are likely to be indistinguishable from each other, especially following aerosol exposure, we have developed a cocktail of chimeric monoclonal antibodies that is capable of neutralizing all three toxins. The efficacy of this cocktail was demonstrated in mouse models of lethal dose toxin challenge.

The development of therapeutics directed against the Select Agents and Toxins poses significant and unique challenges. Foremost, the pathogens and toxins that are currently classified by the Centers for Disease Control and Prevention (CDC) as potential biothreat agents are genetically, evolutionarily, and structurally diverse, thereby necessitating therapeutics tailored against each agent (Mantis et al., 2011). Second, many of these agents, but in particular the toxins, can induce morbidity and even mortality within a matter of hours, which means that therapeutic interventions treatments will likely be initiated in the absence of definitive etiologic diagnosis (Wolfe et al., 2013). In addition, the earliest clinical manifestations of many select agents and toxins are expected to be indistinguishable from each other, which in a clinical setting may necessitate the administration of combinations of therapies (2007).

Ricin toxin, staphylococcal enterotoxin B (SEB), and *Clostridium perfringens* epsilon toxin (ETX) are fast acting, highly toxic and potentially lethal agents for which there are currently no available countermeasures (Mantis, 2005). The toxins are from unrelated sources and share no obvious functional domains or enzymatic activities (**Table 1**; **Figure S1**). Ricin toxin is a 65 kDa heterodimeric glycoprotein from the castor bean plant (*Ricinus communis*). The A subunit of ricin (RTA) is a ribosome-inactivating protein (RIP), while the B subunit (RTB) is a lectin that modulates toxin attachment and entry into mammalian cells. SEB is a 28 kDa superantigen produced by *Staphylococcus aureus* that, when ingested, causes symptoms that are classically associated with food poisoning, including cramps, vomiting and diarrhea (Krakauer and Stiles, 2013). While oral exposure to SEB is debilitating, it is rarely fatal. This is in contrast to SEB aerosol exposure, which in non-human primate models results in pulmonary endema and systemic complications (Lindsay and Griffiths, 2013; Mattix et al., 1995). Finally, ETX is a 33 kDa β-pore-forming toxin (PFT) secreted by *Clostridium perfringens* types B and D, which are

economically important pathogens associated with enterotoxemia in several species of livestock (Stiles et al., 2013; Uzal et al., 2014). All three toxins cross epithelial barriers and can elicit mucosal and systemic damage following ingestion or inhalation (Mantis, 2005). Due to the capacity of these toxins to induce similar clinical signs, morbidity and mortality, and their recognized potential as biological warfare and bioterrorism agents, we reasoned that a tripartite antitoxin cocktail capable of neutralizing ricin, SEB, and ETX would be of significant medical benefit.

Neutralizing mAbs against ricin, SEB, and ETX have been previously described (**Table 1**); mAb PB10 is directed against ricin toxin (Sully et al., 2014), 19F1 against SEB (L.Zeitlin, manuscript in preparation), and 4D7 against ETX (Garcia et al., 2014; Hauer and Clough, 1999). The murine variable domains of each of the mAbs were synthesized (Life Technologies; San Diego, CA) and grafted onto human IgG_1 frameworks, and transformed into *Agrobacterium tumefaciens*, which were then used for vacuum infiltration of *Nicotiana benthamiana* using the rapid antibody-manufacturing platform (RAMP) based on magnICON (Giritch et al., 2006; Hiatt and Pauly, 2006). RAMP makes use of transgenic *N. benthamiana* (Strasser et al., 2008) in which plant-specific glycosyl-transferases have been inhibited by RNAi, so the resulting mAbs contain mammalian N-glycans. The resulting chimeric (c-) derivatives of PB10, 19F1, and 4D7 have each been shown to retain potent toxin-neutralizing activity and to passively protect mice against a cognate toxin challenge (Garcia et al., 2014; Sully et al., 2014).

