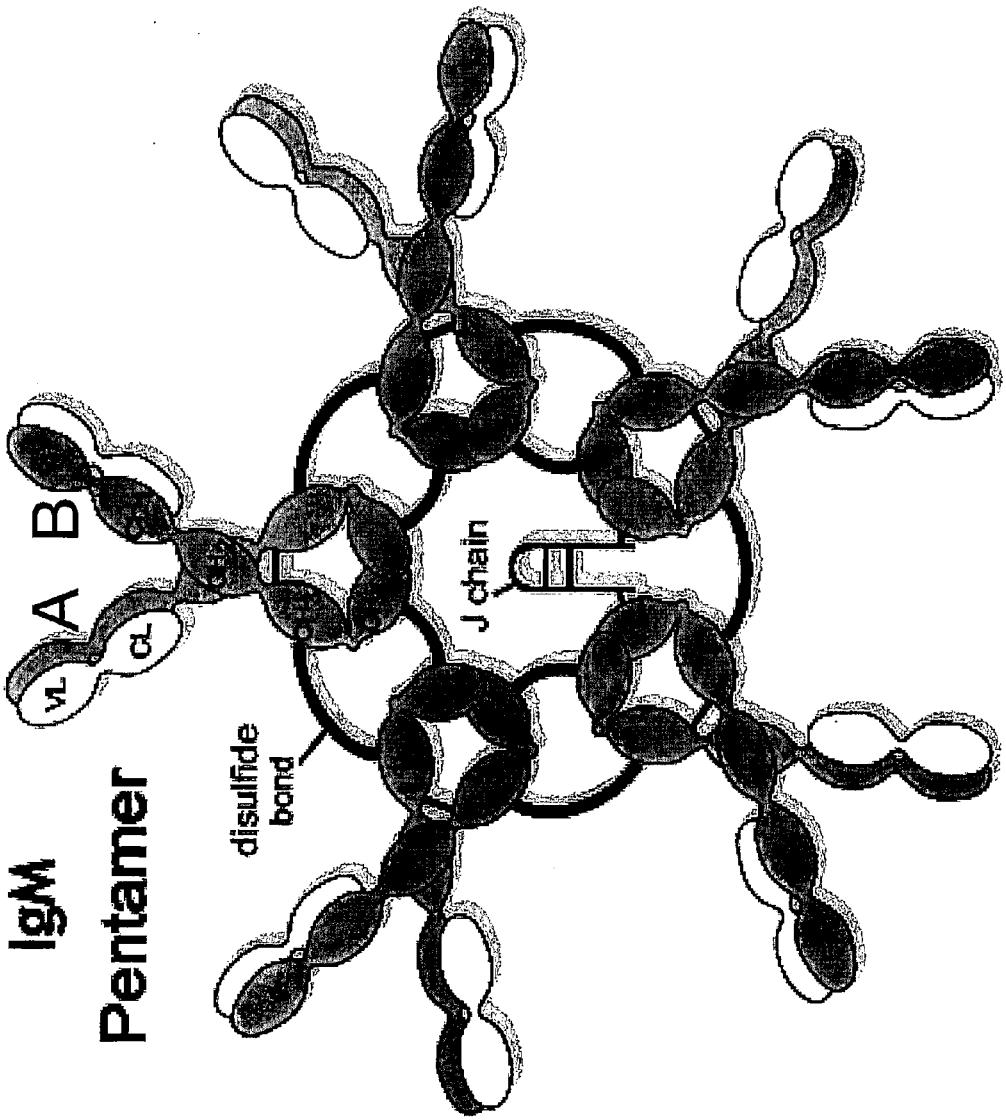


Five (A,B) unit, A = B in natural IgM

FIG. 1

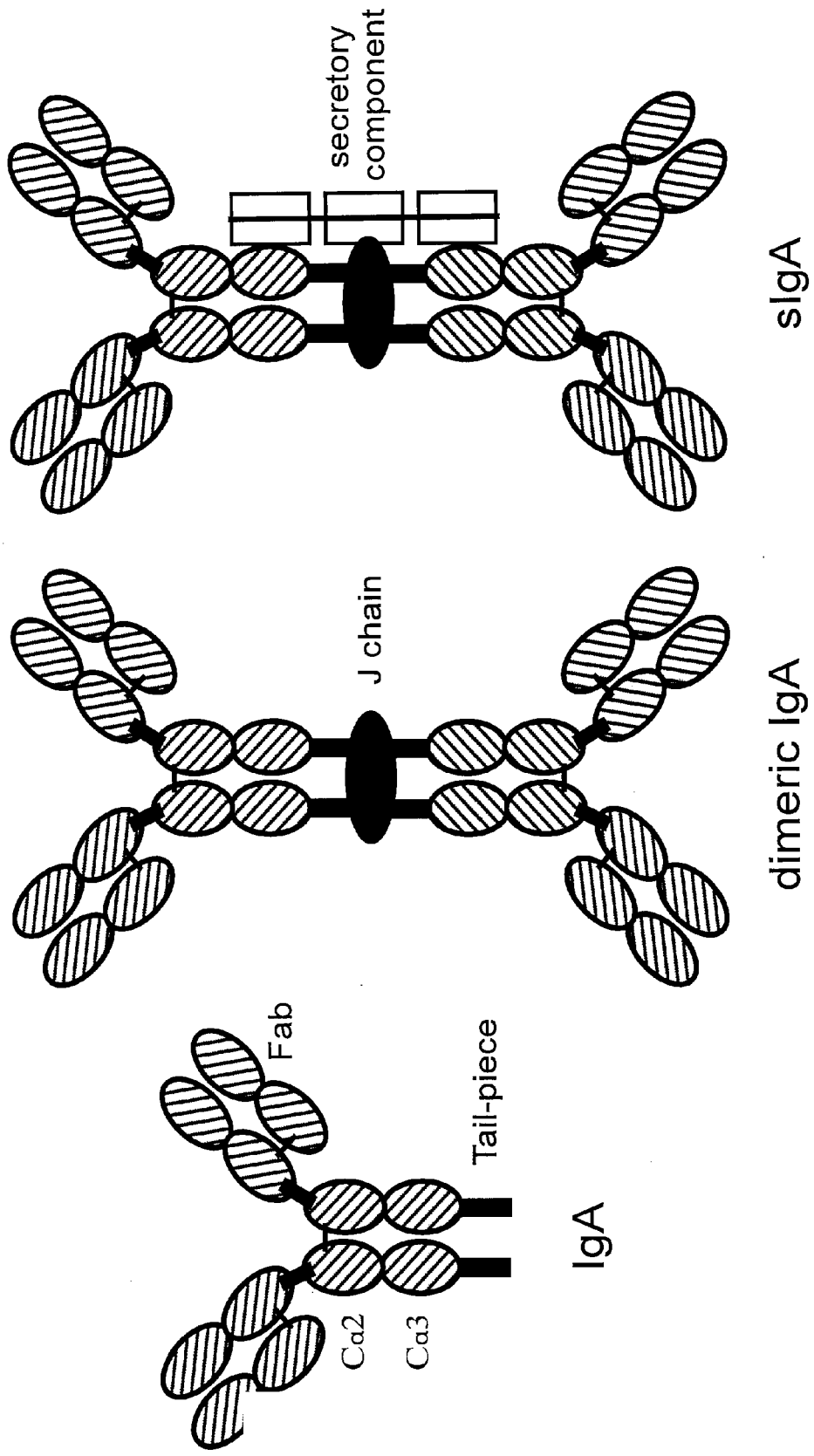

secretory component
sIgA
J chain
dimeric IgA
Fab
Tail-piece
Cα2
Cα3
IgA

FIG. 2

Mature Human J Chain

- QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNI RIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPT EVELDNQIVTATQSNICDEDSATETCYTYDRNKCYT AVVPLVYGGETKMVETALTPDACYPD (SEQ ID NO: 1)
- Number of amino acids: 137
- Molecular weight: 15594.4
- Theoretical pI: 4.59

