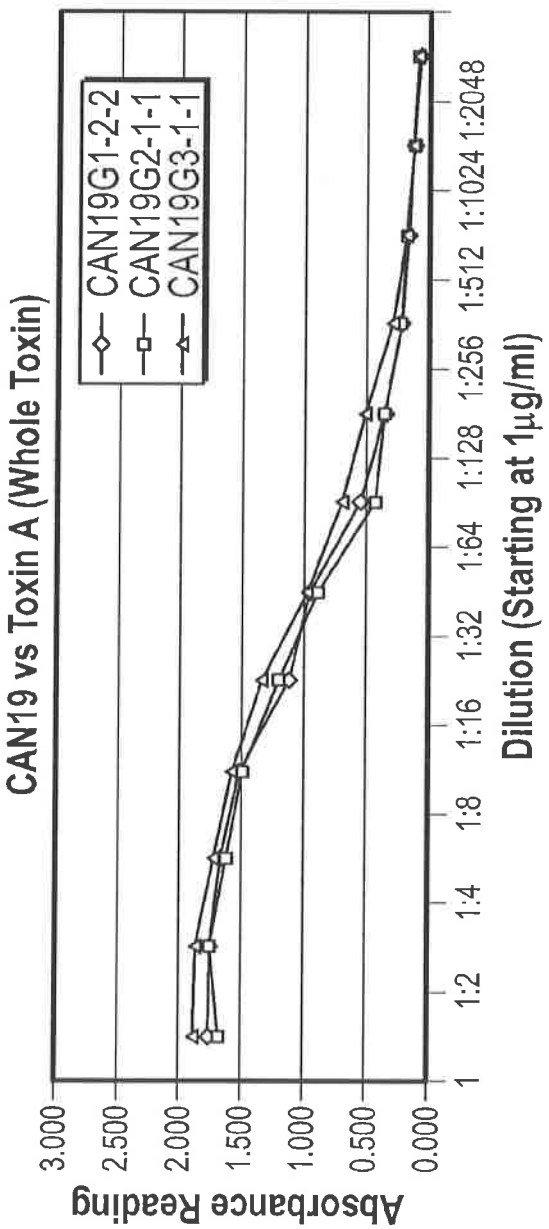

CAN19 vs Toxin A (Whole Toxin)
CAN19G1-2-2
CAN19G2-1-1
CAN19G3-1-1
Absorbance Reading
3.000
2.500
2.000
1.500
1.000
0.500
0.000
1
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
Dilution (Starting at 1µg/ml)

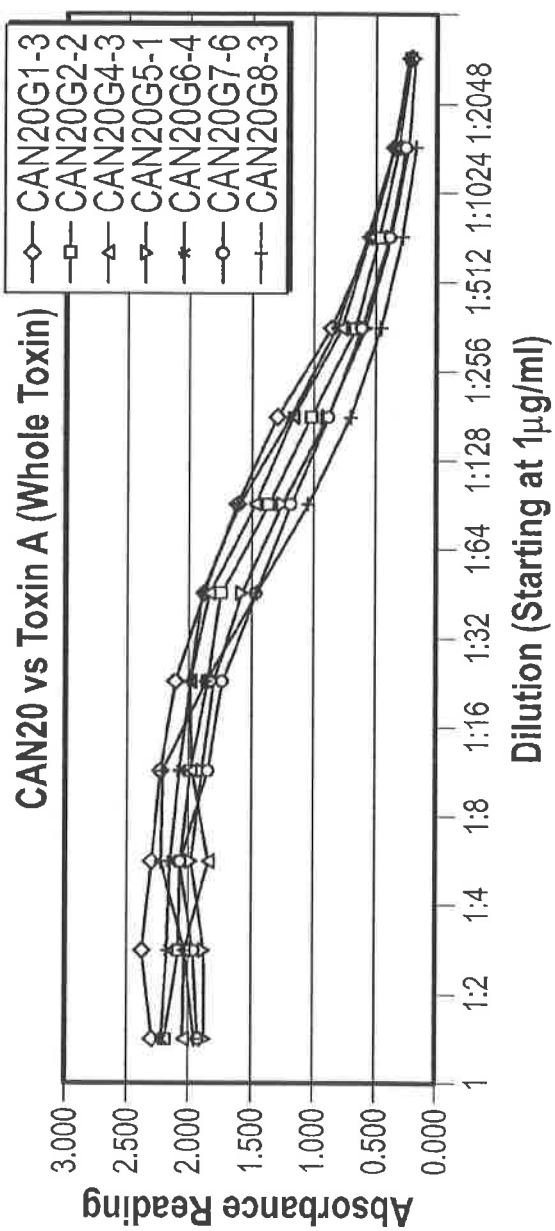

CAN20 vs Toxin A (Whole Toxin)
CAN20G1-3
CAN20G2-2
CAN20G4-3
CAN20G5-1
CAN20G6-4
CAN20G7-6
CAN20G8-3
Absorbance Reading
3.000
2.500
2.000
1.500
1.000
0.500
0.000
1
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
Dilution (Starting at 1µg/ml)

FIG. 1

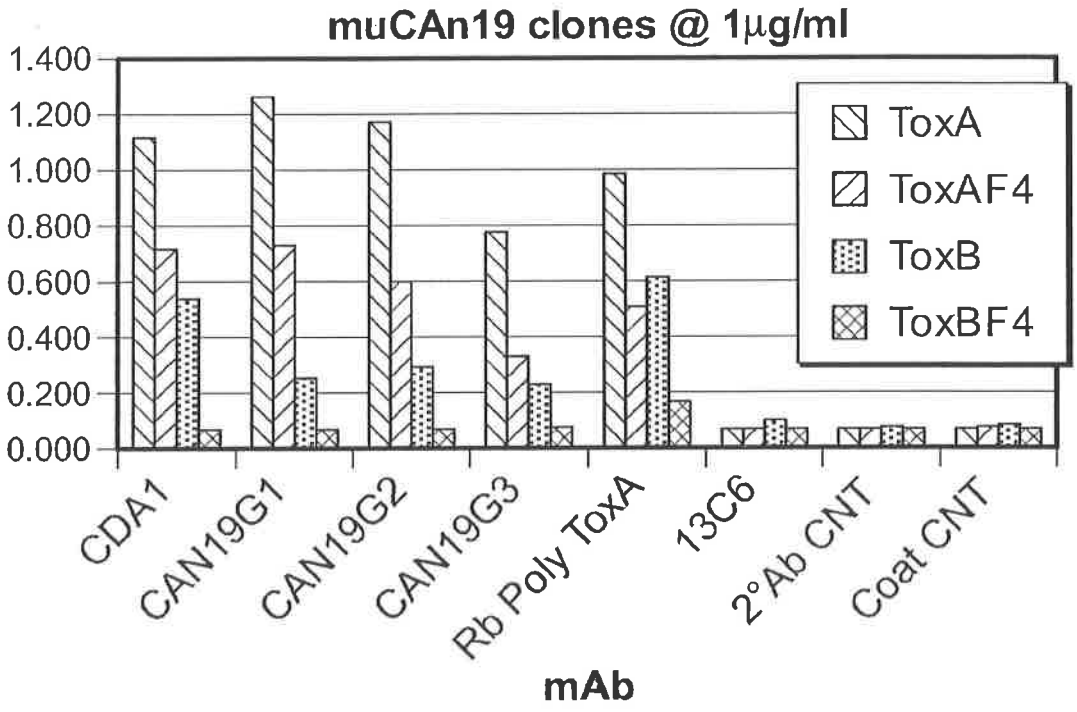

muCAn19 clones @ 1µg/ml
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
ToxA
ToxAF4
ToxB
ToxBF4
CDA1
CAN19G1
CAN19G2
CAN19G3
Rb Poly ToxA
13C6
2°Ab CNT
Coat CNT
mAb

FIG. 2

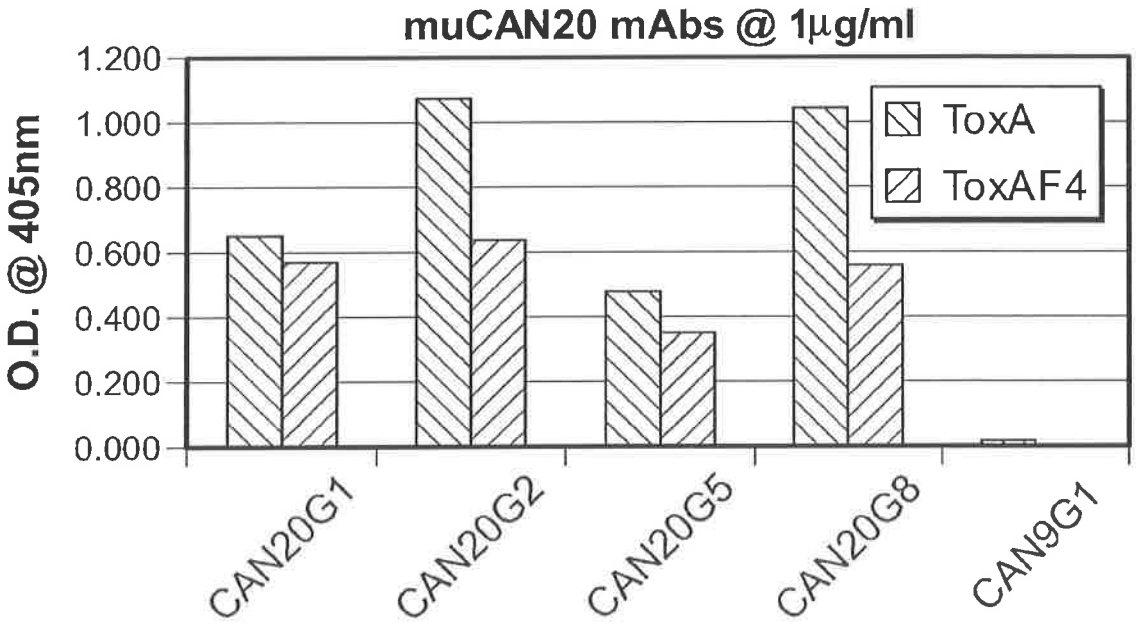
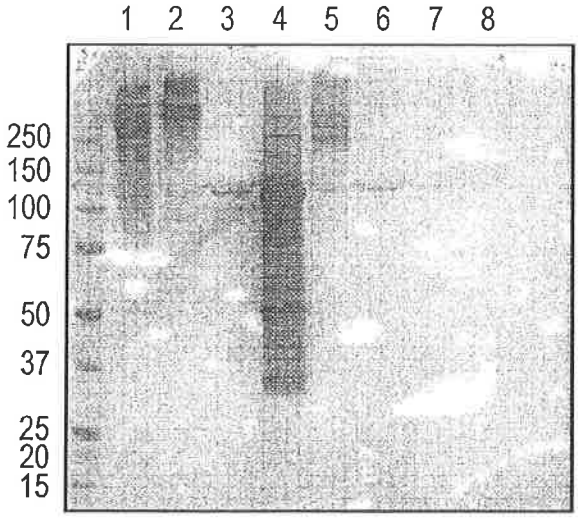
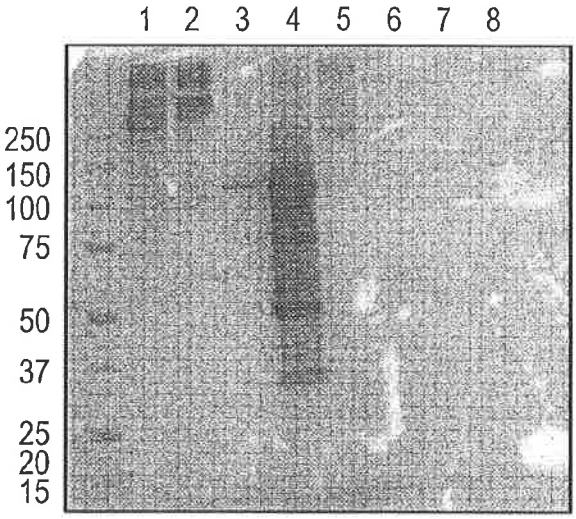

muCAN20 mAbs @ 1µg/ml
O.D. @ 405nm
1.200
1.000
0.800
0.600
0.400
0.200
0.000
ToxA
ToxAF4
CAN20G1
CAN20G2
CAN20G5
CAN20G8
CAN9G1

