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Novel Chimpanzee/Human Monoclonal Antibodies That Neutralize Anthrax Lethal Factor, and Evidence for Possible Synergy with Anti-Protective Antigen Antibody▽

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Three chimpanzee Fabs reactive with lethal factor (LF) of anthrax toxin were isolated and converted into complete monoclonal antibodies (MAbs) with human γ1 heavy-chain constant regions. In a macrophage toxicity assay, two of the MAbs, LF10E and LF11H, neutralized lethal toxin (LT), a complex of LF and anthrax protective antigen (PA). LF10E has the highest reported affinity for a neutralizing MAb against LF (dissociation constant of 0.69 nM). This antibody also efficiently neutralized LT in vitro, with a 50% effective concentration (EC_{50}) of 0.1 nM, and provided 100% protection of rats against toxin challenge with a 0.5 submolar ratio relative to LT. LF11H, on the other hand, had a slightly lower binding affinity to LF (dissociation constant of 7.4 nM) and poor neutralization of LT in vitro (EC_{50} of 400 nM) and offered complete protection in vivo only at an equimolar or higher ratio to toxin. Despite this, LF11H, but not LF10E, provided robust synergistic protection when combined with MAb W1, which neutralizes PA. Epitope mapping and binding assays indicated that both LF10E and LF11H recognize domain I of LF (amino acids 1 to 254). Although domain I is responsible for binding to PA, neither MAb prevented LF from binding to activated PA. Although two unique MAbs could protect against anthrax when used alone, even more efficient and broader protection should be gained by combining them with anti-PA MAbs.

Anthrax is a highly lethal infectious disease caused by the spore-forming bacterium *Bacillus anthracis*. The deliberate distribution of anthrax spores through the U.S. mail system in 2001 resulted in five deaths among the 11 individuals who contracted inhalational anthrax (18). This incident highlighted the great threat posed by the potential use of anthrax in terrorism and warfare. The lethality of inhalational anthrax is primarily due to the action of anthrax toxins. The bacterium produces three toxin components; these are protective antigen (PA) (83 kDa), lethal factor (LF) (85 kDa), and edema factor (EF) (89 kDa) (13, 32). PA binds to host cell anthrax toxin receptors and is cleaved by cell surface furin to produce a 63-kDa peptide, PA63 (activated PA). Anthrax toxin receptor-bound PA63 oligomerizes to a heptamer and translocates up to three molecules of LF or EF from the cell surface via endosomes to the cytosol. Therefore, PA functions as a vehicle to mediate the cellular uptake of LF and EF (for a review, see reference 44). PA with LF forms lethal toxin (LT), and PA with EF forms edema toxin (ET). LF is a zinc-dependent endopeptidase that cleaves mitogen-activated protein kinase kinases and disrupts intracellular signaling (8, 30, 40). LT can replicate symptoms of anthrax disease when injected into animals (27). EF is a calcium-calmodulin-dependent adenylate cyclase that transforms ATP to cyclic AMP, and ET has a range of toxic effects in the host (12, 20). These toxins are the dominant virulence factors for anthrax disease, and vaccination against their common component, PA, is sufficient for protection against anthrax disease.

Currently antibiotics are the only choice for clinical treatment of anthrax disease. Although effective, antibiotics have limitations. Exposure to the bacterium followed by bacterial division leads to production of large quantities of the anthrax toxins. Thus, unless exposure is diagnosed early enough for antibiotic treatment to prevent significant replication, patients will succumb to disease even after the killing of all bacteria. The current PA-based vaccine approved by the U.S. Food and Drug Administration is also not effective postexposure in protecting newly infected individuals, as it requires repeated administration and at least 4 weeks for development of anti-PA protective titers. Thus, in the absence of any small-molecule toxin inhibitors, monoclonal antibodies (MAbs) against toxin components are the only viable candidates for immediate neutralization of the effects of toxin. Although PA has been the primary target for passive protection (5, 25, 31, 35, 41, 43), it has been suggested that immunity to LF and EF can also play an important role in protection (14, 33, 34), and thus these proteins may represent alternative targets for antibody therapy against anthrax. In a previous study, the protective effects of anti-PA and anti-LF antibodies were greatly synergized by their combination (3). Furthermore, concerns that PA may be mutated within currently recognized neutralization epitopes such that anti-PA therapies would no longer be effective against this toxin warrant the further development of antibodies targeting the other toxin components. A cocktail of more