FIG. 3

Hum = Human NP_653247 23-159
Chm = Chimpanzee XP_001160135 39-175
Gor = Gorilla XP_004038830 23-159
Ora = Orangutan NP_001125381 23-159
Gib = Gibbon XP_003265761 39-175
Mar = Marmoset XP_003732538 32-168
Cyn = Cynomolgus Monkey NP_001247815 23-159
Bab = Baboon XP_003898844 23-159
Squ = Squirrel Monkey XP_003931978 23-159
Shr = Tree Shrew XP_006142955 25-160
Dol = Dolphin XP_004328961 25-158
Kil = Killer Whale XP_004283629 25-158
Ele = Elephant XP_003414110 25-158
Sea = Seal XP_006729388 25-158
Rhi = Rhinoceros XP_004419202 25-157
Cat = House Cat XP_003985350 25-158
Wol = Wolf XP_532398 26-159
Pan = Giant Panda EFB23253 1-134
Fer = Ferret XP_004766376 26-158
Hor = Horse NP_001271464 25-158
Gui = Guinea Pig NP_001265705 25-160
Cam = Camel XP_006188738 25-158
Goa = Goat XP_005681786 25-158
Chn = Chinchilla XP_005392838 94-229
Ham = Hamster XP_005068228 24-160
She = Sheep XP_004009937 25-158
BBa = Brown Bat XP_006094475 25-158
Ant = Antelope XP_005983836 25-158
Cow = Cow NP_786967 25-157
Mou = Mouse NP_690052 23-159
Rat = Rat EDL88516 23-159
Hed = Hedgehog XP_004703237 25-157
Rab = Rabbit P23108 1-136
Opo = Opossum XP_003341415 29-162
All = Alligator XP_006270094 26-159
Tur = Turtle XP_005304167 26-159
Tas = Tasmania Devil XP_003772863 27-160
Pla = Platypus XP_003430954 36-160
Par = Parakeet XP_005142787 28-160
Duc = Duck XP_005031370 28-160
Chi = Chicken NP_989594 26-158
Tur = Turkey XP_003205717 27-159
Fal = Falcon XP_005243236 29-160
Spa = Sparrow XP_005492217 29-158