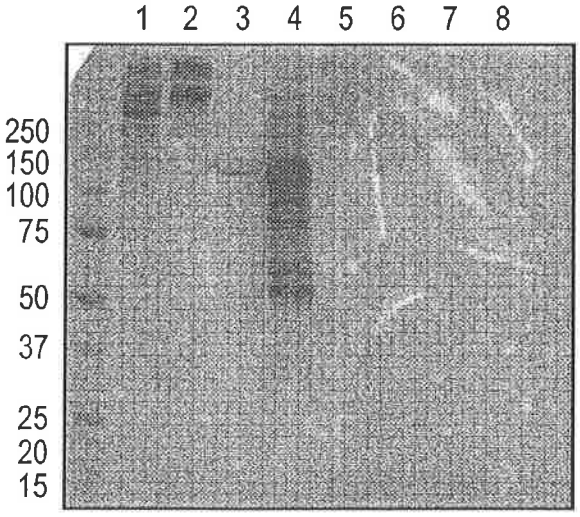
FIG. 3



Blot A: Probed with CAN19G1 at 0.5 µg/ml protein loaded at 2 µg



Blot B: Probed with CAN19G2 at 0.5 µg/ml protein loaded at 2 µg



Blot C: Probed with CAN19G3 at 0.5 µg/ml protein loaded at 2 µg

1. Toxin A
2. Toxoid A
3.
4. Toxin A Fragment 4
5. Toxin B
6.
7. Toxin B Fragment 4
8. PilF (negative CNT)

FIG. 4

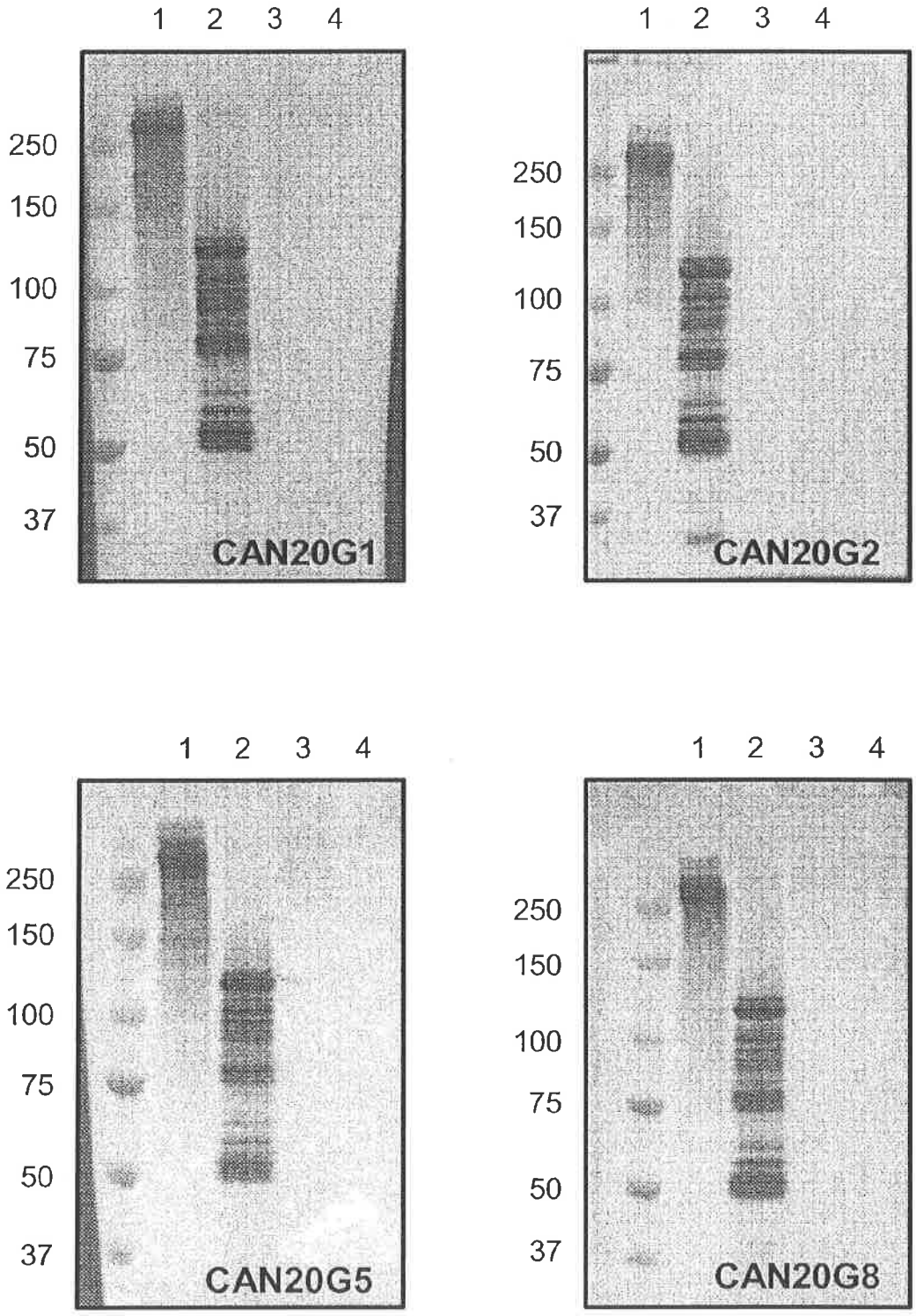

1 2 3 4
250
150
100
75
50
37
CAN20G1
1 2 3 4
250
150
100
75
50
37
CAN20G2
1 2 3 4
250
150
100
75
50
37
CAN20G5
1 2 3 4
250
150
100
75
50
37
CAN20G8

FIG. 5

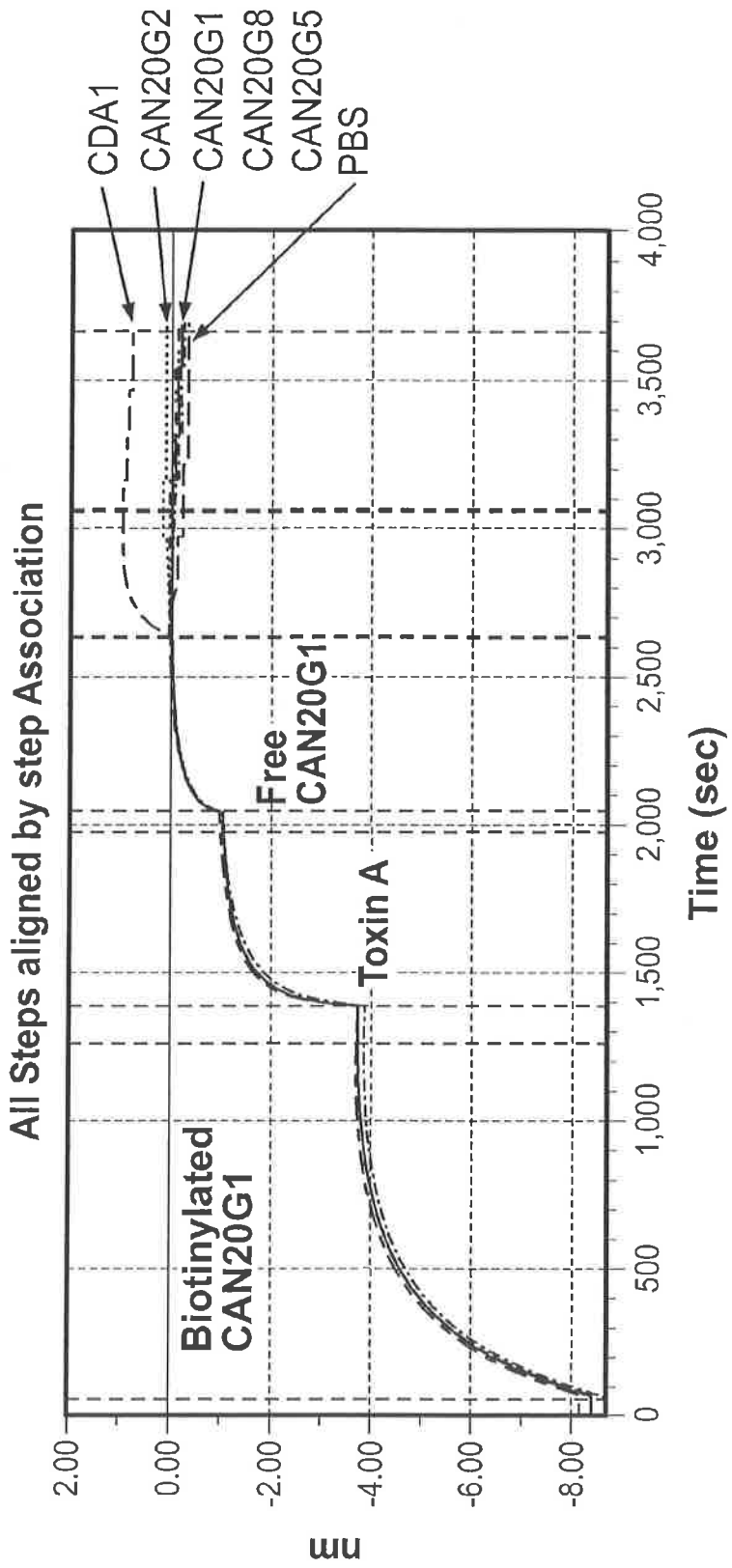

All Steps aligned by step Association
nm
2.00
0.00
-2.00
-4.00
-6.00
-8.00
Time (sec)
0
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
CDA1
CAN20G2
CAN20G1
CAN20G8
CAN20G5
PBS
Biotinylated CAN20G1
Toxin A
Free CAN20G1

FIG. 6A

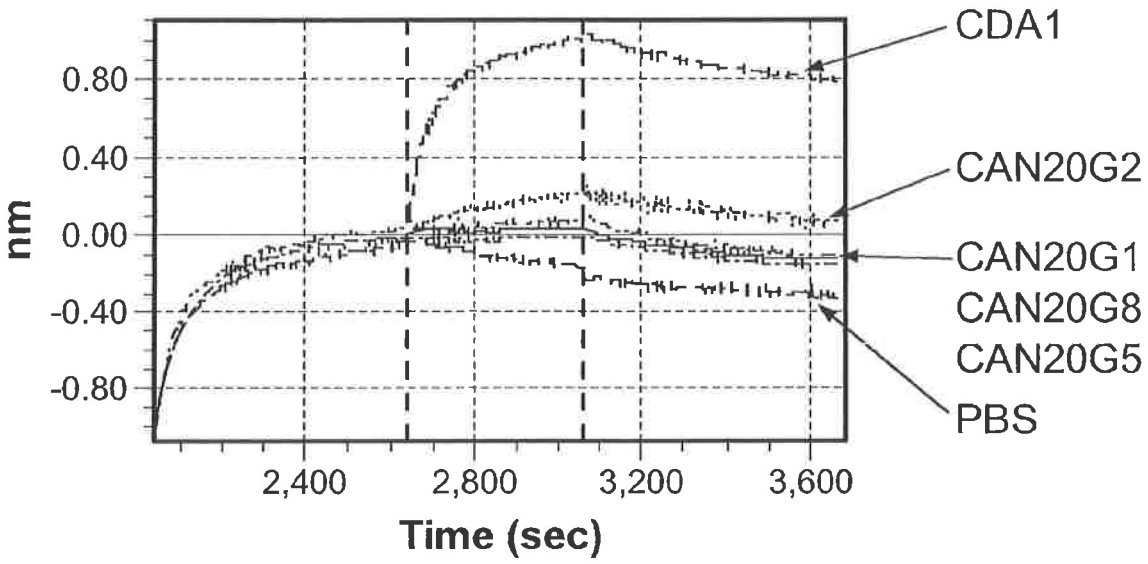

CDA1
CAN20G2
CAN20G1
CAN20G8
CAN20G5
PBS
nm
0.80
0.40
0.00
-0.40
-0.80
2,400
2,800
3,200
3,600
Time (sec)