To examine the functional properties of a cocktail of plant-derived cPB10, c19F1, and c4D7, the three chimeric mAbs were combined at equimolar amounts and evaluated for toxin binding activity by ELISA and for toxin-neutralizing activities in cell-based cytotoxicity assays. We found that the binding profile of cPB10 as part of the tripartite cocktail was identical to cPB10

alone and its parenteral murine counterpart in terms of reactivity with RTA, ricin holotoxin, and its linear peptide epitope (**Figure 1A,C**). Moreover, the 50% inhibitory concentration (IC_{50}) of cPB10 was the same whether cPB10 was tested by itself or combined with c19F1 and c4D7 (**Figure 1B**). The toxin-binding activities (**data not shown**) as well as toxin-neutralizing activities (**Figure 1D,E**) of c19F1 and c4D7 as a cocktail were also indistinguishable from the individual mAbs themselves. These data indicate that there is no evidence to suggest that the different chimeric mAbs interfere with each other's function activities.

We next evaluated the tripartite cocktail for the ability to passively protect mice against ricin, SEB, and ETX in well-established mouse models of toxin challenge. For ricin toxin, mice received 5 μg, 2.5 μg or 1.5 μg of cPB10, by itself or as part of the tripartite cocktail, and were then challenged with 10 x LD_{50} ricin (**Figure 2A**). As expected, control mice succumbed to ricin intoxication within 48 h. Protection afforded by cPB10 was dose-dependent and was identical whether cPB10 was administered alone or in combination with c19F1 and c4D7, demonstrating that neither the anti-SEB or anti-ETX mAb interferes with cPB10. The reciprocal passive protection studies were done with the cocktail using mouse models of SEB and ETX intoxication. The SEB challenge model consisted of i.p. injection of 5 x LD_{50} SEB followed 4 h later by a potentiating dose of lipopolysaccharide (40 μg;List Biological Laboratories, Campbell, CA). Protection afforded by c19F1 was dose-dependent with complete survival observed in mice receiving 100 μg of c19F1, alone or in combination with c4D7 and cPB10 (**Figure 2B**). Finally, mAb c4D7 was able to fully protect mice when administered as part of the tripartite cocktail. The ETX challenge model involved i.p. administration of the cocktail to mice 24 h prior to intravenous injection of 3 x LD_{50} ETX, as described previously (Garcia et al., 2014). Control mice succumbed to toxin-induced death within 12 h, whereas cocktail-treated mice

117 survived without showing any clinical abnormalities (**Figure 2C**). Additional control mice
118 treated only with c4D7 also survived without showing any clinical abnormalities. These data
119 demonstrate the potential of a mixture of cPB10, c19F1 and c4D7 to protect mice against lethal
120 challenge doses of ricin, SEB, and ETX.

121 We next wished to further evaluate the tripartite antitoxin cocktail in a mucosal challenge
122 model and as a possible therapeutic. We chose ricin toxin for these studies since cPB10 has been
123 recently evaluated in respiratory tract challenge model and its therapeutic window has been
124 established (Sully et al., 2014). Groups of mice received 120 μg, 60 μg or 20 μg of cPB10 in the
125 context of the cocktail and then challenged with the same dose of ricin as above, but delivered
126 via the intranasal (i.n.) route. Protection afforded by the tripartite cocktail was dose-dependent
127 (**Figure 3A**); mice that received 120 μg cPB10 were protected from ricin challenge and
128 experienced a transient reduction in blood glucose levels (**Figure 3B**); half the mice that received
129 60 μg cPB10 were protected against ricin, whereas the control mice (no cPB10) or mice that
130 received 20 μg of cPB10 experienced a rapid onset of hypoglycemia and succumbed to toxin-
131 induced death within 48 h. These data demonstrate cPB10 within the context of the cocktail is
132 protective against respiratory tract challenge but that the amount of antibody required for
133 protection was 10-20 times that required for systemic challenge. Because the exact LD_{50} for i.n.
134 challenge is unknown, this requirement for increased dosing of mAb could be due to i.n.
135 challenge being more lethal than systemic challenge or due to a need for higher mAb
136 concentrations for protection on mucosal surfaces. In future studies, this observation will be
137 validated in an aerosol challenge model as it may have important implications for ricin-based
138 antibody prophylactics.

Finally, to assess the therapeutic potential of the tripartite cocktail, mice were challenged with 10 x LD_{50} ricin and then administered the cocktail at hourly intervals thereafter at amounts equivalent to 25 μg cPB10 per mouse (**Figure 3C**). In agreement with what we reported recently for cPB10 alone (Sully et al., 2014), the tripartite cocktail was able to fully rescue mice from toxin-induced death if administered within 4 h following challenge. It should be underscored that the mouse model is particularly stringent and that the actual therapeutic window in humans may in fact be greater than 4 h depending on the dose.