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than one MAb that could recognize distinct epitopes on multiple toxin proteins (PA, LF, and EF) could certainly broaden the spectrum of protection against anthrax. In recent years, several anti-LF neutralizing MAbs have been reported (1, 21, 24, 37, 46). However, only one of them was a human antibody; the others were rodent MAbs that would need further manipulation before use in humans.

Chimpanzee immunoglobulins (Igs) are virtually identical to human Igs and may have clinically useful applications (9). As part of a larger study (5), we recovered chimpanzee MAbs specific for LF from a combinatorial cDNA library of antibody genes developed from chimpanzees that had been immunized with anthrax toxins. In this work we describe the detailed characterization of these anti-LF antibodies.

MATERIALS AND METHODS

Materials. Rabbit polyclonal antibodies to PA and LF were generated in one of our laboratories. The anti-LF antibody cross-reacts with EF and can be used for detection of either toxin by Western blotting. MEK1 N-terminal (NT) antibody was purchased from Calbiochem (San Diego, CA). Anti-PA MAbs 14B7 and W1 have been described in detail previously (5, 23). The infrared dye-conjugated secondary antibody (IRDye800CW IgG) used in Western blotting was purchased from Rockland Immunochemicals (Gilbertsville, PA).

Toxins. PA and LF were made from *Bacillus anthracis* in our laboratory as previously described (39). The recombinant LF used in this study has the N-terminal sequence HMAGG. FP59 (a fusion of the N terminus of LF [amino acids 1 to 254] and *Pseudomonas aeruginosa* exotoxin A domain III) was made from *Escherichia coli* as previously described (2). For cytotoxicity or neutralization assays, toxin was prepared in Dulbecco's modified Eagle medium (Invitrogen, Carlsbad, CA) prior to addition to cells. Toxin for animal injections was prepared in sterile phosphate-buffered saline (PBS) with or without antibody. Concentrations and doses of LT refer to the amounts of each component (e.g., 100 ng LT/ml is 100 ng PA plus 100 ng LF/ml, and 10 μg LT is 10 μg PA plus 10 μg LF).

Phage library construction and selection. The combinatorial cDNA library of chimpanzee γ1/κ antibody genes was constructed by cloning heavy and light chains into pComb3H at XhoI/SpeI and SacI/XbaI sites as described previously (4). The library was panned against immobilized recombinant LF protein that had been blocked in solution with two previously isolated nonneutralizing anti-LF Fabs (5). The panning procedure was performed three times, and the LF-specific clones were identified by 96-well phage enzyme-linked immunosorbent assay (ELISA) as described previously (15).

Fab and IgG production and purification. The phagemid encoding soluble Fab was generated by removal of the phage coat protein III-encoding region from phagemid DNA through restriction enzyme digestion and religation. The soluble Fab was expressed and purified on a nickel-charged column as described previously (4). The conversion of Fab to IgG and the expression of IgG were carried out as described previously (4).

The purities of Fab and IgG were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (NuPAGE MOP; Invitrogen, Carlsbad, CA). Protein concentrations were determined both by dye binding assay (Pierce, Rockford, IL) and by measurement of optical density at 280 nm, assuming that an optical density at 280 nm of 1.35 is equivalent to 1.0 mg/ml.

ELISA analysis of IgG specificity. Ninety-six-well ELISA plates were coated with recombinant LF (5 μg/ml) or unrelated proteins (bovine serum albumin [BSA], thyroglobulin, lysozyme, and phosphorylase *b*) (Sigma, St. Louis, MO) (10 μg/ml) in carbonate buffer (pH 9.5). ELISAs were performed as previously described (5).