FIG. 6B

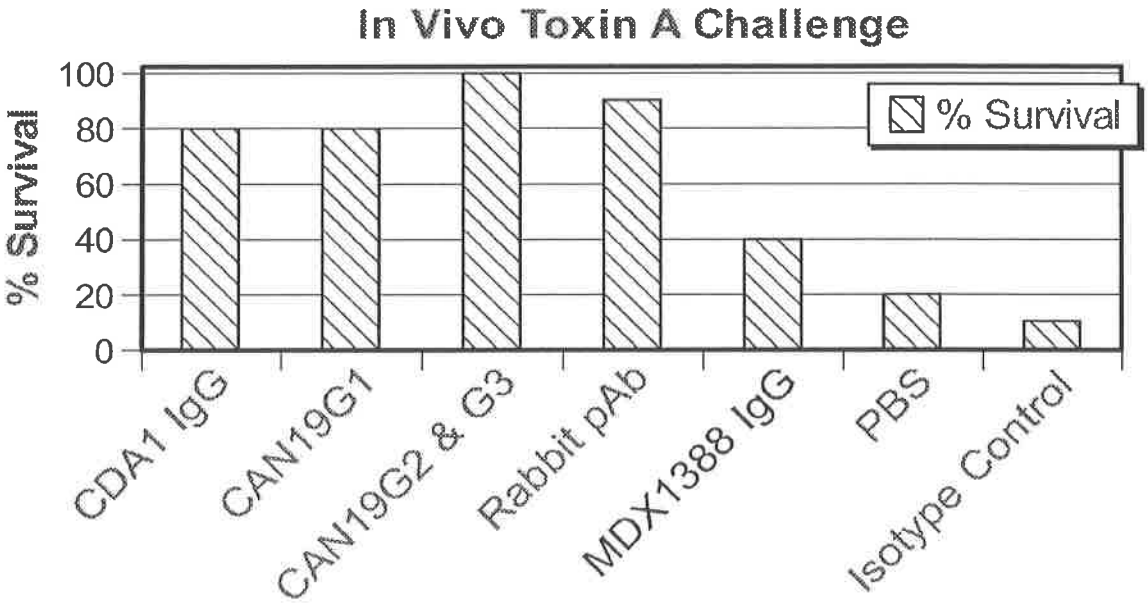

In Vivo Toxin A Challenge
% Survival
100
80
60
40
20
0
% Survival
CDA1 IgG
CAN19G1
CAN19G2 & G3
Rabbit pAb
MDX1388 IgG
PBS
Isotype Control

FIG. 7

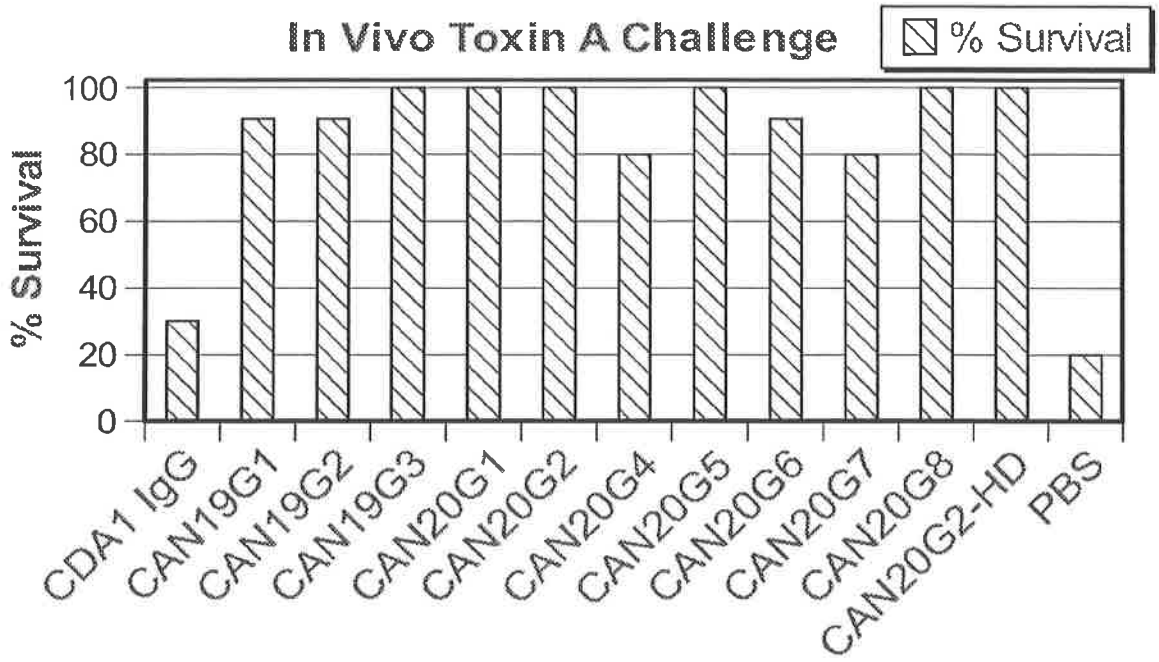

In Vivo Toxin A Challenge
% Survival
% Survival
100
80
60
40
20
0
CDA1 IgG
CAN19G1
CAN19G2
CAN19G3
CAN20G1
CAN20G2
CAN20G4
CAN20G5
CAN20G6
CAN20G7
CAN20G8
CAN20G2-HD
PBS

FIG. 8

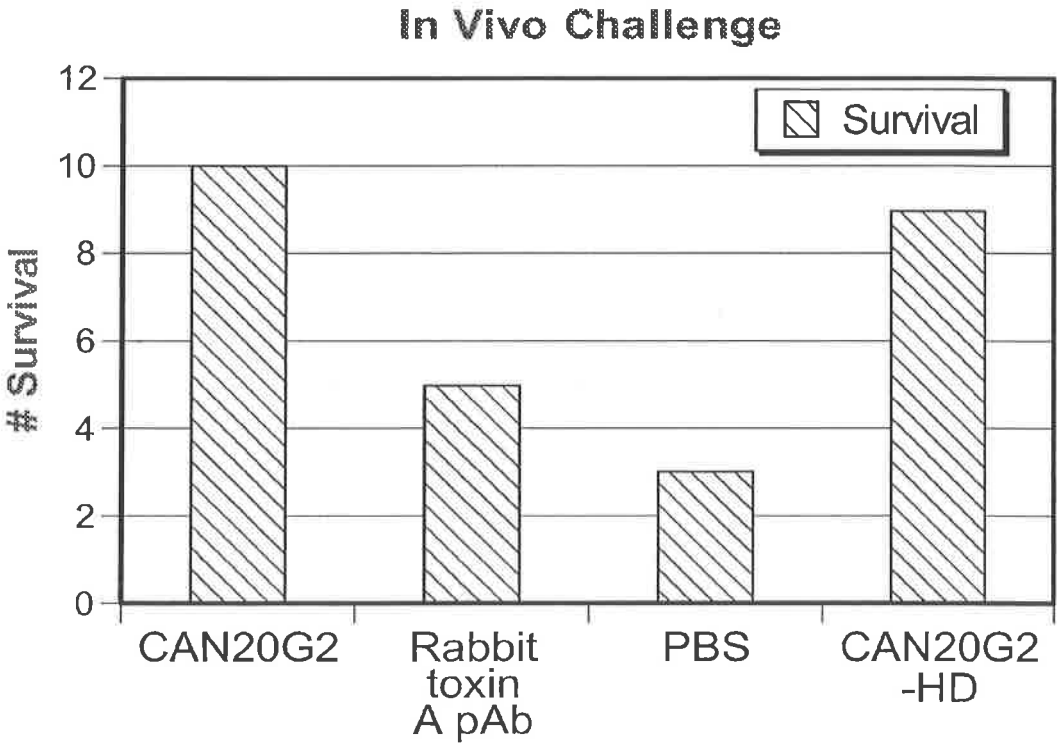

In Vivo Challenge
Survival
Survival
12
10
8
6
4
2
0
CAN20G2
Rabbit toxin A pAb
PBS
CAN20G2 -HD

FIG. 9

Primer Name	Category	Primer Sequence
5'mVK-Lead-1	Subgroup Specific	GGTGCAGATTTTCAGCTTCC
3'KappaConstRT		GTGCTGTCTTTGCTGTCCTG
5'mVH-Lead-2		BTNCTYYTCTKCCTGRT
5'mVH-Lead-2A		TGGSTGTGGAMCTTGCTATT
3'mIG1-2C RT		AGGASAGCTGGGAAGGTGTG
5'mVK-Lead-3	Non-Subgroup Specific	CTWKGRSTKCTGCTKYTCTG
5'mVK-Lead-3A		CCTGTTAGGCTGTTGGTGCT
5'mVH-IGHV1-Lead		RKCARCARCTRCAGGTGTCC
5'mVH-Lead-1		CCYWNTTTTAMAWGGTGTCCAKTGT
5'mVH-Lead-3		GGATGGAGCTRTATCATBCTC
5'mVH-Lead-4		GRTCTTTMTYTTHHTCCTGTCA
5'mVH-Lead-5		VCCTTWMMTGGTATCCWGTST

W = A or T
S = C or G
R = A or G
Y = C or T
K = G or T
M = A or C
B = C, G, or T (not A)
D = A, G, or T (not C)
H = A, C, or T (not G)
V = A, C, or G (not T)
N = A, C, G, OR T

FIG. 10

Result summary:	**Productive IGK rearranged sequence** (no stop codon and in-frame junction)		
V-GENE and allele	Musmus IGKV4-74*01	score = 1324	identity = **96,81%** (273/282 nt)
J-GENE and allele	Musmus IGKJ1*01	score = 180	identity = 100,00% (36/36 nt)
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[26.17.36.10]	[7.3.9]	CLQYHRSPRTF

FIG. 11

FR1-IMGT CDR1-IMGT FR2-IMGT CDR2-IMGT FR3-IMGT CDR3-IMGT FR4-IMGT
(1-26) (27-38) (39-55) (56-65) (66-104) (105-117) (118-128)
A B BC C C' C'C" C" D E F FG G
(1-15) (16-26) (27-38) (39-46) (47-55) (56-65) (66-74) (75-84) (85-96) (97-104) (105-117) (118-128)
1 10 15 16 23 26 27 38 3941 46 47 55 56 65 66 74 75 80 84 85 89 96 97 104 105 11112 117 118 128
QIQLVQSGP.EVKKP GETVKISCKAS GYTF....TNQG MNWVKQAP GKGLKWMGW INTN..TGEP TYTEEFK.G RFAFSLETSA STAYLQINNLKN EDTATYFC YVNYDY..YTMDF WGQGTSVTVSS
QVQLVQSGS.ELKKP GASVKVSCKAS GYTF....TSYA MNWVRQAP GQGLEWMGW INTN..TGNP TYAQGFT.G RFVFSLDTSV STAYLQISSLKA EDTAVYYC AR YYYYYGMDV WGQGTTVTVSS
I P V ET I NQG K K K E TEE K A E A NN N T F YV D T F S
V-REGION
N-
J-REGION
Vh
IGHV2-
4-1*02
(Homo
sapiens)

FIG. 12

IGHJ6*01
FR1-IMGT CDR1-IMGT FR2-IMGT CDR2-IMGT FR3-IMGT CDR3-IMGT
FR4-IMGT
(1-26) (27-38) (39-55) (56-65) (66-104) (105-117)
(118-128)
A B BC C C' C'C" C" D E F FG
G
(1-15) (16-26) (27-38) (39-46) (47-55) (56-65) (66-74) (75-84) (85-96) (97-104) (105-117)
(118-128)
1 10 15 16 23 26 27 38 3941 46 47 55 56 65 66 74 75 80 84 85 89 96 97 104 105 11112
117 118 128
vk .WLTQSPAIMSASL GDRVTMTCTAS SSVI.....STY LHWYQQKP GSSPKLWIY ST.....S TLASGVP.P RFSGSG..SG TSYSLTISSMEA EDAATYYC
LQYHR.....SPRT FGGGTKLEIK.
IGKV1-39*01 DIQMTQSPSSLSASV GDRVTITCRAS QSI.....SSY LNWYQQKP GKAPKLLIY AA.....S SLQSGVP.S RFSGSG..SG TDFTLTISSLQP EDFATYYC QQSYS~P
LT FGGGTKVEIK.
(Homo sapiens)
L
V-REGION
|||-J-REGION-|
IGKJ4*01

FIG. 12 (Cont.)