FIG. 4

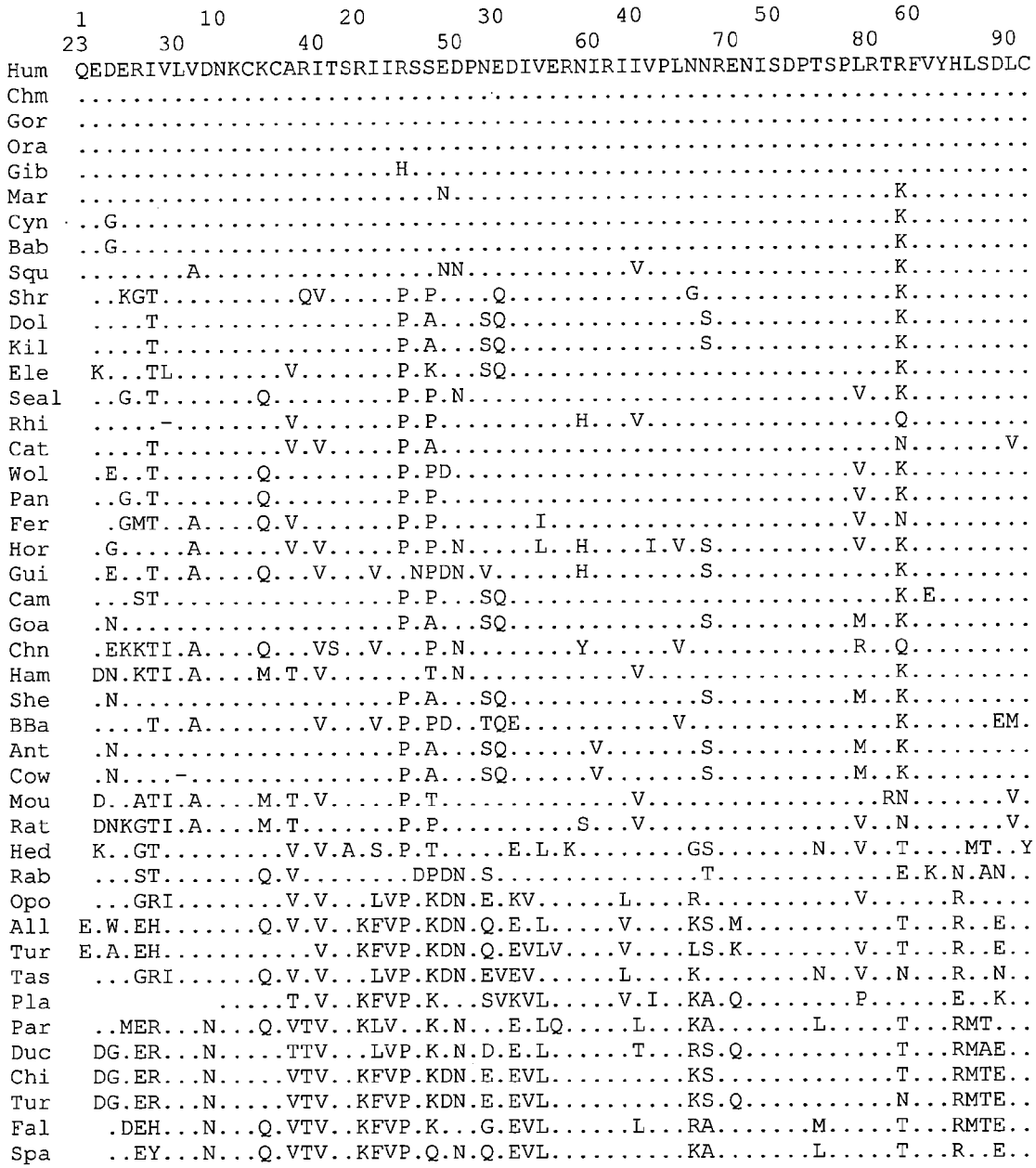

1 10 20 30 40 50 60
23 30 40 50 60 70 80 90
Hum QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLC
Chm
Gor
Ora
Gib
Mar
Cyn
Bab
Squ
Shr
Dol
Kil
Ele
Seal
Rhi
Cat
Wol
Pan
Fer
Hor
Gui
Cam
Goa
Chn
Ham
She
BBa
Ant
Cow
Mou
Rat
Hed
Rab
Opo
All
Tur
Tas
Pla
Par
Duc
Chi
Tur
Fal
Spa

FIG. 4 (Cont.)