In summary, we have generated a cocktail of chimeric mAbs against three putative biothreat toxins derived from common, readily accessible plant (ricin) and bacteria (ETX and SEB). Due to their excellent safety profile and efficacy, mAbs are a rapidly growing class of therapeutic drugs (Reichert et al., 2005). Moreover, passive immunization with antibodies has been shown to be effective against a wide variety of toxins (Froude et al., 2011; Wang et al., 2013). We therefore envision that the chimeric mAbs (or fully humanized derivatives) could be used as a means of providing passive immunity to first responders, laboratory staff or military personnel in the event that they may be at risk of toxin exposure. As alluded to above, future improvements to the cocktail may include humanization of the mAbs and the engineering of point mutations in the Fc gamma chain constant regions that result in extended serum half-life with the possibility of using the cocktail as a prophylactic and provide passive protection for greater than six months (Robbie et al., 2013; Zalevsky et al., 2010). Such a cocktail would constitute a significant resource within the public health and biodefense community.

We also envision the possibility that the cocktail could be used as a post-exposure therapeutic, although it is important to underscore that in this study we have only examined the potential of the tripartite cocktail to rescue mice following ricin challenge. We did not

investigate whether the combination of cPB10, c19F1 and c4D7 had any therapeutic activity against SEB or ETX. Indeed, rescuing an individual following *C. perfringens* ETX exposure may be a particularly formidable challenge considering that the toxin exerts its effects on host cells virtually instantaneously. In the ETX-challenge model employed in this study, control mice succumbed to intoxication with 12 h, indicating that the therapeutic window (in rodents, at least) is likely to be very narrow. However, as is the case for ricin and SEB, the actual therapeutic potential of any antibody against ETX is going to be dependent on the amount and route (i.e., systemic versus mucosal) of toxin exposure. From the results of our limited *in vitro* and *in vivo* analysis of the combination of cPB10, c19F1 and c4D7 antibodies, we can only speculate that the tripartite cocktail has therapeutic utility in humans in an actual clinical setting.

ACKNOWLEDGEMENTS

The authors thank Drs. Yuri Gleba and Victor Klimyuk for access to the Icon Genetics' magnICON system and Dr. Herta Steinkellner for access to the transgenic *N. benthamiana*. This work was supported by NIH grants U01AI082276 (LZ), R01AI098774 (LZ).

REFERENCES

2007. NIAID Strategic Plan for Biodefense Research - 2007 Update.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The Protein Data Bank. Nucleic Acids Res 28, 235-242.

Froude, J.W., 2nd, Stiles, B., Pelat, T., Thullier, P., 2011. Antibodies for biodefense. MAbs 3.

Garcia, J.P., Beingesser, J., Bohorov, O., Bohorova, N., Goodman, C., Kim, D., Pauly, M., Velasco, J., Whaley, K., Zeitlin, L., Roy, C.J., Uzal, F.A., 2014. Prevention and treatment of Clostridium perfringens epsilon toxin intoxication in mice with a neutralizing monoclonal antibody (c4D7) produced in Nicotiana benthamiana. Toxicon 88C, 93-98.

Giritch, A., Marillonnet, S., Engler, C., van Eldik, G., Botterman, J., Klimyuk, V., Gleba, Y., 2006. Rapid high-yield expression of full-size IgG antibodies in plants coinfected with noncompeting viral vectors. Proc Natl Acad Sci U S A 103, 14701-14706.

Hauer, P.J., Clough, N.E., 1999. Development of monoclonal antibodies suitable for use in antigen quantification potency tests for clostridial veterinary vaccines. Developments in biological standardization 101, 85-94.

Hiatt, A., Pauly, M., 2006. Monoclonal antibodies from plants: A new speed record. Proceedings of the National Academy of Sciences 103, 14645-14646.

Karauzum, H., Chen, G., Abaandou, L., Mahmoudieh, M., Boroun, A.R., Shulenin, S., Devi, V.S., Stavale, E., Warfield, K.L., Zeitlin, L., Roy, C.J., Sidhu, S.S., Aman, M.J., 2012. Synthetic human monoclonal antibodies toward staphylococcal enterotoxin B (SEB) protective against toxic shock syndrome. J Biol Chem 287, 25203-25215.