IGVH

CAN20G2 Heavy
Closest Human framework insertion.
Kozak Seq and HAVT20 Leader
IGHV7-4-1*02
CAN20G CDRs
IGHJ6*01
Altered HpaI GTT-AAC RE site for cloning

<----------Kozak Seq and HAVT20 Leader----------
GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCTGTCTT

----------><----------FR1----------
GAATTTTCCATGGCTCAGGTGCAGCTGGTGCAATCTGGGTCTGAGTTGAAGAAGCC

----------><----------CDR1----------
TGGGGCCTCAGTGAAGGTTTCCTGCAAGGCTTCTGGGTATACCTTCACAAACCAAG

---><----------FR2----------><---
GAATGAATTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGAT

----------CDR2----------><----------
AAACACCAACACTGGAGAGCCAACGTATGCCCAGGGCTTCACAGGACGGTTTGTC

----------FR3----------
TTCTCCTTGGACACCTCTGTCAGCACGGCATATCTGCAGATCAGCAGCCTAAAGGC

----------><----------CDR3----------
TGAGGACACTGCCGTGTATTACTGTTATGTCAATTACGATTATTATACTATGGACTT

-><----------FR4---------->
CTGGGGGCAAGGGACCACGGTCACCGTCTCCTCA

Translation:

<---------- FR1----------><----CDR1-----><----------FR2----------><---
QVQLVQSGSELKKPGASVKVSCKASGYTFTNQGMNWVRQAPGQGLEWMGWIN

---CDR2--><----------FR3---------- ><---------CDR3-------
TNTGEPTYAQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCYVNYDYYTMD

-><----------FR4------>
FWGQGTTVTVSS

FIG. 13A

CAN20G2 Kappa
IGKV1D-39*01
CAN20G2 CDRs
IGKJ4*01

<----------Kozak Seq and HAVT20 Leader----------
GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCTGTCTT

----------><----------FR1----------
GAATTTTCCATGGCTGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCT

----------><----------CDR1----------
GTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTTCAAGTGTAATTTCCACTTA

-><----------FR2----------><---------
CTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATAGCA

--CDR2-><----------FR3----------
CATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTG

-><----------CDR3----------><----------FR4----------
TCTCCAGTATCACCGTTCCCCACGGACGTTCGGCGGAGGGACCAAGGTGGAGATCA

--->
AA

Translation:

<----------FR1----------><-----CDR1-----><----------FR2----------
D I Q M T Q S P S S L S A S V G D R V T I T C R A S S S V I S T Y L N W Y Q Q K P G

----------><CDR2><----------FR3----------
K A P K L L I Y S T S S L Q S G V P S R F S G S G S G T D F T L T I S S L Q P E

----------><----------CDR3----------><----------FR4---------->
D F A T Y Y C L Q Y H R S P R T F G G G T K V E I K

FIG. 13B

CAN20G2 Heavy Total: 8 Resurfaced AA
Kozak and HAVT20 Leader
CAN20G2 Framework Sequence
CAN20G2 CDR

<----------Kozak and HAVT20 Leader----------
GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCTGTCTT

----------><----------FR1----------
GAATTTTCCATGGCTCAGATCCAGTTGGTGCAGTCTGGACCTGAGCTGAAGAAGCC

----------><----------CDR1----------
TGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGGTATACCTTCACAAACCAAG

--->><----------FR2----------><---
GAATGAACTGGGTGAAACAGGCTCCAGGAAAGGGTTTAAAGTGGATGGGCTGGAT

----------CDR2----------><----------
AAACACCAACACTGGAGAGCCAACATATACTGCCGATTTCACAGGACGGTTTGCC

----------FR3----------
TTCTCTTTAGAAACCTCTGTGAGCACTGCCTATTTGCAGATCAACTCCCTCAAAGC

----------><----------CDR3----------
TGAGGACACGGCTACATATTTCTGTTATGTCAATTACGATTATTATACTATGGACT

--->><----------FR4---------->
TCTGGGGTCAAGGAACCCTGGTCACCGTCTCCTCA

Translation:

<----------FR1----------><-----CDR1----><----------FR2----------><---
QIQLVQSGPELKKPGETVKISCKASGYTFTNQGMNWVKQAPGKGLKWMGWIN

--CDR2----><----------FR3----------><---------CDR3-----
TNTGEPTYTADFTGRFAFSLETSVSTAYLQINSLKAEDTATYFCYVNYDYYTM

--->><----------FR4--------->
DFWGQGTLVTVSS

FIG. 14A

CAN20G2 Kappa Total: 7 Resurfaced AA

<----------KozakandHAVT20 Leader----------
GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCTGTCTT

----------><----------FR1----------
GAATTTTCCATGGCTGACGTTCAGCTCACCCAGTCTCCAAGCATCATGTCTGCATC

----------><----------CDR1----------
TCTAGGGGATCGGGTCACCATGACCTGCACTGCCAGCTCAAGTGTAATTTCCACTT

---><----------FR2----------><------
ACTTGCACTGGTATCAGCAGAAGCCAGGATCCTCCCCCAAACTCTGGATTTATAGC

---CDR2---><----------
ACATCCACCCTGGCTTCTGGAGTCCCAAGCCGCTTCAGTGGCAGTGGGTCTGGGAC

----------FR3----------
CGACTACTCTCTCACAATCAGCAGCATGGAGCCTGAAGATGCTGCCACTTATTACT

----><----------CDR3----------><----------FR4----------
GCCTCCAGTATCACCGTTCCCCACGGACGTTCGGTGGAGGCACCAAGGTGGAAAT

---------->
CAAA

Translation:

<----------FR1----------><-------CDR1-------><----------
D V Q L T Q S P S I M S A S L G D R V T M T C T A S S S V I S T Y L H W

----------FR2----------><CDR2><----------FR3----------
Y Q Q K P G S S P K L W I Y S T S T L A S G V P P R F S G S G S G T D Y S

----------><----------CDR3----------><----------FR4---------->
L T I S S M E P E D A A T Y Y C L Q Y H R S P R T F G G G T K V E I K

FIG. 14B

CAN20G2 Heavy Resurfaced

GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCTGTCTTGAATTTTCCATG

<----------FR1----------

GCTCAGATCCAGTTGGTGCAGTCTGGACCTGAGCTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCT

---------><---------CDR1---------><----------FR2----------

GCAAGGCTTCTGGGTATACCTTCACAAACCAAGGAATGAACTGGGTGAAACAGGCTCCAGGAAAGGG

----------><---------CDR2---------><---------FR3----------

TTTAAAGTGGATGGGCTGGATAAACACCAACACTGGAGAGCCAACATATACTGCCGATTTCACAGGA

CGGTTTGCCTTCTCTTTAGAAACCTCTGTGAGCACTGCCTATTTGCAGATCAACTCCCTCAAAGCTGA

----------><----------CDR3----------><-----FR4------

GGACACGGCTACATATTTCTGTTATGTCAATTACGATTATTATACTATGGACTTCTGGGGTCAAGGAAC

---------->

CCTGGTCACCGTCTCCTCAGGTGAGTGCGGCCGCGAGCCCAGACACTGGACGCTGAACCTCGCGGAC
AGTTAAGAACCCAGGGGCCTCTGCGCCCTGGGCCCAGCTCTGTCCCACACCGCGGTCACATGGCACCA
CCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTG
TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAG
GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT
CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGGTGAGAGGCCAGCA
CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAG
TCCCAGTCCAGGGCAGCAAGGCAGGCCCCGTCTGCCTCTTCACCCGGAGGCCTCTGCCCGCCCCACTC
ATGCTCAGGGAGAGGGTCTTCTGGCTTTTTCCCCAGGCTCTGGGCAGGCACGGGCTAGGTGCCCCTAA
CCCAGGCCCTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGAC
CCTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGACACCTTCTCTCC
TCCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGAGCCCAAATCTTGTGACAAAACTCACACAT
GCCCACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAG

FIG. 15

AGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGACACGTCCACCTCCATCTCTTCCTCAGCA
CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGA
TCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAA
GTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCA
GTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGC
AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCA
AAGCCAAAGGTGGGACCCGTGGGGTGCGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTG
CCCTGAGAGTGACCGCTGTACCAACCTCTGTCCCTACAGGGCAGCCCCGAGAACCACAGGTGTACA
CCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGG
CTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAA
GACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC
ACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGATGAGCTAGC

Translation:

<----------FR1----------><-CDR1--><------FR2------><--CDR2-><----------
QIQLVQSGPELKKPGETVKISCKASGYTFTNQGMNWVKQAPGKGLKWMGWINTNTGEPTYTADFTGRFA
-----FR3------------------><---CDR3--><--FR4----->
FSLETSVSTAYLQINSLKAEDTATYFCYVNYDYYTMDFWGQGTLVTVSS

FIG. 15 (Cont.)

CAN20G2 Kappa Resurfaced

ALTERED BamHI SITE ggaTCC→ggcAGC

<----------Kozak and HAVT20 Leader----------
GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCT

----------><----------FR1----------
GTCTTGAATTTTCCATGGCTGACGTTCAGCTCACCCAGTCTCCAAGCATCAT

----------><---CDR1---
GTCTGCATCTCTAGGGGATCGGGTCACCATGACCTGCACTGCCAGCTCAAGT

----------><----------FR2----------
GTAATTTCCACTTACTTGCACTGGTATCAGCAGAAGCCAGGCAGCTCCCCCA

----------><-----CDR2------><----------FR3----------
AACTCTGGATTTATAGCACATCCACCCTGGCTTCTGGAGTCCCAAGCCGCTT

CAGTGGCAGTGGGTCTGGGACCGACTACTCTCTCACAATCAGCAGCATGGA

----------><----------CDR3----------
GCCTGAAGATGCTGCCACTTATTACTGCCTCCAGTATCACCGTTCCCCACGG

------><----------FR4---------->
ACGTTCGGTGGAGGCACCAAGGTGGGAAATCAAACGTAAGTGCACTTTGCGGCCGCTAGGA
AGAAACTCAAAACATCAAGATTTTAAATACGCTTCTTGGTCTCCTTGCTATAATTATCTGGGATAAGCATGC
TGTTTTCTGTCTGTCCCTAACATGCCCTGTGATTATCCGCAAACAACACACCCAAGGGCAGAACTTTGTTAC
TTAAACACCATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCAT
CTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAG
GCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGC
AGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAA
ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC
AGGGGAGAGTGTTGATAGTTAACG

Translation:

<----------FR1----------><------CDR1-----><----------
D V Q L T Q S P S I M S A S L G D R V T M T C T A S S S V I S T Y L H W Y

----------FR2----------><CDR2><----------FR3----------
Q Q K P G S S P K L W I Y S T S T L A S G V P P R F S G S G S G T D Y S L

----------><----------CDR3---------><----------FR4---------->
T I S S M E P E D A A T Y Y C L Q Y H R S P R T F G G G T K V E I K R T V A A P S V F I F P
P S D E Q L K S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T
L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G E C

FIG. 16

Alignment with the closest gene and allele from the IMGT V domain directory: All species (All species)
FR1-IMGT
CDR1-IMGT
FR2-IMGT
CDR2-IMGT
FR3-IMGT
CDR3-IMGT
FR4-IMGT
(1-26)
(27-38)
(39-55)
(56-65)
(66-104)
(105-117)
(118-128)
A
B
BC
C
C'
C'C"
C"
D
E
F
FG
G
(1-15)
(16-26)
(27-38)
(39-46)
(47-55)
(56-65)
(66-74)
(75-84)
(85-96)
(97-104)
(105-117)
(118-128)
1 10 15 16 23 26 27 38 39 41 46 47 55 56 65 66 74 75 80 84 85 89 96 97 104 105 111 112 117 118 128
Avastin KappaChain
DIQMTQSPSSLSASV
GDRVTITCSAS QDI.....SNY LNWYQQKP
GKAPKVLIY
FT.....S
S:HSGVP.S
RFSGSG..SG
TDFTLTISSLQP
EDFATYYC
QQYST....VPWT FGQGTKVEIK.
IGKV1D-33*01 (Homo sapiens)
DIQMTQSPSSLSASV
GDRVTITCQAS QDI.....SNY LNWYQQKP
GKAPKLLIY
DA.....S
N:ETGVP.S
RFSGSG..SG
TDFTFTISSLQP
EDIATYYC
QQYDNLP
WT FGQGTKVEIK.
S
V
FT
S HS
L
F
ST
V-REGION
J-REGION
IGKJ1*01

FIG. 17

Region(s) and domain(s) identified in the sequence (by comparison with the closest genes and alleles Homo sapiens IGKV1D-33*01 and IGKJ1*01)

>M64855|IGKV1D-33*01|Homo sapiens|F|V-REGION|321..607|287 nt|1| | | |95 AA|95+0=287| | |
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYDNLP

>M64855|IGKV1D-33*01|Homo sapiens|F|V-REGION|321..607|287 nt|1| | | | |287+0=287| | |
GACatcCAGatgACCcagTCTccaTCCtccCTGtctGCAtctGTAggaGACagaGTCaccATCactTGCcagGGagtCAGgacATTagcAACtatTTAaatTGGtatCAGcagAAAccaGGGaaaGCCcctA
AGctcCTGatcTACgatGCAtccAATttgGAAacaGGGgtcCCAtcaAGttcAGTggaAGTggaTCTgggACAgatTTTactTTCaccATCagcAGCctgCAGcctGAAgatATTgcaACAtatTACtgtCA
AcagTATgatAATctcCCT

>J00242|IGKJ1*01|Homo sapiens|F|J-REGION|251..288|38 nt|2| | | |12 AA|12+0=38| | |
WTFGQGTKVEIK

>J00242|IGKJ1*01|Homo sapiens|F|J-REGION|251..288|38 nt|2| | | | |38+0=38| | |
Tggacgttcggccaagggaccaaggtggaaatcaaa

WTFGQGTKVEIK

FIG. 17 (Cont.)