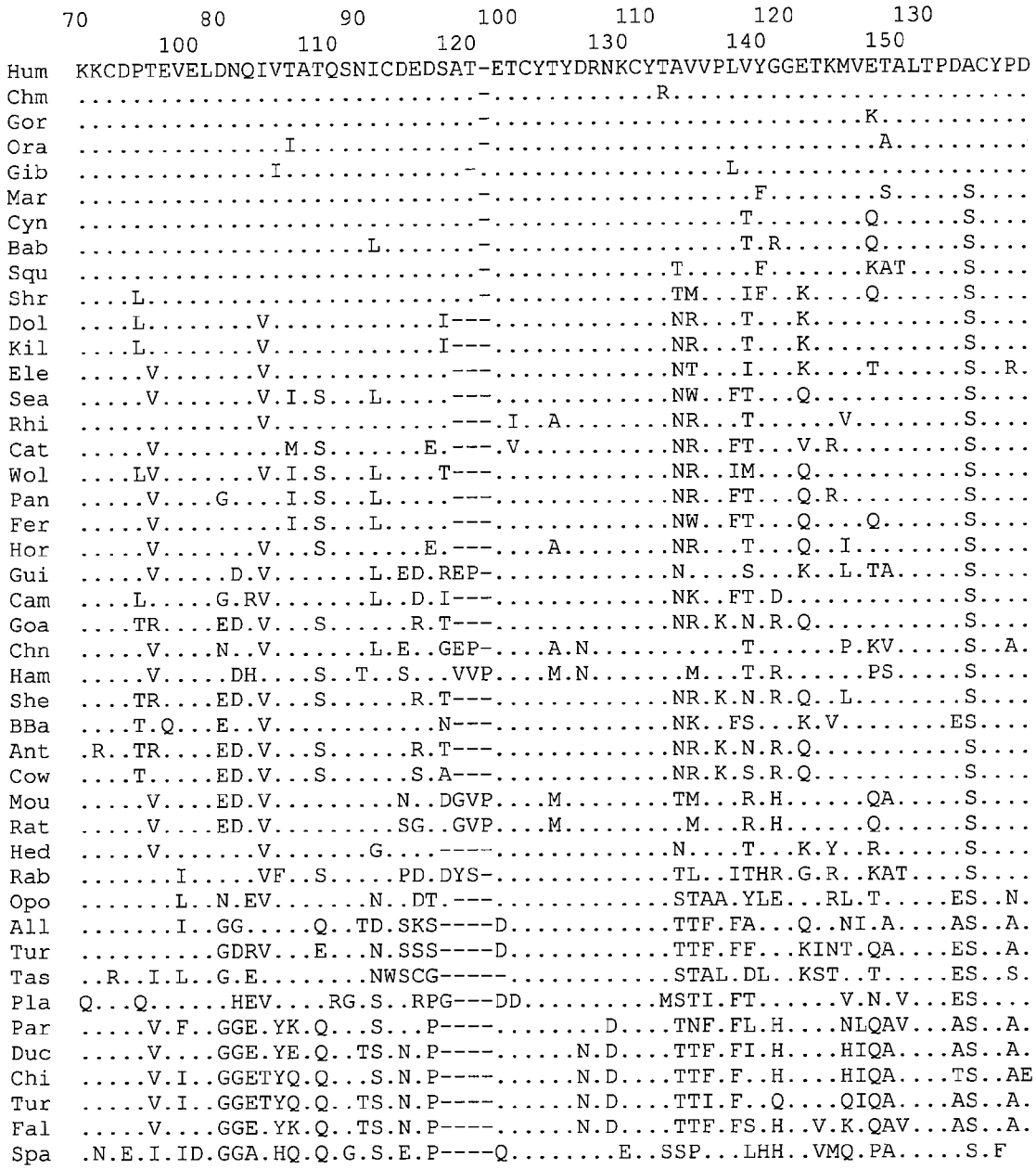

70 80 90 100 110 120 130
100 110 120 130 140 150
Hum KKCDPTEVELDNQIVTATQSNICDEDSAT-ETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD
Chm
Gor
Ora
Gib
Mar
Cyn
Bab
Squ
Shr
Dol
Kil
Ele
Sea
Rhi
Cat
Wol
Pan
Fer
Hor
Gui
Cam
Goa
Chn
Ham
She
BBa
Ant
Cow
Mou
Rat
Hed
Rab
Opo
All
Tur
Tas
Pla
Par
Duc
Chi
Tur
Fal
Spa

FIG. 4 (Cont.)

Human vs Bat J Chain

```
Query  2    EDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRII--------------------  41
            EDERI+LVDNKCKCARITSRII S EDP++DIVERNIRI
Sbjct  25   EDERILLVDNKCKCARITSRIIPSPEDPSQDIVERNIRIKLGDEKSMVNLWNCKLLCTAG  84

Query  42   ----------------VPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTA  86
                            VPLNNRENISDPTSP RT FVYHLSDLCK CDPTEVELDNQ+VT
Sbjct  85   LQSKEGGMVADTAGKVPLNNRENISDPTSPRRTNFVYHLSDLCKNCDPTEVELDNQVVTV  144

Query  87   TQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD  137
            TQSNICDED+  ETCY YDRNKCYT  VPL+YGG+T MVETALTPD+CYPD
Sbjct  145  TQSNICDEDN--ETCYAYDRNKCYTNRVPLLYGGKTIMVETALTPDSCYPD  193
```