Nucleic acid sequence analysis of LF-specific Fab clones. The genes coding for the variable regions of heavy (VH) and light (VL) chains of LF-specific clones were sequenced, and their corresponding amino acid sequences were aligned. The presumed family usage and germ line origin were determined for each VH and VL gene by search of V-Base, which is a compilation of all of the available human variable-region Ig germ line sequences (6).

Affinity measurement. The kinetic analysis of the LF-neutralizing Fabs was performed on a BIAcore 3000 instrument (GE Healthcare, Piscataway, NJ). The LF was immobilized onto a CM3 chip using *N*-hydroxysuccinimide–1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide coupling chemistry to achieve 1,420 resonance units. Anti-LF Fabs at concentrations ranging from 0.4 nM to 2000 nM were injected onto the chip surface. The kinetic interaction between Fab and immobilized LF was displayed in the sensorgram and evaluated by globally modeling kinetic data as a continuing distribution of affinity and rate constants (38).

LT neutralization. LT (100 ng/ml) was prepared in Dulbecco's modified Eagle medium in a 96-well plate. Antibodies were diluted serially directly into the toxin mixture and incubated for 1 h at 37°C. Anti-PA antibody 14B7 was used on each plate as a positive control in all neutralization assays. The LT-MAb mixtures were then transferred to RAW264.7 macrophage cells grown to 80 to 90% confluence in 96-well plates. Cells were incubated for 4 h, and cell viability was assessed by incubation with 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, MO) at a final concentration of 0.5 mg/ml for 40 min. The blue pigment produced by viable cells was solubilized by aspirating the medium and adding 50 μl/well of 0.5% (wt/vol) SDS and 25 mM HCl in 90% (vol/vol) isopropanol and shaking the plates for 5 min prior to reading at 570 nm using a microplate reader. Results were plotted, and the effective concentration that produced 50% neutralization (EC_{50}) was calculated with Prism software (Graphpad Software Inc., San Diego, CA).

PA binding, LF binding, and MEK cleavage assays. LT was incubated with each MAb at a 100-fold weight excess or with PBS for 1 h prior to addition to CHO (Chinese hamster ovary) cells or RAW264.7 cells in six-well plates. In other experiments, LF was incubated with each MAb at a 100-fold weight excess or with PBS for 1 h before addition of PA. LT alone (without antibody) and LF alone (without antibody and PA) served as positive and negative controls, respectively. Cells were treated with the toxin-antibody mixture at 37°C for 1 h. After the medium was aspirated, cells were washed five times in ice-cold PBS and lysed in radioimmunoprecipitation assay buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS in PBS plus Complete protease inhibitor cocktail [Roche Applied Science, Indianapolis, IN]). Protein concentrations in lysates were quantified using the bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL) for equal loading on gels. Western blot analysis was performed using rabbit polyclonal anti-LF (1:1,000), anti-PA (1:5,000), or anti-MEK1 N-terminal (1:5,000) antibodies. Infrared dye-conjugated secondary goat anti-rabbit IgG (1:20,000) was used for detection with the Odyssey infrared imaging system (Li-Cor Biosciences, Lincoln, NE).

Animal studies. Two chimpanzees, no. 1603 and 1609, were immunized with recombinant PA, LF, and EF, and the bone marrows were used for library construction as described previously (4). A classic Fischer 344 rat toxin challenge model was used for assessing the protective efficacy of antibodies in vivo (23). Female Fischer 344 rats at 160 to 180 g (Taconic Farms, Germantown, NY) were injected via the tail vein with a mixture of antibody and PA plus LF (LT) at different molar ratios, prepared in sterile PBS, or with LT alone. The LT dose was in all cases 10 μg/rat, and injection volumes were 200 μl/rat. A group of six or more rats was used for each treatment. Animals were observed continuously for the first 8 h, then at 16 h, and throughout the second day. Animals were monitored for signs of malaise and mortality. All animal experiments were performed under protocols approved by the NIAID Animal Care and Use Committee.