Hybrid (closest humanized Avastin Kappa Chain) IGKV1D-33-01 Kappa Chain and CAN20G2 Kappa CDRs

Kozak and HAVT20 Leader
Framework 1-2-3
CAN20G2 Kappa CDR1-2-3
IGKJ1-01
Altered nucleotides (codons) bold and underlined and capitalized to match Avastin.

<----------Kozak and HAVT20 Leader----------
GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCT

----------><----------FR1----------
GTCTTGAATTTTCCATGGCTGACATCCAGATGACCCAGTCTCCATCCTCCCTGTCT

----------><----------CDR1---------
GCATCTGTAGGAGACAGAGTCACCATCACTTGC**AGC**GCGAGTTCAAGTGTAATTTC

----------><----------FR2----------
CACTTACTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAG**GTG**CTGATCTA

-><----CDR2-----><----------FR3----------
CAGCACATCC**AGC**TTG**CACAGC**GGGGTCCCATCAAGGTTCAGTGGAAGTGGATCTG

GGACAGATTTTACT**CTG**ACCATCAGCAGCCTGCAGCCTGAAGAT**TTC**GCAACATATT

----------><----------CDR3----------><----------FR4----------
ACTGTCTCCAGTATCACCGTTCCCCACGGACGTTCGGCCAAGGGACCAAGGTGG

---------->
AAATCAAACGTAAGTGCACTTTGCGGCCGCTAGGAAGAAACTCAAAACATCAAGATTTTAA
ATACGCTTCTTGGTCTCCTTGCTATAATTATCTGGGATAAGCATGCTGTTTTCTGTCTGTCCCT
AACATGCCCTGTGATTATCCGCAAACAACACACCCAAGGGCAGAACTTTGTTACTTAAACAC
CATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCC
ATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCT
ATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTC
CCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCAC
CCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC
CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTGATAGT
TAACG

FIG. 18

Kozak and HAVT20 Leader
CAN20G2 Heavy V-Region
Human IgG1 Constant with Introns
Double Stop

<----------Kozak and HAVT20 Leader----------
GCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCT
----------><---------- CAN20G2 Heavy V-Region---------
GTCTTGAATTTTCCATGGCTCAGATCCAGTTGGTGCAGTCTGGACCTGAGGT

GAAGAAGCCTGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGGTATAC

CTTCACAAACCAAGGAATGAACTGGGTGAAACAGGCTCCAGGAAAGGGTTT

AAAGTGGATGGGCTGGATAAACACCAACACTGGAGAGCCAACATATACTGA

AGAGTTCAAGGGACGGTTTGCCTTCTCTTTAGAAACCTCTGCCAGCACTGCC

TATTTGCAGATCAACAACCTCAAAAATGAGGACACGGCTACATATTTCTGTT

ATGTTAACTACGATTATTATACTATGGACTTCTGGGGTCAAGGAACCTCGGT
----------><---------- Human IgG1 Constant with Introns----------
CACCGTCTCCTCAGGTGAGTGCGGCCGCGAGCCCAGACACTGGACGCTGAA

CCTCGCGGACAGTTAAGAACCCAGGGGCCTCTGCGCCCTGGGCCCAGCTCT

GTCCCACACCGCGGTCACATGGCACCACCTCTCTTGCAGCCTCCACCAAGG

GCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG

CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG

ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCC

CGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGAC

CGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT

CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGGTGAGAGGCCAG

FIG. 19A

CACAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTCCTGCCTGG

ACGCATCCCGGCTATGCAGTCCCAGTCCAGGGCAGCAAGGCAGGCCCCGTC

TGCCTCTTCACCCGGAGGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGGG

TCTTCTGGCTTTTTCCCCAGGCTCTGGGCAGGCACGGGCTAGGTGCCCCTAA

CCCAGGCCCTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAG

CCATATCCGGGAGGACCCTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAA

CTCTCCACTCCCTCAGCTCGGACACCTTCTCTCCTCCCAGATTCCAGTAACT

CCCAATCTTCTCTCTGCAGAGCCCAAATCTTGTGACAAAACTCACACATG

CCCACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGC

GGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGT

GCTGACACGTCCACCTCCATCTCTTCCTCAGCACCTGAACTCCTGGGGGGA

CCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT

CCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAG

ACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA

TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGT

GGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAG

TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA

CCATCTCCAAAGCCAAAGGTGGGACCCGTGGGGTGCGAGGGCCACATGGA

CAGAGGCCGGCTCGGCCCACCCTCTGCCCTGAGAGTGACCGCTGTACCAAC

CTCTGTCCCTACAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC

FIG. 19A (Cont.)

CATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGT

CAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG

GCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGAC

GGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGC

AGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAA

CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGATGA

Translation Introns Removed:

<-------- Kozak and HAVT20 Leader----------><---------- CAN20G2 Heavy V-Region----------
AATMACPGFLWALVISTCLEFSMAQIQLVQSGPEVKKPGETVKISCKASGYTFTNQG

MNWVKQAPGKGLKWMGWINTNTGEPTYTEEFKGRFAFSLETSASTAYLQINNLKNE

----------><----------Human IgG1 Constant with Introns----------
DTATYFCYVNYDYYTMDFWGQGTSVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL

VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN

HKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV

VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK

EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS

DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL

HNHYTQKSLSLSPGK

FIG. 19A (Cont.)

Kozak and HAVT20 Leader
CAN20G2 Kappa V-Region
Altered BamHI Site
Kappa Constant with Introns
Double Stop



FIG. 19B

TGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGA

TGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAG

AGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTC

ACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGC

AGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCG

>

TCACAAAGAGCTTCAACAGGGGAGAGTGTTGATAG

Translation Introns Removed:

<--------Kozak and HAVT20 Leader-------><----------CAN20G2 Kappa V-Region----------
AATMACPGFLWALVISTCLEFSMAQVVLTQSPAIMSASLGDRVTMTCTASSSVISTYLHWYQ

QKPGSSPKLWIYSTSTLASGVPPRFSGSGSGTSYSLTISSMEAEDAATYYCLQYHRSPRTFG

----------><----------Kappa Constant with Introns----------
GGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE

>
SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

FIG. 19B (Cont.)

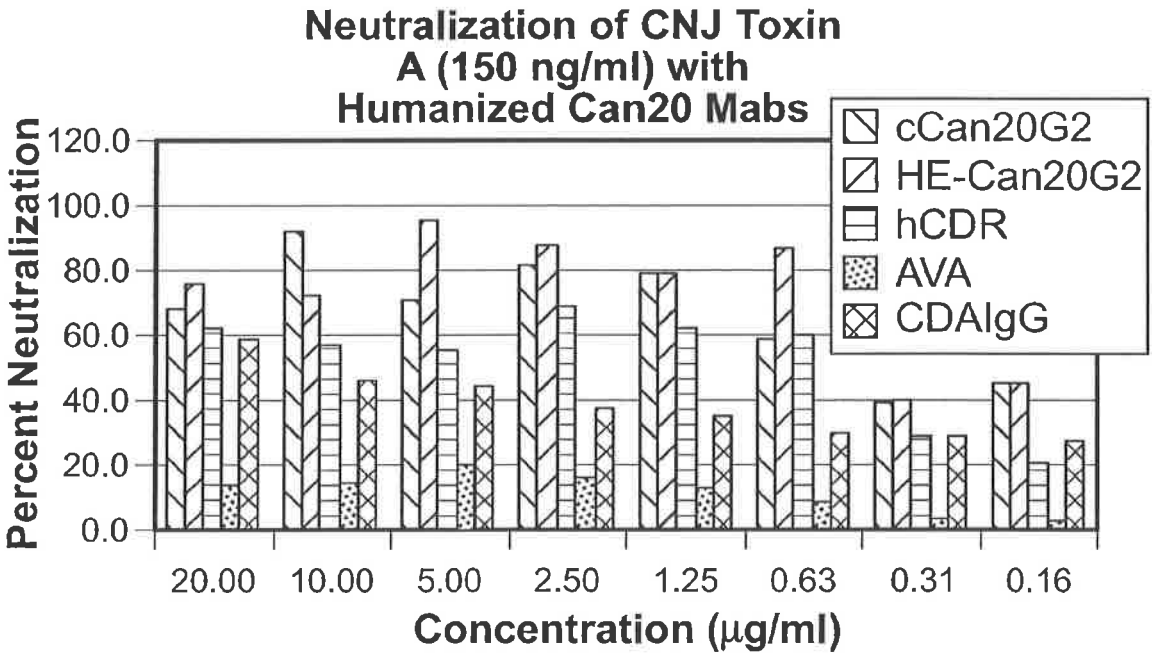

Neutralization of CNJ Toxin A (150 ng/ml) with Humanized Can20 Mabs
Percent Neutralization
120.0
100.0
80.0
60.0
40.0
20.0
0.0
20.00
10.00
5.00
2.50
1.25
0.63
0.31
0.16
Concentration (µg/ml)
cCan20G2
HE-Can20G2
hCDR
AVA
CDAIgG

FIG. 20A

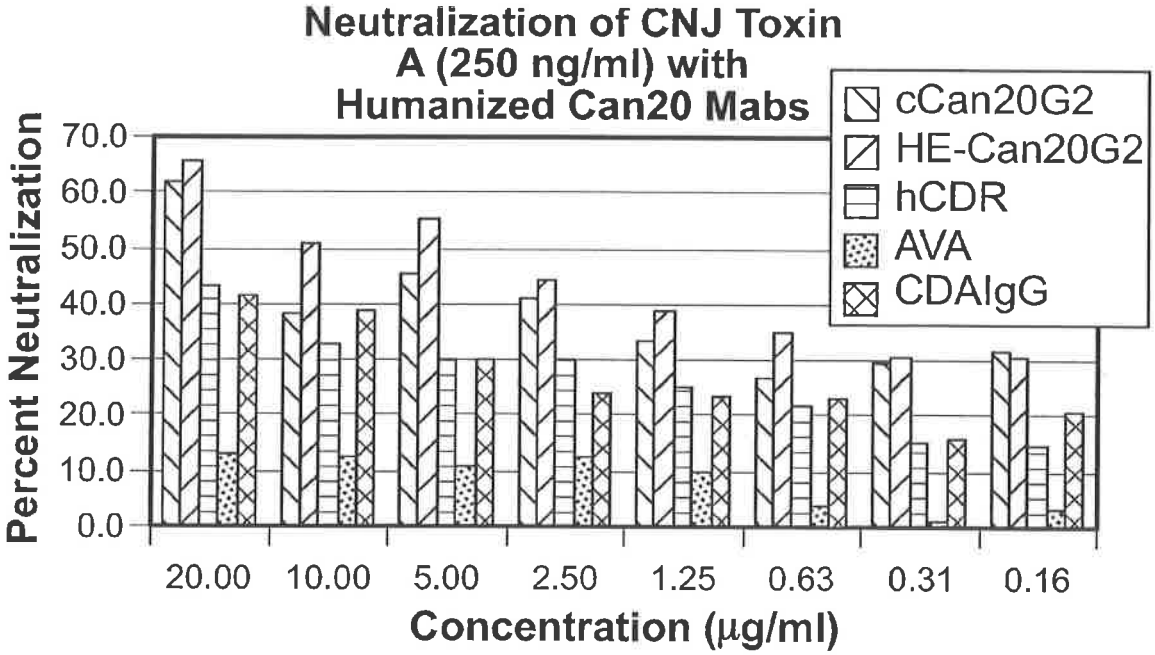

Neutralization of CNJ Toxin
A (250 ng/ml) with
Humanized Can20 Mabs
cCan20G2
HE-Can20G2
hCDR
AVA
CDAIgG
Percent Neutralization
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
20.00
10.00
5.00
2.50
1.25
0.63
0.31
0.16
Concentration (µg/ml)