Krakauer, T., Stiles, B.G., 2013. The staphylococcal enterotoxin (SE) family: SEB and siblings. Virulence 4, 759-773.

Lindsay, C.D., Griffiths, G.D., 2013. Addressing bioterrorism concerns: options for investigating the mechanism of action of Staphylococcus aureus enterotoxin B. Human & experimental toxicology 32, 606-619.

Mantis, N.J., 2005. Vaccines against the category B toxins: Staphylococcal enterotoxin B, epsilon toxin and ricin. Adv Drug Deliv Rev 57, 1424-1439.

Mantis, N.J., Morici, L.A., Roy, C.J., 2011. Mucosal Vaccines for Biodefense. Curr Top Microbiol Immunol.

Mattix, M.E., Hunt, R.E., Wilhelmsen, C.L., Johnson, A.J., Baze, W.B., 1995. Aerosolized staphylococcal enterotoxin B-induced pulmonary lesions in rhesus monkeys (Macaca mulatta). Toxicol Pathol 23, 262-268.

O'Hara, J.M., Brey, R.N., Mantis, N.J., 2013. Comparative Efficacy in Mice of Two Lead Candidate Ricin Toxin A Subunit (RTA) Vaccine. Clinical and Vaccine Immunology in press.

Reichert, J.M., Rosensweig, C.J., Faden, L.B., Dewitz, M.C., 2005. Monoclonal antibody successes in the clinic. Nat Biotechnol 23, 1073-1078.

Robbie, G.J., Criste, R., Dall'acqua, W.F., Jensen, K., Patel, N.K., Losonsky, G.A., Griffin, M.P., 2013. A novel investigational Fc-modified humanized monoclonal antibody, motavizumab-YTE, has an extended half-life in healthy adults. Antimicrob Agents Chemother 57, 6147-6153.

Robertson, S.L., Li, J., Uzal, F.A., McClane, B.A., 2011. Evidence for a prepore stage in the action of Clostridium perfringens epsilon toxin. PLoS One 6, e22053.

Stiles, B.G., Barth, G., Barth, H., Popoff, M.R., 2013. Clostridium perfringens epsilon toxin: a malevolent molecule for animals and man? Toxins (Basel) 5, 2138-2160.

Strasser, R., Stadlmann, J., Schähs, M., Stiegler, G., Quendler, H., Mach, L., Glössl, J., Weterings, K., Pabst, M., Steinkellner, H., 2008. Generation of glyco-engineered Nicotiana benthamiana for the production of monoclonal antibodies with a homogeneous human-like N-glycan structure. Plant Biotechnology Journal 6, 392-402.

Sully, E.K., Whaley, K.J., Bohorova, N., Bohorov, O., Goodman, C., Kim do, H., Pauly, M.H., Velasco, J., Hiatt, E., Morton, J., Swope, K., Roy, C.J., Zeitlin, L., Mantis, N.J., 2014. Chimeric Plantibody Passively Protects Mice against Aerosolized Ricin Challenge. Clin Vaccine Immunol 21, 777-782.

Uzal, F.A., Freedman, J.C., Shrestha, A., Theoret, J.R., Garcia, J., Awad, M.M., Adams, V., Moore, R.J., Rood, J.I., McClane, B.A., 2014. Towards an understanding of the role of Clostridium perfringens toxins in human and animal disease. Future microbiology 9, 361-377.

Vance, D.J., Mantis, N.J., 2012. Resolution of two overlapping neutralizing B cell epitopes within a solvent exposed, immunodominant alpha-helix in ricin toxin's enzymatic subunit. Toxicon 60, 874-877.

Wang, X.Z., Coljee, V.W., Maynard, J.A., 2013. Back to the future: recombinant polyclonal antibody therapeutics. Current opinion in chemical engineering 2, 405-415.

Wolfe, D.N., Florence, W., Bryant, P., 2013. Current biodefense vaccine programs and challenges. Human vaccines & immunotherapeutics 9.

Zalevsky, J., Chamberlain, A.K., Horton, H.M., Karki, S., Leung, I.W., Sproule, T.J., Lazar, G.A., Roopenian, D.C., Desjarlais, J.R., 2010. Enhanced antibody half-life improves in vivo activity. Nat Biotechnol 28, 157-159.