Statistics. The protective efficacy of antibody against toxin challenge in rats was analyzed between groups with and without antibody treatment by Fisher's exact test (two sided) (GraphPad Prism 5, San Diego, CA), as used by others previously (16, 17, 22). A P value of <0.05 was considered statistically significant.

RESULTS

Three LF-specific Fabs were isolated and characterized. Following three rounds of panning against LF, 96 individual clones were screened for binding to LF and to BSA by phage ELISA. Ninety-two Fab clones were found to bind to LF but not to BSA. Sequence analysis identified three unique anti-LF Fab clones (LF9D, LF10E, and LF11H) with distinct VH and VK sequences (Fig. 1A and B). The closest human V-gene germ line origin of the three clones was determined from a sequence similarity search of all the known human Ig genes (Table 1). The three Fab clones were subsequently converted to chimpanzee/human IgG1 chimeras as described previously (4). The binding specificities of the IgGs were examined by ELISA. All three IgG clones were specific for LF, since they

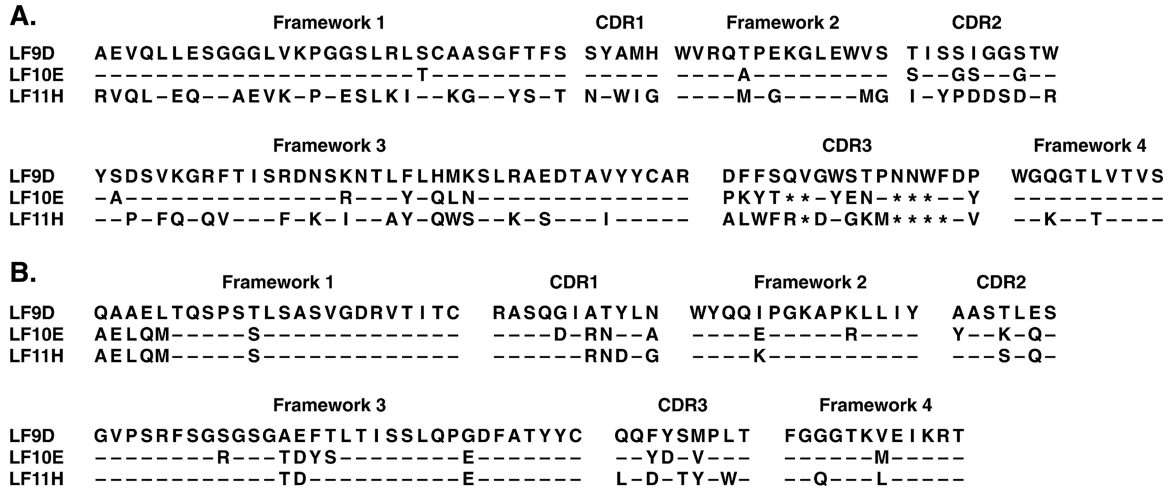

FIG. 1. Alignment of the deduced amino acid sequences of the variable domains of the heavy (A) and kappa (B) chains of anti-LF clones. Substitutions relative to LF9D are shown as single amino acid letters. Identical residues are indicated by dashes. The absence of corresponding residues relative to the longest sequence is indicated by asterisks. Complementarity-determining regions (CDR1, CDR2, and CDR3) and framework regions (1, 2, 3, and 4) are indicated above the sequence alignments.

bound to LF (Fig. 2) but not to BSA, thyroglobulin, lysozyme, or phosphorylase *b* (data not shown).

Anti-LF antibodies LF10E and LF11H neutralize LT with high affinity. We tested the anti-LF MAbs in the standard macrophage toxicity neutralization assays used for assessing anti-PA and anti-LF antibodies and compared the EC_{50}s for neutralization by these antibodies to those for the well-characterized monoclonal anti-PA MAbs 14B7 and W1 (Fig. 3). LF10E protected macrophages with an average EC_{50} of 15.6 ± 7.5 ng/ml (~0.1 nM), comparable to the value for the highly potent W1 anti-PA MAb (5). Thus, it was 10-fold more potent than MAb 14B7. LF11H had a much lower in vitro neutralization activity, with an EC_{50} of 62.6 ± 28.3 μg/ml (~400 nM). LF9D did not neutralize at all (data not shown).