FIG. 20B

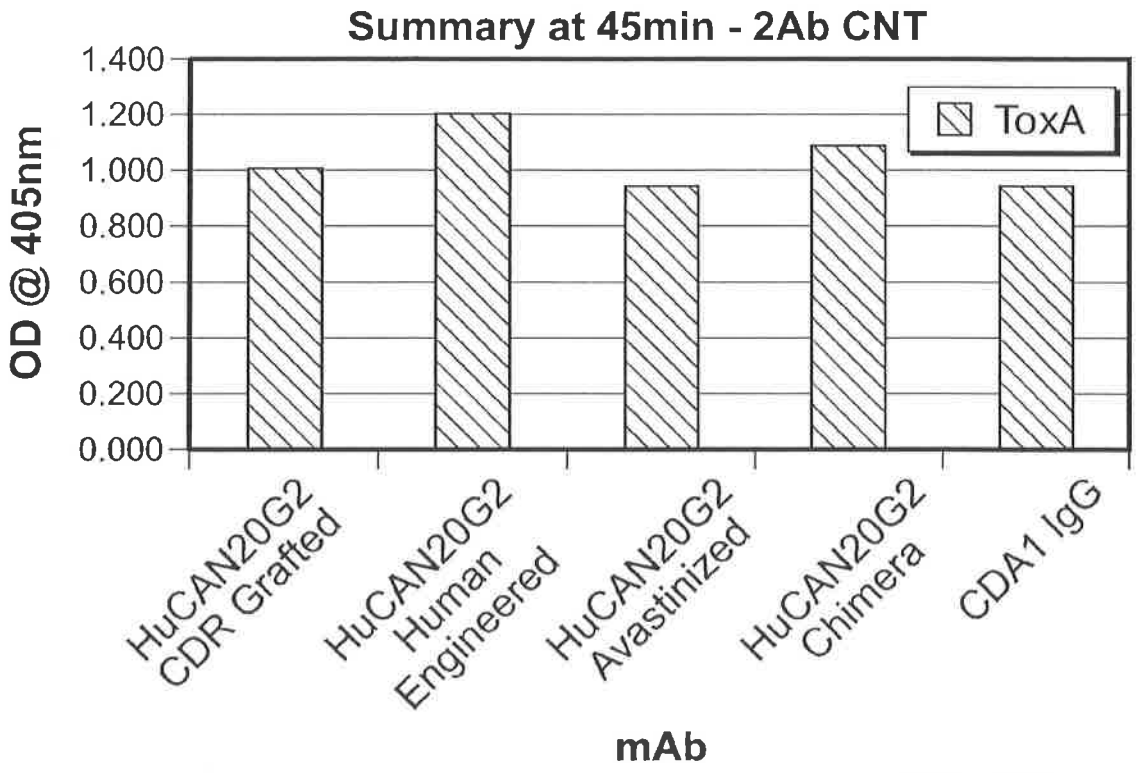


FIG. 21A

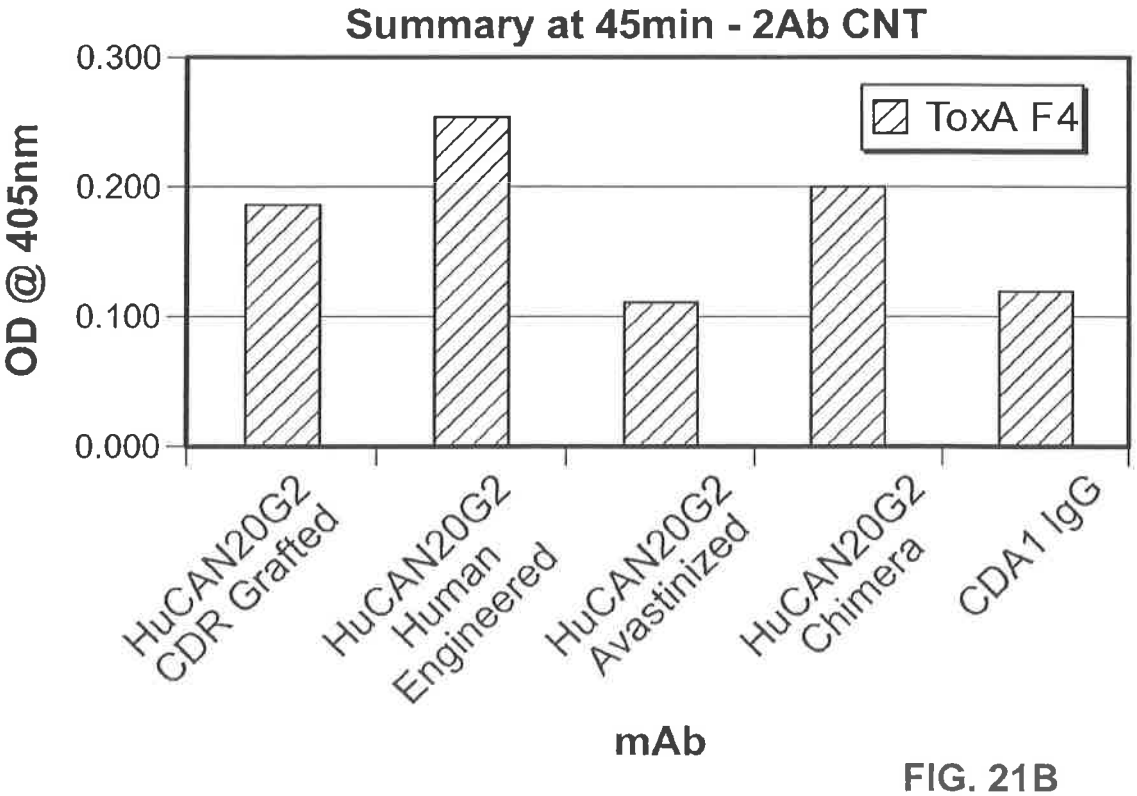


FIG. 21B

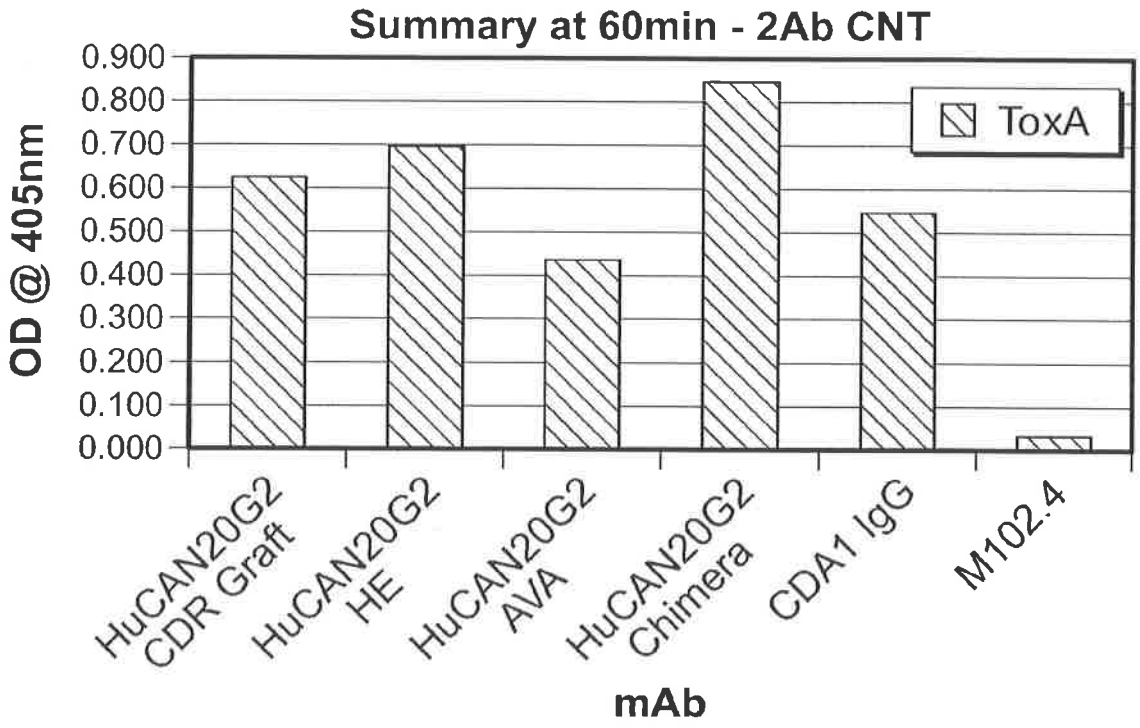


FIG. 21C

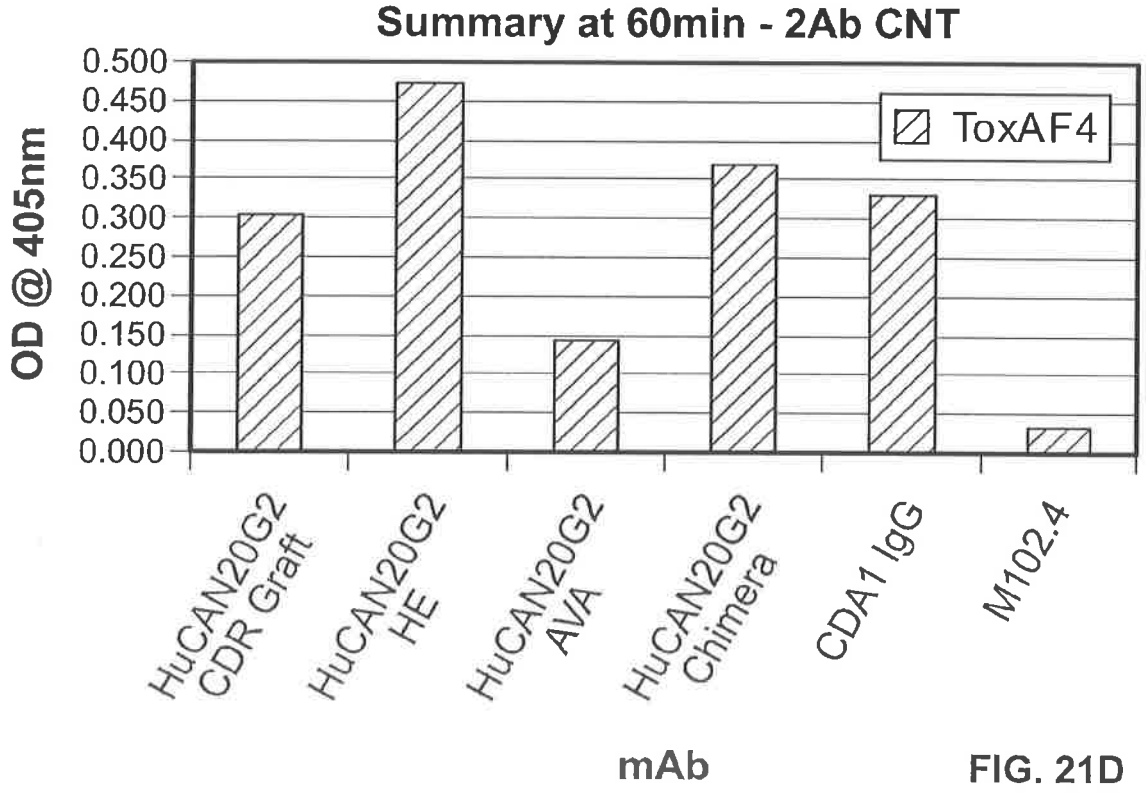


FIG. 21D

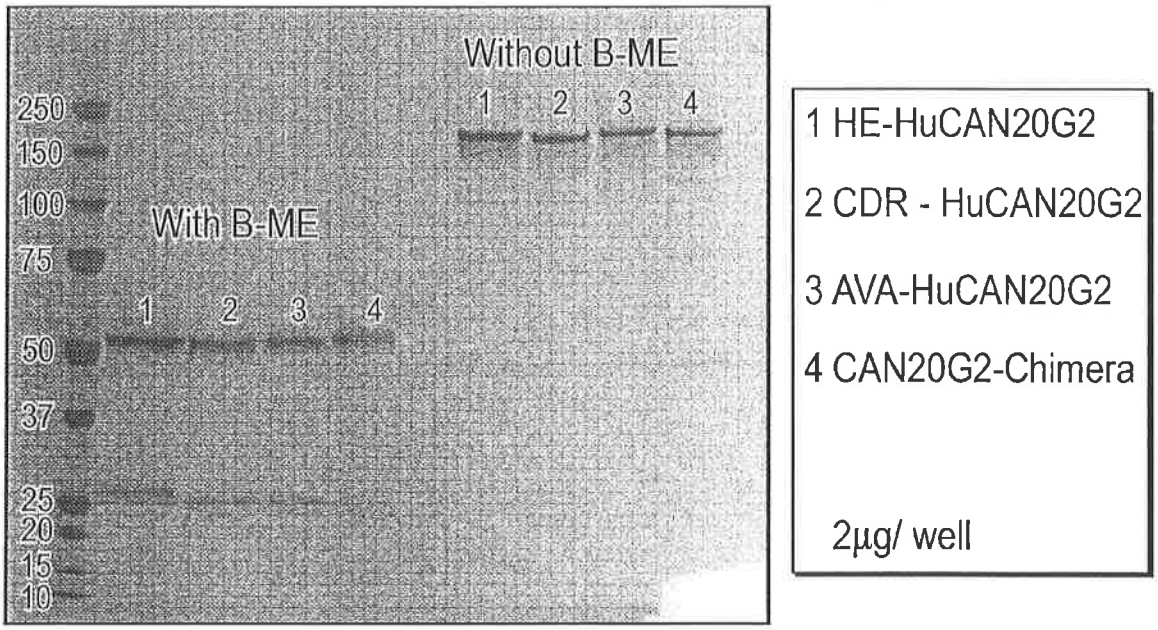

Without B-ME
1 2 3 4
250
150
100
With B-ME
75
1 2 3 4
50
37
25
20
15
10
1 HE-HuCAN20G2
2 CDR - HuCAN20G2
3 AVA-HuCAN20G2
4 CAN20G2-Chimera
2µg/ well