TABLE(S)

Table 1. Characteristics of SEB, ETX and ricin toxin and their respective mAbs.

Toxin	kDa	Toxin Class	mAb	Reference
SEB	28	super antigen	19F	*in preparation*
ETX	33	β-pore forming	4D7	(Garcia et al., 2014)
Ricin	65	ribosome-inactivating	PB10	(Sully et al., 2014)

FIGURE LEGENDS

Figure 1. Toxin binding and neutralizing activities associated with the chimeric mAbs in the context of the tripartite cocktail. The tripartite cocktail was assessed for specificity for ricin (**panels A-C**), SEB **(panel D**) and ETX (**panel E**). (**A**) cPB10 (alone or in cocktail) reactivity with ricin holotoxin and subunits by ELISA. Nunc Maxisorb F96 microtiter plates (ThermoFisher Scientific) were coated by overnight incubation with 1 μg/ml ricin, RTA, RTB or BSA. Plates were developed using horseradish peroxidase (HRP)-labeled goat anti-human IgG (Invitrogen) and 3,3',5,5' tetramethylbenzidine (Kirkegaard & Perry Labs, Gaithersburg, MD), as described (Sully et al., 2014). (**B**) Toxin-neutralizing activity of cPB10. Serial dilutions (in triplicate) of cPB10, alone or the cocktail were mixed with ricin (10 ng/ml) and then applied to Vero cells, as described (Sully et al., 2014). Cell viability was assessed 48 h later; (**C**) cPB10 (alone or in cocktail) reactivity with RTA-peptide array. Overlapping 18-mer peptides spanning the length of RTA (O'Hara et al., 2013) were used to coat Nunc Maxisorb F96 microtiter plates (ThermoFisher Scientific) before being probed with cPB10. ELISA plates were developed as described in panel A. Peptide A11 (RTA residues Y91-F108) co corresponds to PB10's known epitope (Vance and Mantis, 2012). (**D**) The neutralizing activity of c19F1 was determined using peripheral blood mononuclear cells (PBMCs) and SEB toxin, as described (Karauzum et al., 2012). The resulting inhibition of INF-γ production by c19F1 or the antibody cocktail were indistinguishable (**E**) ETX neutralizing assays were performed by incubating ETX with indicated concentrations of c4D7, alone or in the context of the cocktail. Neutralizing assays were done using Madin-Darby Canine Kidney (MDCK II) cells, as described (Garcia et al., 2014; Robertson et al., 2011). ETX was obtained from BEI Resources (Manassas, VA).

Figure 2. Protection afforded by the tripartite mAb cocktail in mice upon challenge with ricin, SEB and ETX. The tripartite mAb cocktail was assessed for the ability to protect mice against ricin (**panels A, D-F**), SEB (**panel B**) and ETX (**panel C**). All studies involving mice were done in strict compliance the Institutional Animal Care and Use Committees (IACUC) at the Wadsworth Center, Iowa State University, and University of California, Davis. (**A**) BALB/c mice (female, 6-8 weeks of age; Taconic Labs, Hudson, NY) were housed under conventional, specific pathogen-free conditions. cPB10, alone or in the cocktail was administered to mice

(n=10/group) by intraperitoneal (i.p.) injection 24 h prior to challenge with 10x LD_{50} ricin (~2 μg mouse; Vector Laboratories, Burlingame, CA), also by i.p. injection. Survival was monitored over a period of five days. (**B**) To evaluate c19F1, the chimeric mAb alone or in the context of the cocktail was mixed with SEB (1 μg) for 1 hr and then injected into BALB/c mice (Karauzum et al., 2012). Four hours later the animals received a potentiating dose of lipopolysaccharide (40 μg; List Biological Laboratories, Campbell, CA) and were monitored for survival for 5 days. (**C**) To evaluate c4D7, the chimeric mAb in the context of the cocktail was administered to female BALB/c mice by i.p. injection, as described previously (Garcia et al., 2014). Twenty-four hours later, the animals were challenged by intravenous injection 3xLD_{50} ETX.