The affinities of the two neutralizing Fabs, LF10E and LF11H, were determined by surface plasmon resonance. We found that LF10E had an affinity for LF in the subnanomolar range, which was about 10-fold higher than the affinity of LF11H (Table 2). This suggests that factors other than affinity contribute to the 4,000-fold difference in in vitro neutralizing activity between LF10E and LF11H.

LF10E and LF11H bind to LFn and prevent LT-mediated MEK cleavage in cells but do not prevent LF binding to cleaved PA. To determine the region of LF to which LF10E and LF11H bind, we tested the reactivity in Western blots of these MAbs with LF and with a fusion protein consisting of the N-terminal 254 amino acids of LF (LFn) coupled to *Pseudomonas aeruginosa* exotoxin A (FP59). Purified PA, EF, and a fusion protein consisting of the N-terminal region of EF (amino acids 1 to 260) coupled to diphtheria toxin (FP119) were used as negative controls. This region of EF has very high homology to LFn, and all available polyclonal antibodies to LF cross-react with EF through binding to this domain. Both MAb LF10E and LF11H bound only to LF and FP59 and did not cross-react with EF (Fig. 4A and B). Thus, the epitope for LF10E and LF11H is located within the N-terminal region of LF, which was previously identified as the PA binding domain (26). To determine if the anti-LF MAbs interfered with the binding of LF to cleaved PA through blocking of this domain,

TABLE 1. Assignment of three chimpanzee anti-LF Fab clones to their closest human germ line counterparts, based on nucleotide sequence homology

MAb	Germ line gene in[a]:						
	VH family	Segment			VK family	Segment	
		VH	D	JH		VK	JK
LF9D	3	DP-47	D3	J5b	I	DPK8	J4
LF10E	3	DP-47	D5-12	J4b	I	DPK3	J4
LF11H	5	D7-73	D7-27	J6c	I	DPK3	J1

[a] The closest human V-gene germ lines were identified by search of the V-Base database.

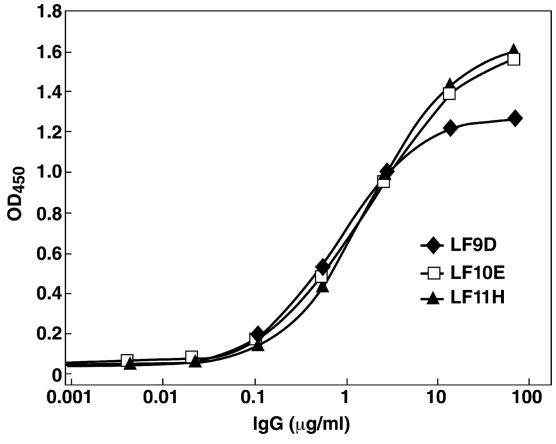

FIG. 2. ELISA titration of anti-LF MAbs. Recombinant LF was used to coat ELISA plates. Wells were then incubated with various dilutions of LF9D, LF10E, and LF11H IgGs, and the bound IgGs were detected by the addition of peroxidase-conjugated anti-human Fc antibody followed by tetramethylbenzidine substrate. OD_{450}, optical density at 450 nm.

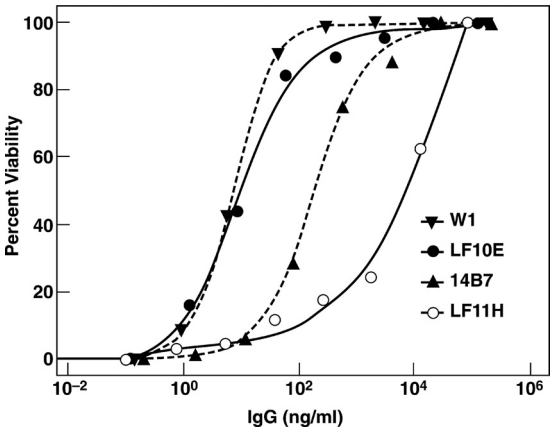

FIG. 3. LF10E and LF11H neutralize LT toxicity. LT (100 ng/ml) was incubated with serial dilutions of each antibody (1 h, 37°C) prior to treatment of RAW264.7 macrophage cells with LT-MAb mixtures for 4 h. Cell viability was assessed by 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide staining and is presented as a percentage of the value for untreated controls.