FIG. 22

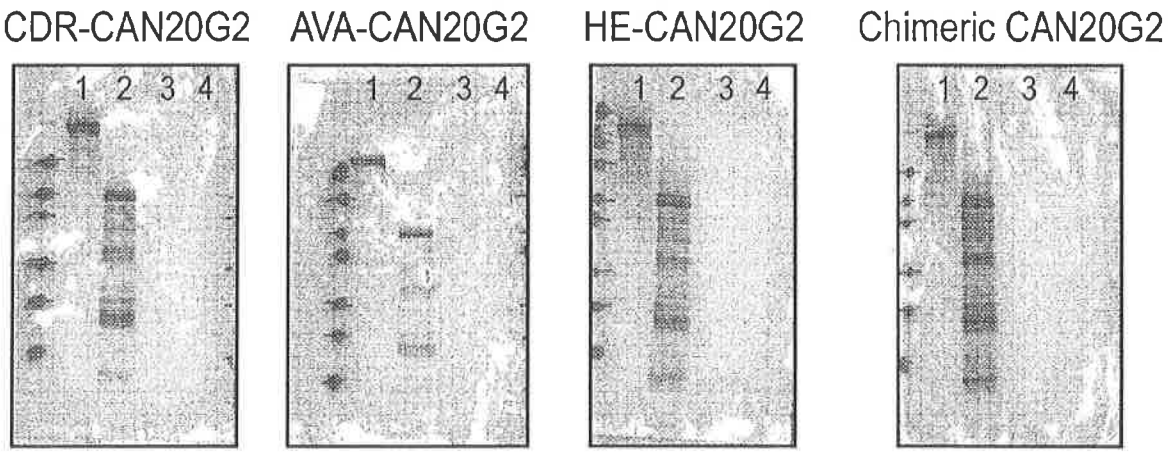

CDR-CAN20G2
AVA-CAN20G2
HE-CAN20G2
Chimeric CAN20G2
1 2 3 4
1 2 3 4
1 2 3 4
1 2 3 4

FIG. 23

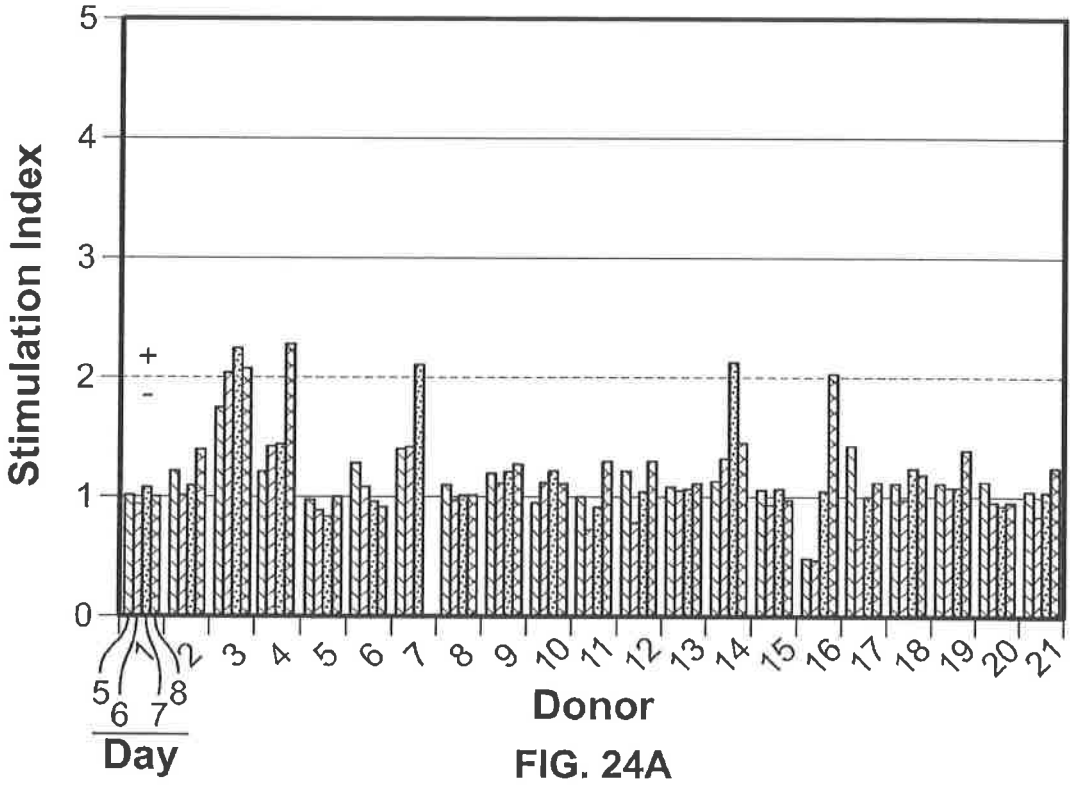

Stimulation Index
5
4
3
2
1
0
+
-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Donor
5 6 7 8
Day

FIG. 24A

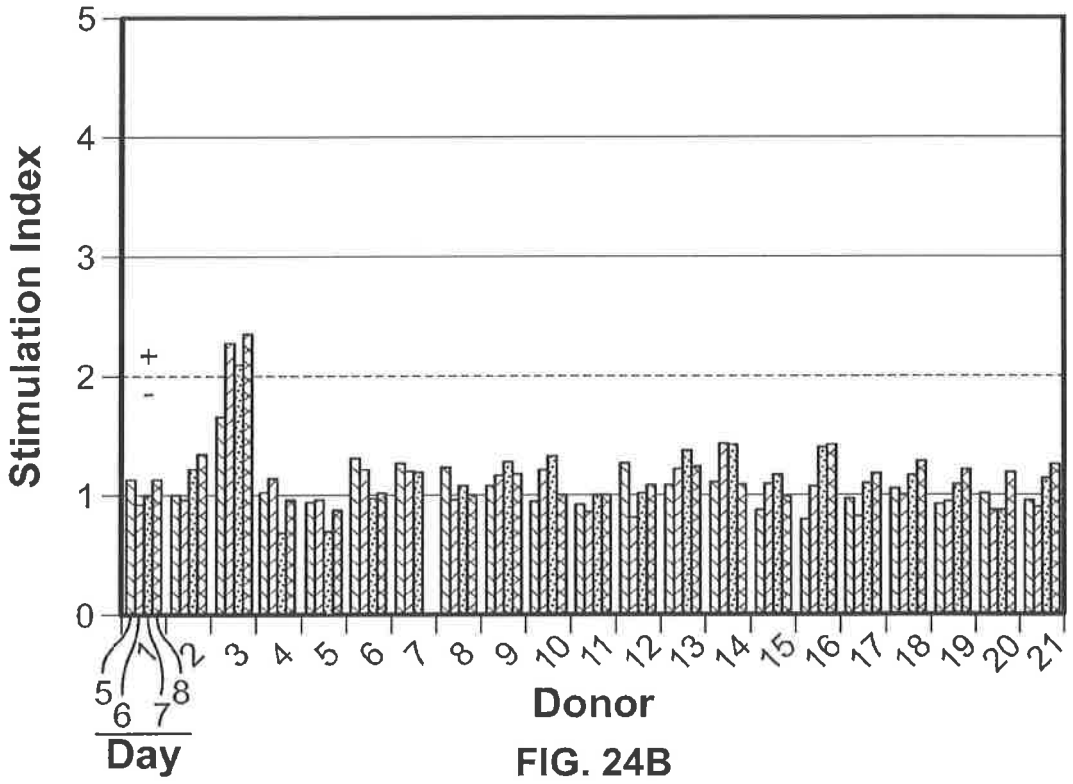

Stimulation Index
5
4
3
2
1
0
+
-
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
Donor
5
6
7
8
Day

FIG. 24B

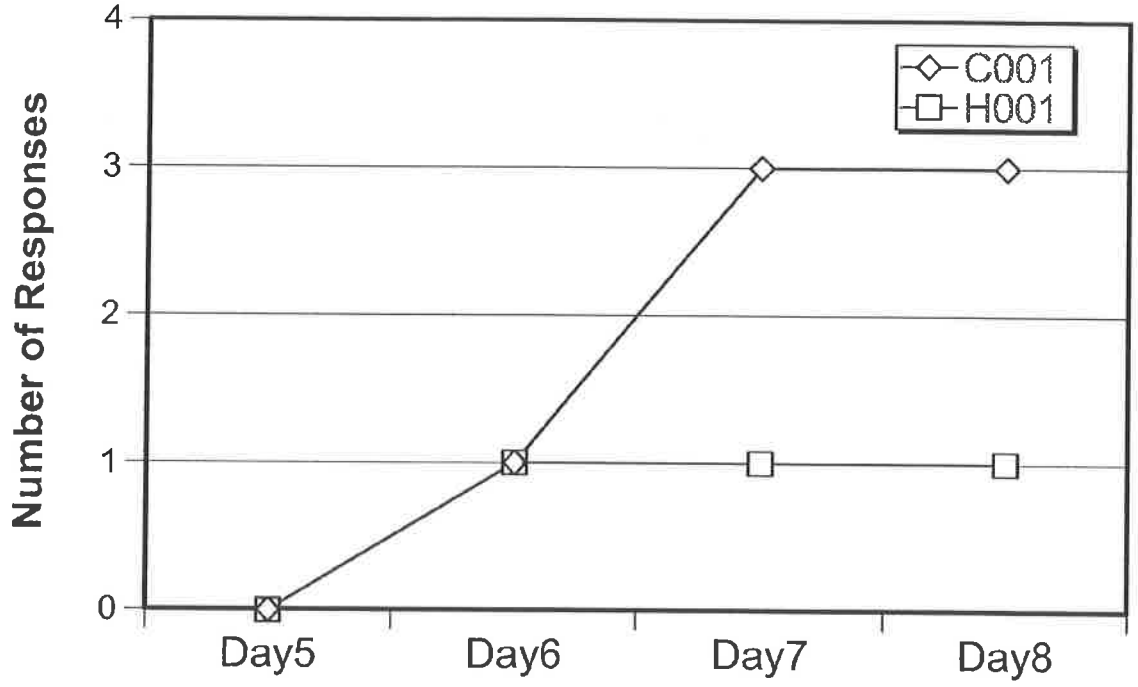

Number of Responses
0
1
2
3
4
Day5
Day6
Day7
Day8
C001
H001

FIG. 25

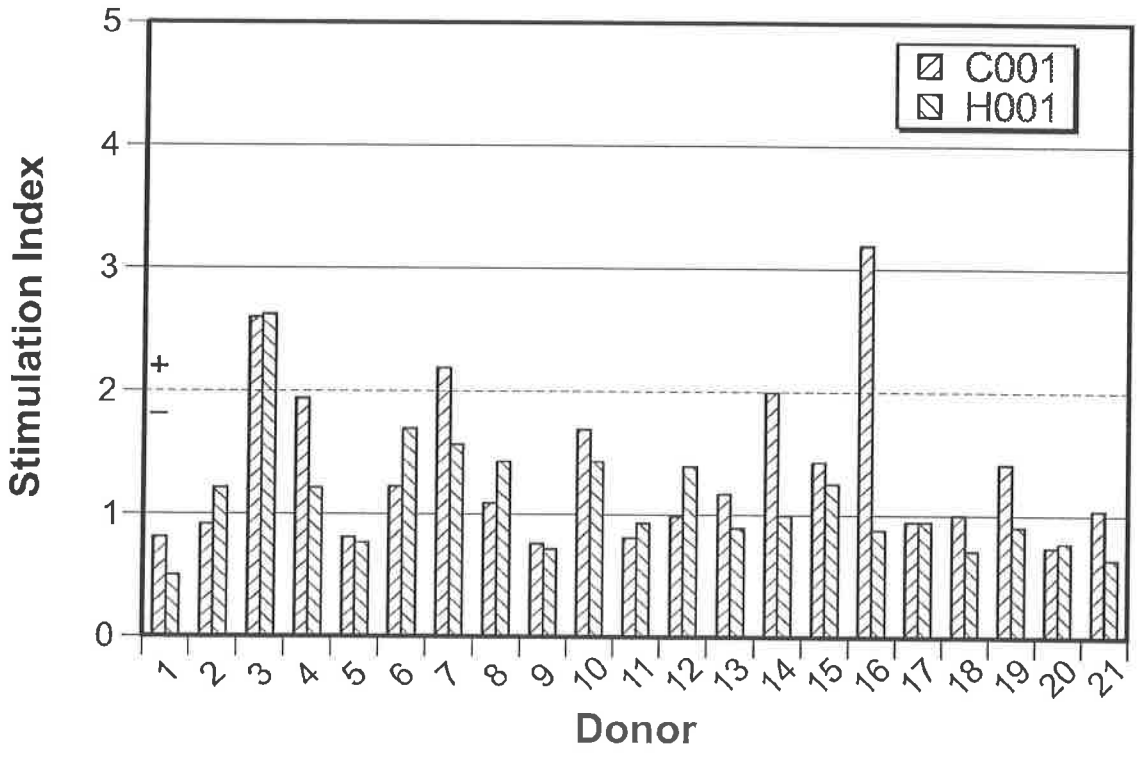

Stimulation Index
0
1
2
3
4
5
+
–
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Donor
C001
H001