Figure 3. Mucosal protection and therapeutic potential of the tripartite cocktail against ricin toxin. (**A-B**). Capacity of cPB10 to protect against intranasal ricin challenge. The tripartite cocktail was administered to mice (n=8 mice per group) by intraperitoneal (i.p.) injection 24 h prior to intranasal 10 x LD_{50} ricin challenge. Mice were monitored for survival (panel A) and morbidity (panel B), as determined by blood glucose levels (Sully et al., 2014); (**C**) Groups of BALB/c mice (n=8 per group) were challenged with 10 x LD_{50} ricin and then administered (by i.p. injection) the tripartite cocktail (25 μg of cPB10/mouse) at indicated times (hours). Survival was monitored for five days.

SUPPLEMENTARY FIGURE LEGEND

Figure S1. Structures of ricin, SEB and *C. perfrigens* ETX. Biological assembly images from the Protein Data Bank (PDB) (Berman et al., 2000). The images correspond to the following PBD identifiers: (A) ricin, 2AAI; (B) SEB, 3SEB; (C) ETX, 1UYJ.

Figure 1

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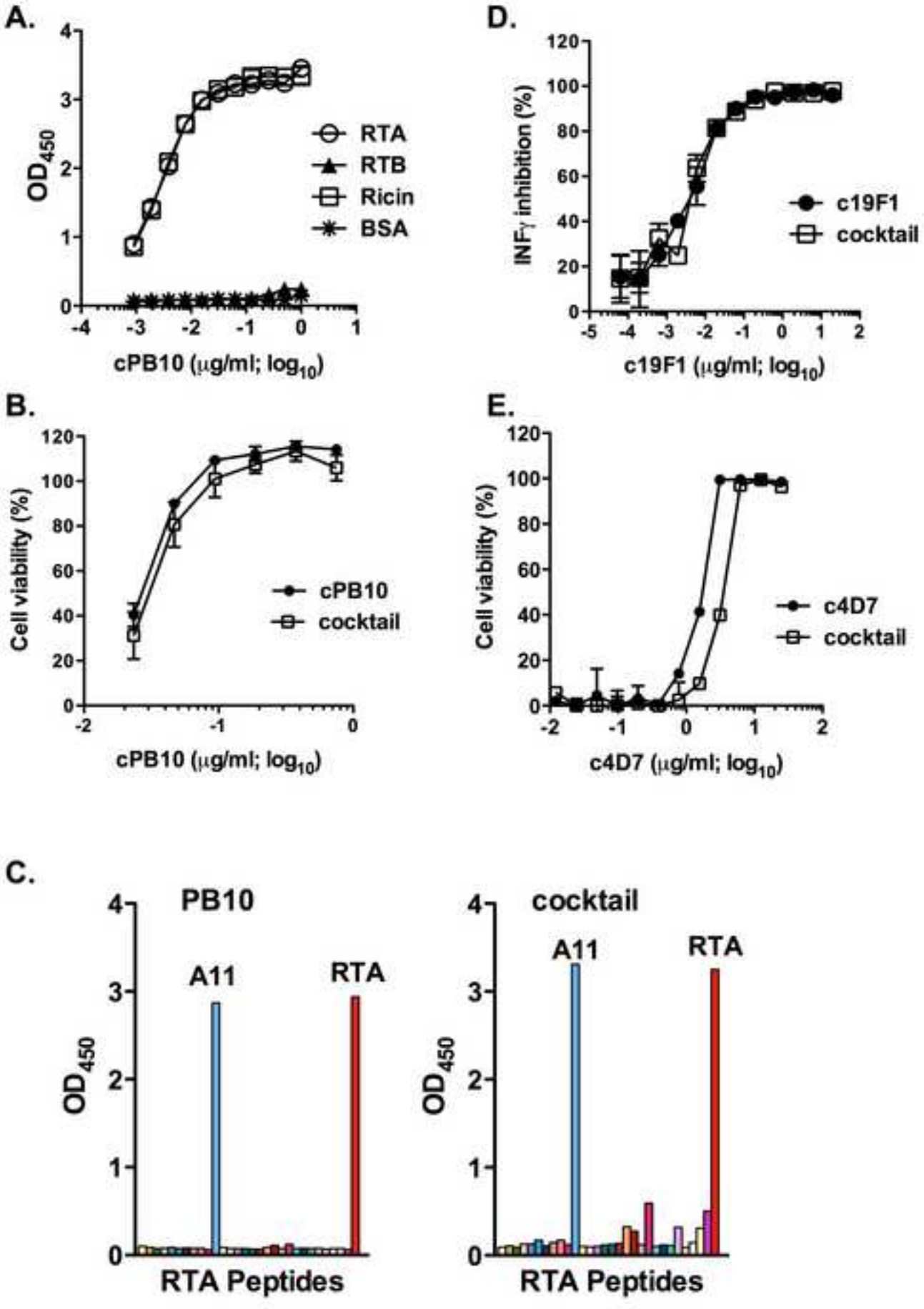