FIG. 5

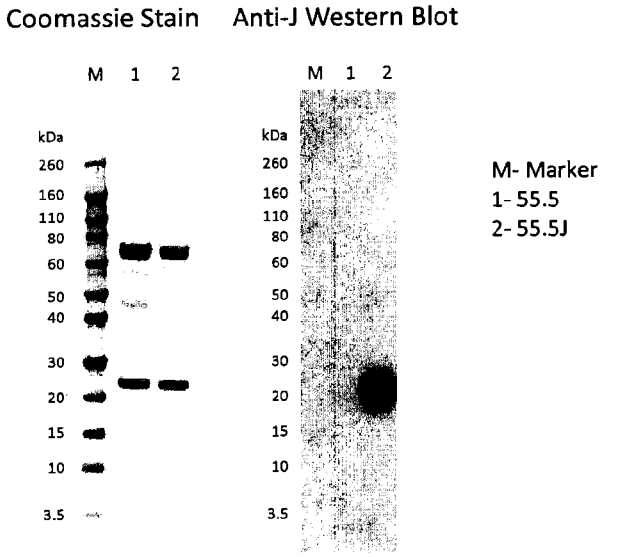

Coomassie Stain
Anti-J Western Blot
M 1 2
M 1 2
kDa
260
160
110
80
60
50
40
30
20
15
10
3.5
kDa
260
160
110
80
60
50
40
30
20
15
10
3.5
M- Marker
1- 55.5
2- 55.5J

FIG. 6

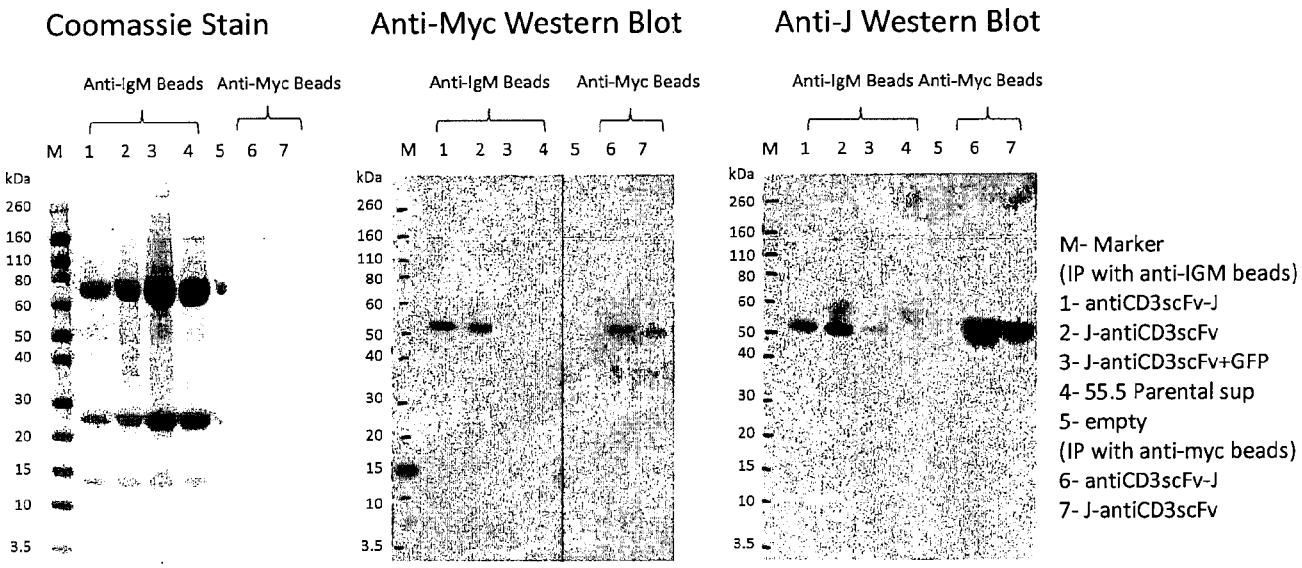

Coomassie Stain
Anti-Myc Western Blot
Anti-J Western Blot
Anti-IgM Beads
Anti-Myc Beads
M 1 2 3 4 5 6 7
kDa
260
160
110
80
60
50
40
30
20
15
10
3.5
M- Marker
(IP with anti-IGM beads)
1- antiCD3scFv-J
2- J-antiCD3scFv
3- J-antiCD3scFv+GFP
4- 55.5 Parental sup
5- empty
(IP with anti-myc beads)
6- antiCD3scFv-J
7- J-antiCD3scFv