FIG. 26

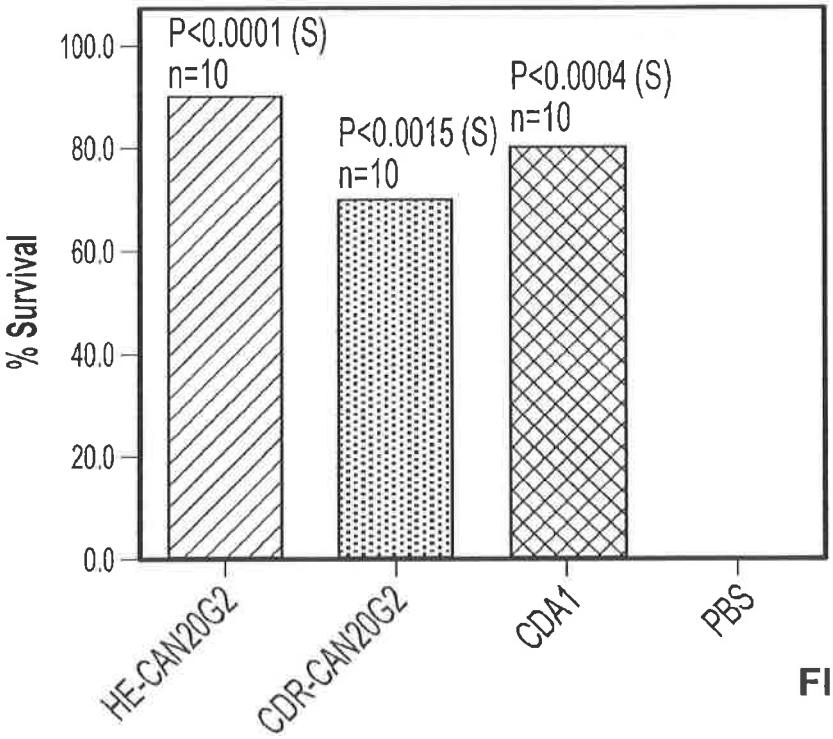

P<0.0001 (S)
n=10
P<0.0015 (S)
n=10
P<0.0004 (S)
n=10
% Survival
100.0
80.0
60.0
40.0
20.0
0.0
HE-CAN20G2
CDR-CAN20G2
CDA1
PBS

FIG. 27

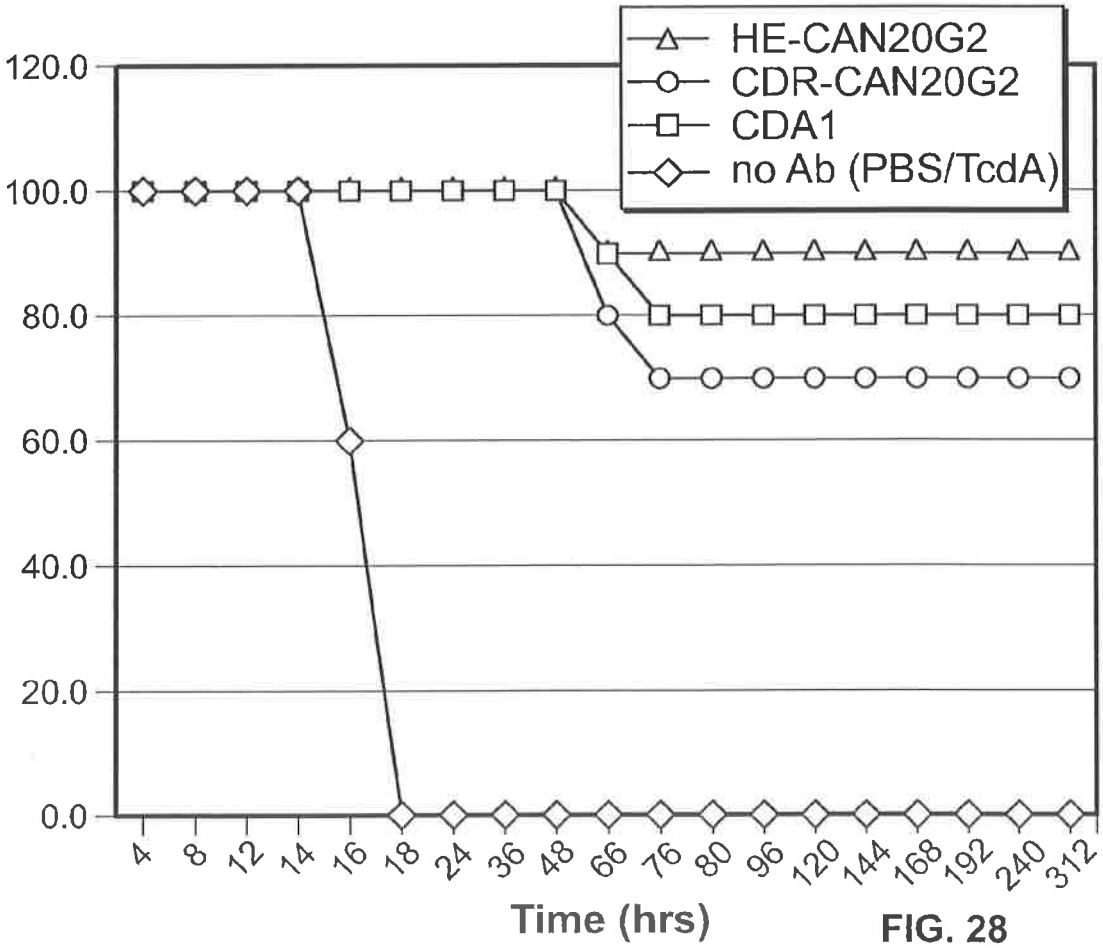

HE-CAN20G2
CDR-CAN20G2
CDA1
no Ab (PBS/TcdA)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
4
8
12
14
16
18
24
36
48
66
76
80
96
120
144
168
192
240
312
Time (hrs)

FIG. 28

SERUM Concentrations (μM) of Antibody CDR-huCAN20G2 in 10 mg/kg IV Dosed RAT SPRAGUE DAWLEY

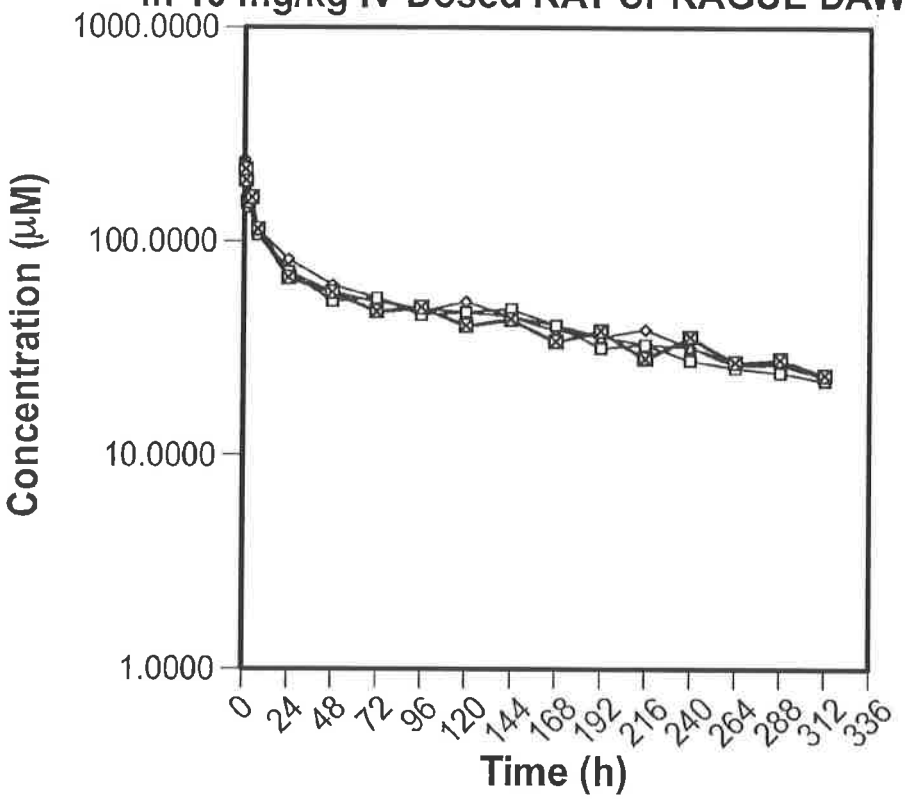


FIG. 29A

SERUM Concentrations (μM) of Antibody HE-huCAN20G2 in 10 mg/kg IV Dosed RAT SPRAGUE DAWLEY

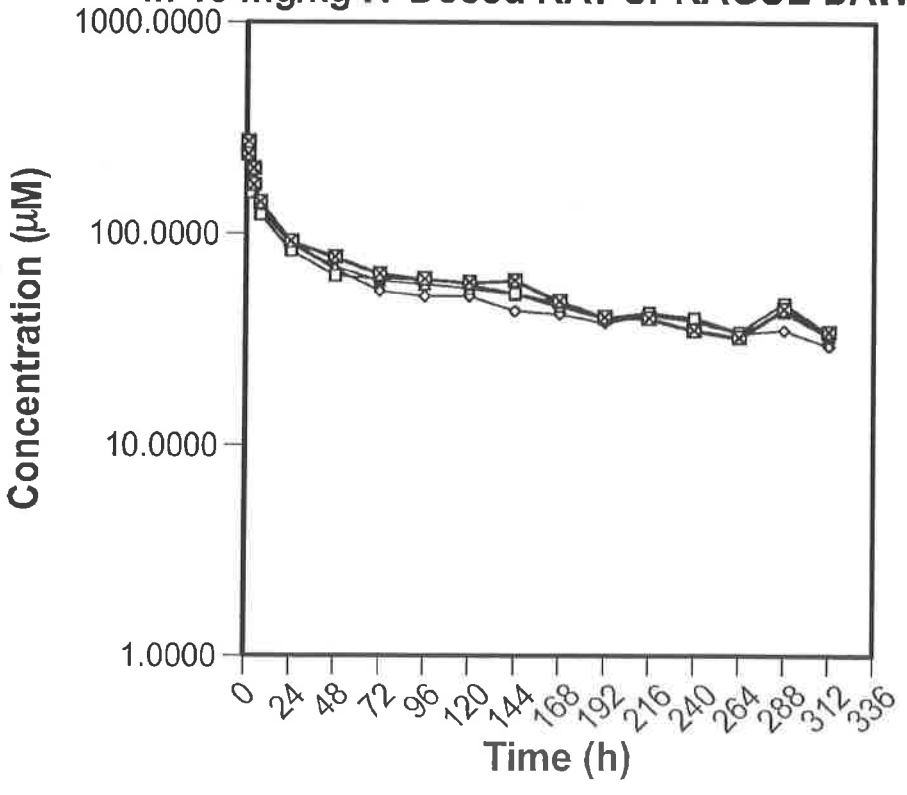


FIG. 29B