Figure 2

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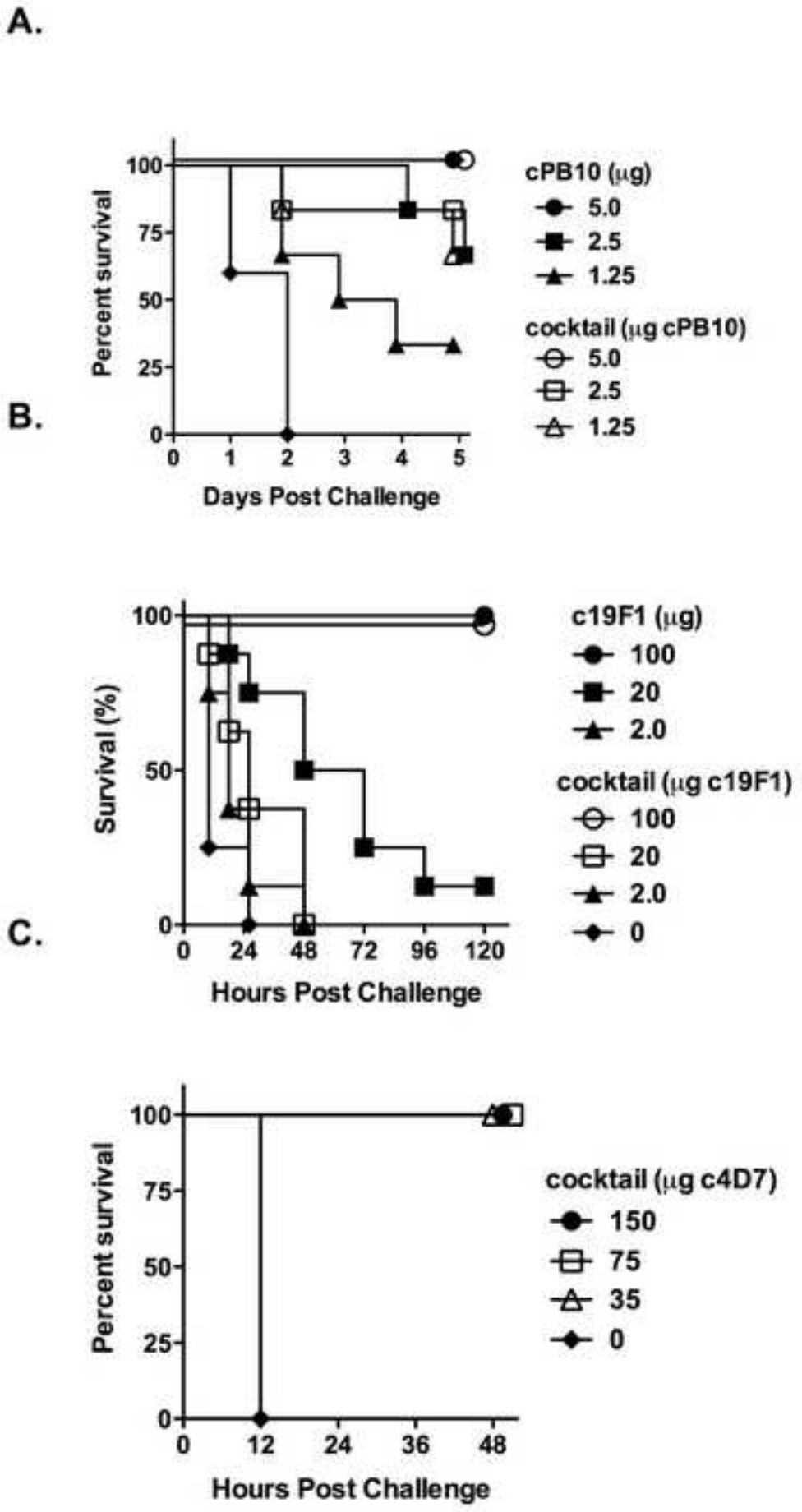