we incubated LT (both PA and LF) with a 100-fold (weight) excess of each antibody or with PBS for 1 h prior to adding these mixtures to the cells. Alternatively, we first incubated LF with a 100-fold (weight) excess of each antibody or with PBS for 1 h prior to adding PA. The binding assay was repeated four times, and the same results were obtained. A representative result is shown in Fig. 4C. We were surprised to discover that LF10E and LF11H did not inhibit the binding of LF to the PA63 on cells, since LF was found in equivalent amounts in lysates from cells treated with LT alone or with LT prebound with either antibody (Fig. 4C). The same result was observed when LF was prebound to either antibody (data not shown). The detected LF was not due to the nonspecific binding of LF to the cells, because LF was either undetectable or barely detectable when LF alone was added to the cells (data not shown). However, each antibody clearly inhibited LF-mediated MEK cleavage in the cytosol (Fig. 4D). This suggests that the anti-LF MAbs are unique in that they act at a translocation step subsequent to LF binding to PA but prior to LF delivery to the cytosol.

LF10E and LF11H protect rats from LT challenge. The MAbs were next tested for protection in the classic Fischer rat model for LT challenge (24). Antibodies were premixed at different molar ratios with LT (10 μg/rat), starting with equimolar amounts of antibody and toxin and reducing antibody to submolar concentrations relative to toxin, and administered intravenously. The mean time to death of control rats challenged with 10 μg of LT in PBS was 82 min. LF10E protected 100% of the rats at an antibody/LF ratio of 1:2, but further reducing the molar ratio to 1:3 resulted in only 67% protection (Table 3). In previous experiments we had established that anti-PA MAb W1 protected rats at an antibody/PA molar ratio of 1:4 but not at lower concentrations. Therefore, we investigated whether the combination of anti-LF 10E with anti-PA MAbs could provide synergistic protection. Combinatorial studies mixing various concentrations of LF10E with a nonprotective dose of anti-PA MAb W1 produced a slight additive effect: five of six animals were protected when nonprotective concentrations of LF10E (1:4 antibody/LF ratio) and W1 (1:6 antibody/PA ratio) were combined, but the level of protection rapidly declined at lower doses of either antibody (Table 3). Similar experiments with LF11H demonstrated that LF11H provided full protection at equimolar ratios of MAb and toxin and 50% protection at a 1:2 molar ratio (Table 4). Interestingly, MAb LF11H, which exhibited far lower neutralizing ability than LF10E in vitro, produced a robust synergistic protection when combined with nonprotective doses of anti-PA MAb W1. This antibody, which was unable to protect at lower-than-equimolar ratios when used alone, was now able to protect at an antibody/LF submolar ratio (1:4) when combined with a nonprotective dose (1:10 antibody/PA ratio) of MAb W1 (Table 4). Although the combination of antibody and LF (1:4) and antibody and PA (1:10) gave 100% survival, the survival with the antibody-LF (1:3) and antibody-PA (1:10) combinations was 83%.