FIG. 7

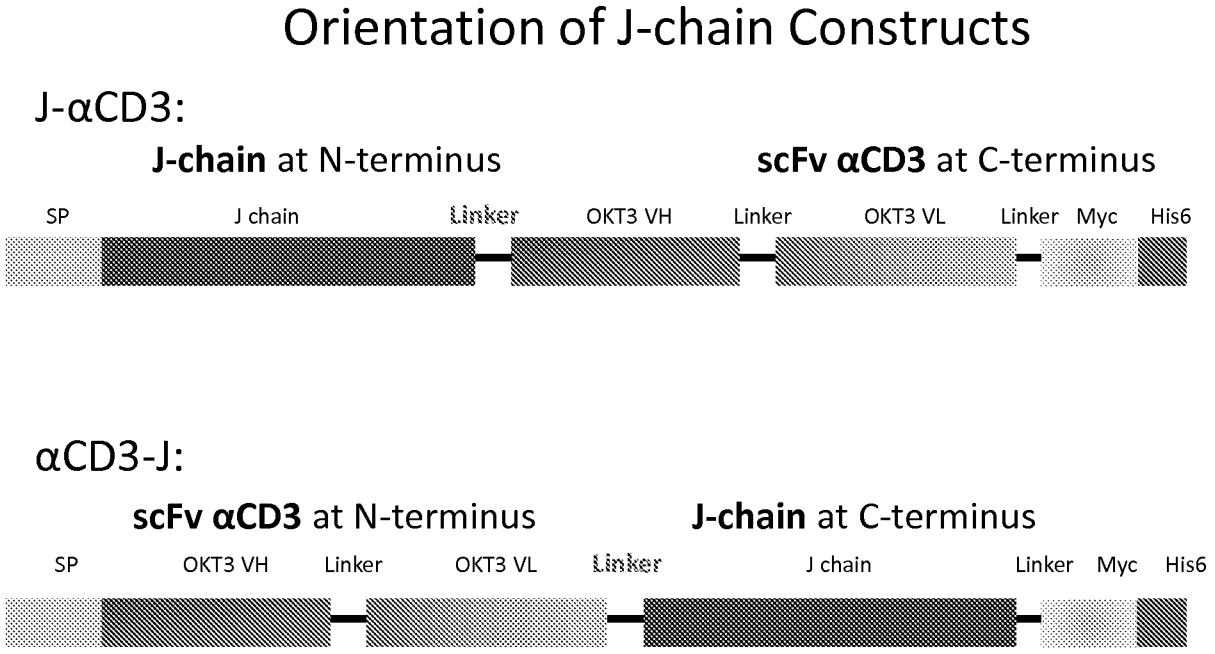

Orientation of J-chain Constructs
J-αCD3:
J-chain at N-terminus
scFv αCD3 at C-terminus
SP
J chain
Linker
OKT3 VH
Linker
OKT3 VL
Linker
Myc
His6
αCD3-J:
scFv αCD3 at N-terminus
J-chain at C-terminus
SP
OKT3 VH
Linker
OKT3 VL
Linker
J chain
Linker
Myc
His6

FIG. 8

antiCD3scFv-J

- MGWSYIILFLVATATGVHS**QVQLQQSGAELARPGASVKMSCKASGYTFTR YTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYM QLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSGGGGSGGGGSGG GGS**QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWI YDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTF GSGTKLEIK**GGGGSGGGGSGGGGS*QEDERIVLVDNKCKCARITSRIIRSS EDPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTE VELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVE TALTPDACYPD*GGGSEQKLISEEDLNSAVD**HHHHHH**-



FIG. 9

J-antiCD3scFv

- MKNHLLFWGVLAVFIKAVHVKA***QEDERIVLVDNKCKCARITSRIIRSSED PNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVE LDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETA LTPDACYPD***GGGGSGGGGSGGGGS**QVQLQQSGAELARPGASVKMSCKASG YTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSS STAYMQLSSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSS**GGGGSGG GGSGGGGS**QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTS PKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSS NPFTFGSGTKLEIK**GGGSEQKLISEEDL**NSAVD**HHHHHH**-