Figure 3

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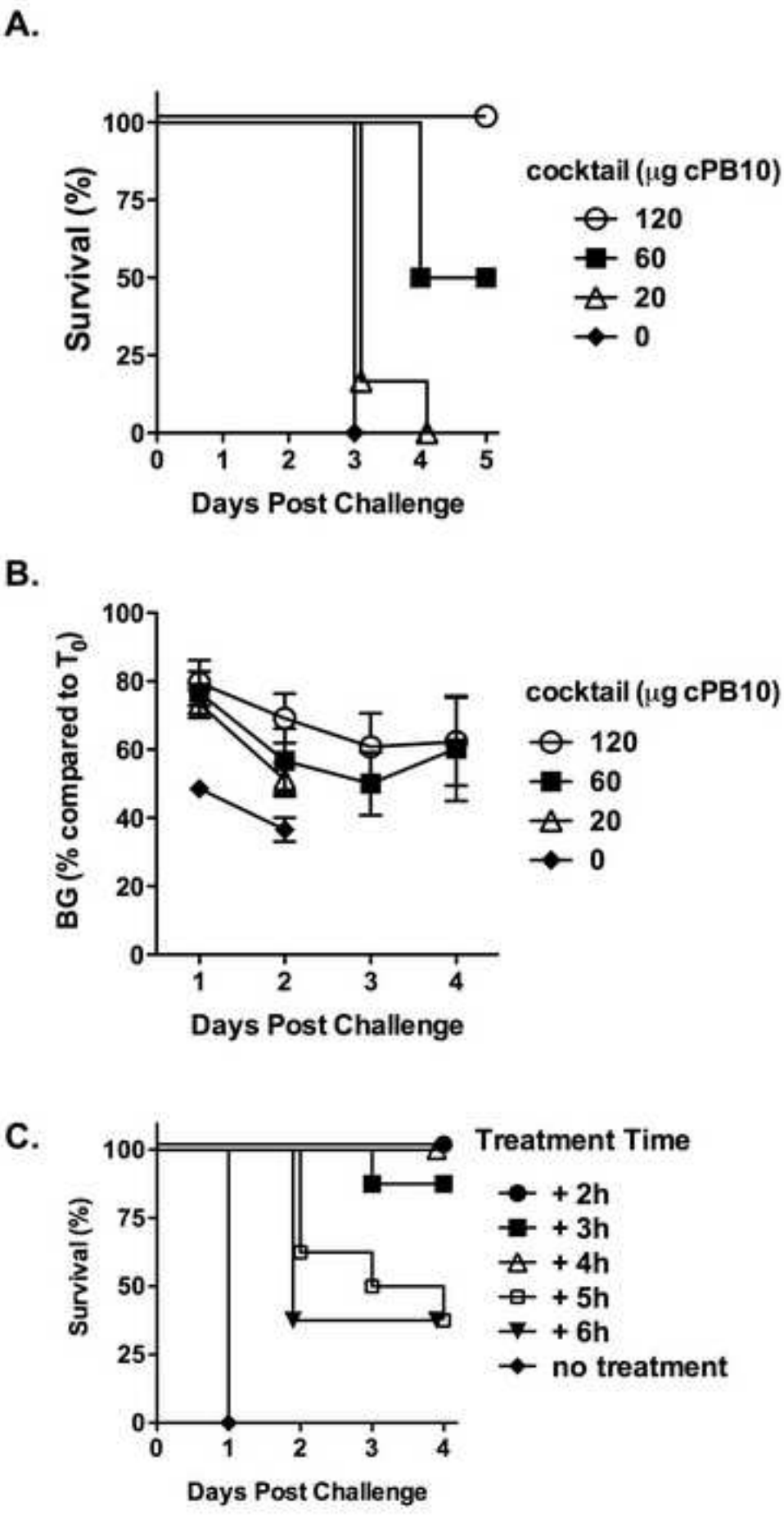

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