DISCUSSION

We have generated two unique chimpanzee/human neutralizing anti-LF MAbs. One of them, LF10E has the highest reported affinity and impressive in vitro neutralization activity (EC_{50} in the picomolar range) (1, 21, 24, 37, 46). Although it is difficult to compare the in vivo protection efficacies of MAb LF10E and other known anti-LF MAbs because different animal models have been used, MAb LF10E demonstrated the most effective in vivo protection among MAbs that have been tested in the rat toxin challenge model (21, 24). However, the protective efficacy was at best minimally enhanced by combining this antibody with the anti-PA W1 antibody, compared with each antibody alone. A similar lack of synergy has been observed with some other anti-LF antibodies (1, 37). On the other hand, anti-LF neutralizing antibody LF11H had relatively lower affinity (dissociation constants in the nanomolar range), poor in vitro neutralizing activity, and inferior protective efficacy in vivo compared to LF10E when used alone. However, the protective efficacy was remarkably enhanced when this antibody was combined with anti-PA W1. Such synergism has been reported previously for combinations of some antibodies to PA and LF (3, 29). In an attempt to determine why these two MAbs behaved differently, we mapped their binding sites in LF. Previously, two neutralization epitopes were identified for anti-LF MAbs; they are located in domain I (37) and domain III (21). By Western blot analysis, we determined that our two MAbs recognize domain I of LF. Since LF domain I is responsible for binding to the activated PA (7, 19), it has been suggested that anti-LF MAbs that recognize

TABLE 2. Binding affinities of anti-LF MAbs[a]

MAb	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	Dissociation constant (nM)
LF10E	2.7×10^5	1.9×10^{-4}	0.69
LF11H	1.3×10^5	1.0×10^{-3}	7.4

[a] Recombinant LF was immobilized on the surface of the plasmon resonance sensor chip CM3 by a standard *N*-hydroxysuccinimide–1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide amine coupling method. The binding responses to LF-protein were collected at a range of Fab concentrations between 0.4 and 2,000 nM. The kinetic and equilibrium constants were determined by modeling the surface binding kinetics as a distribution of rate and affinity constants.

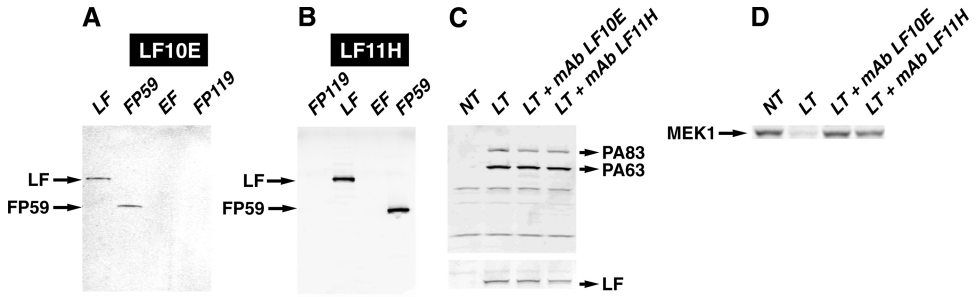

FIG. 4. LF10E and LF11H bind to LF amino acids 1 to 254 and prevent LT-mediated MEK cleavage in cells but do not prevent LF binding to cleaved PA. Purified toxin proteins LF, PA, and EF and fusion proteins FP59 and FP119 were loaded on SDS-polyacrylamide gels (2 ng/well for panel A and 10 ng/well for panel B) and probed with LF10E (A) or LF11H (B) by Western blotting. LT (1 μg/ml) was preincubated with MAbs (100 μg/ml) for 1 h prior to addition of LT or LT-MAb mixtures to CHO cells (C) or RAW 264.7 cells (D). Toxins were then allowed to bind to cells for 1 h prior to Western blotting as described in Materials and Methods. No treatment (NT) was used as a negative control.

domain I neutralize by preventing LF from binding to active PA (37). However, our binding assay demonstrated that neither LF10E nor LF11H blocked LF binding to PA (Fig. 4C). Furthermore, since LF domain I shares 35% sequence identity with domain I of EF, which has a very similar structure (36), and since both LF and EF have comparably high affinities (~1 nM) for PA heptamer (10), it has been suggested that an antibody binding to this region would bind and neutralize both LF and EF. Indeed, sera from animals immunized with EF domain I can neutralize both LT and ET (45). Our rabbit anti-LF polyclonal antibodies and some anti-LF MAbs have been shown to cross-react with EF (24), presumably through binding to this region. However, LF10E and LF11H antibodies bound only to domain I of LF, did not bind to EF, and had no inhibitory effect on PA association (Fig. 4A and B). Therefore, binding to domain I of LF does not necessarily mean that binding to the activated PA will be blocked. An alternative mechanism for neutralization by this antibody may involve prevention of proper translocation and release of LF from the heptamer into the cytoplasm. Neither affinity data nor epitope mapping data provided an explanation as to why our two neutralizing anti-LF antibodies behaved differently, and further work is required to decipher the mechanisms of the unique neutralization by these antibodies.

Various animal models have been used for assessing the efficacy of anthrax vaccine and of neutralizing MAbs (11, 31, 41). However, challenge of rats with anthrax toxin is the classic and most extensively used model. This model is often used for initial evaluation of in vivo protective efficacy of MAbs. An alternative spore challenge model, due to its highly restricted use, is often employed at a later stage for further characterization. So far, the data obtained from these two models have

TABLE 3. Efficacy of LF10E and its combination with anti-PA W1 in protection of rats against LT challenge[a]

Relative molar amt in passive immunization			Survival			Mean time to death (min)[c]
LT (PA + LF)	LF10E	W1	% Surviving	No. surviving/ total	P^b	
1	0.50		100	6/6	0.002	NA
1	0.33		67	4/6	0.06	136
1	0.25		0	0/14		101
1		0.20	33	2/6	0.455	>140[d]
1		0.17	0	0/20		175
1		0.14	0	0/7		150
1	0.25	0.17	83	5/6	0.015	266
1	0.25	0.14	33	2/6	0.455	190
1	0.20	0.17	33	2/6	0.455	249
1			0	0/22		82

[a] Fischer F344 rats were injected intravenously with 10 μg LT/rat with or without antibodies at various relative molar amounts, and rats were monitored for time to death and percent survival.

[b] Fisher's exact test (two-sided) was used to analyze the difference in protection between antibody-treated and control (PBS) groups. A P value of <0.05 was considered statistically significant. A total six animals for each group was used for the analysis.

[c] Mean time to death for six or more rats in each group. NA, not available for group with 100% protection.

[d] Three rats died with a mean time to death of 140 min, and one died overnight.

TABLE 4. Efficacy of LF11H and its combination with anti-PA W1 in protection of rats against lethal toxin challenge[a]

Relative molar amt in passive immunization			Survival			Mean time to death (min)[c]
LT (PA + LF)	LF11H	W1	% Surviving	No. surviving/ total	P^b	
1	1		100	6/6	0.002	NA
1	0.50		50	3/6	0.182	>166[d]
1	0.33		0	0/8		172
1	0.25		0	0/6		126
1		0.20	33	2/6	0.455	>140[e]
1		0.17	0	0/20		175
1		0.14	0	0/7		150
1	0.33	0.17	100	6/6	0.002	NA
1	0.33	0.14	100	6/6	0.002	NA
1	0.33	0.10	83	5/6	0.015	371
1	0.33	0.07	67	4/6	0.061	218
1	0.25	0.14	100	6/6	0.002	NA
1	0.25	0.10	100	6/6	0.002	NA
1			0	0/22		82

[a] Fischer F344 rats were injected intravenously with 10 μg LT /rat with or without antibodies at various relative molar amounts, and rats were monitored for time to death and survival.

[b] Fisher's exact test (two-sided) was used to analyze the difference in protection between antibody-treated and control (PBS) groups. A P value of <0.05 was considered statistically significant. A total six animals for each group was used for the analysis.

[c] Mean time to death for six or more rats in each group. NA, not available for groups with 100% protection.

[d] One rat died in 166 minutes, and two died overnight.

[e] Three rats died with a mean time to death of 140 min, and one rat died overnight.

correlated well (25, 28, 31, 35, 42, 43). Therefore, we think that it is appropriate to use the rat toxin challenge model for initial evaluation of our antibodies for in vivo protective activity and synergy between two antibodies.

In summary, we have generated two unique chimpanzee/human neutralizing MAbs against the N terminus of LF which act at a step subsequent to PA binding. These MAbs could potentially be used, either alone or in combination with anti-PA MAbs, in treating anthrax infection. We believe that combination will improve the efficacy and broaden the spectrum of protection.

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