FIG. 10

Asymmetric IgM Pentamer with modified J-chain

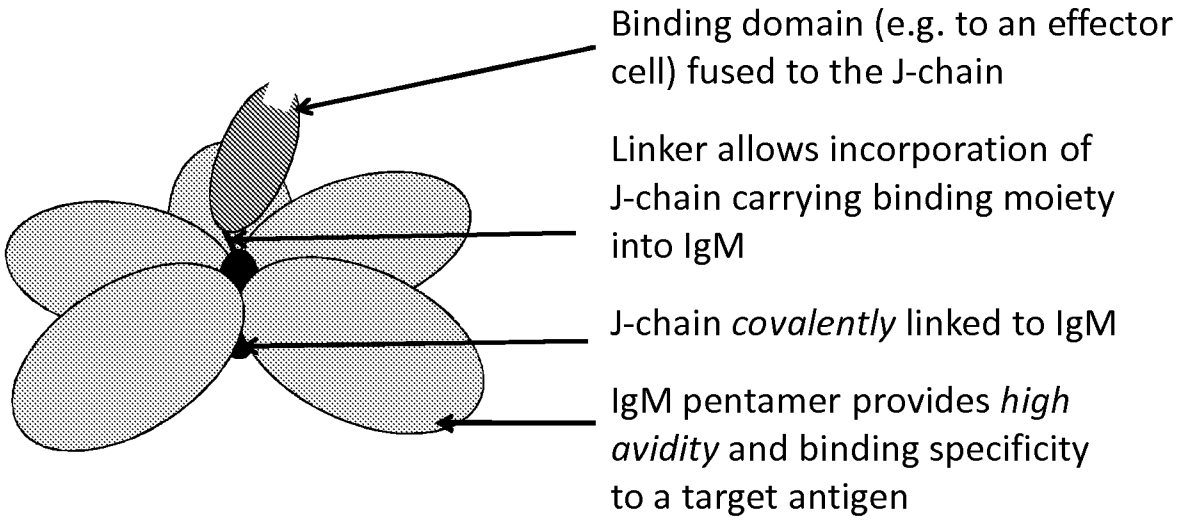
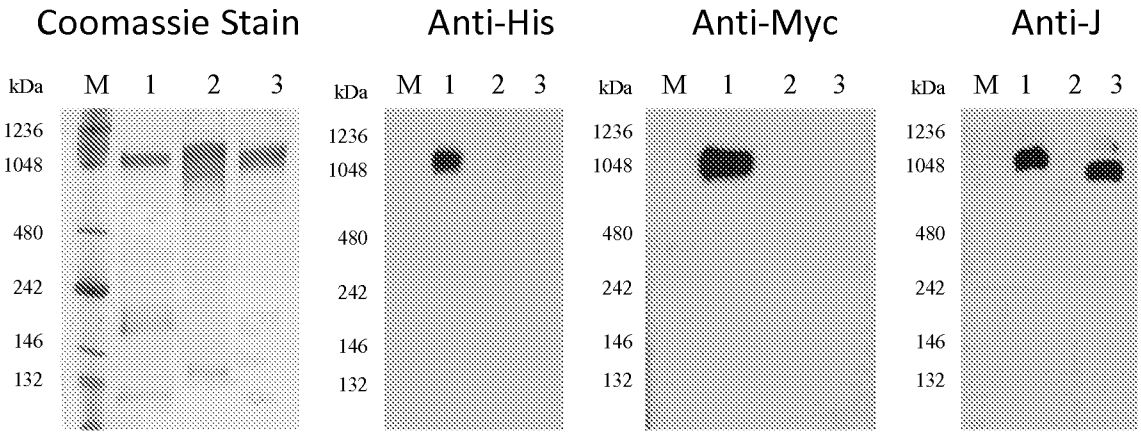


FIG. 11

Modified J Chain with αCD3 scFv Incorporated into αCD20 IgM pentamer



M- Marker
1) IgM + αCD3-J chain
2) 55.5 IgM
3) 55.5 IgM + wt J

FIG. 12

αCD20 $_{IgM}$ x αCD3$_{J}$ bi-specific IgM expressed

- Transient co-transfections: HC, LC, and JC
- All 5 J variant are pentamers

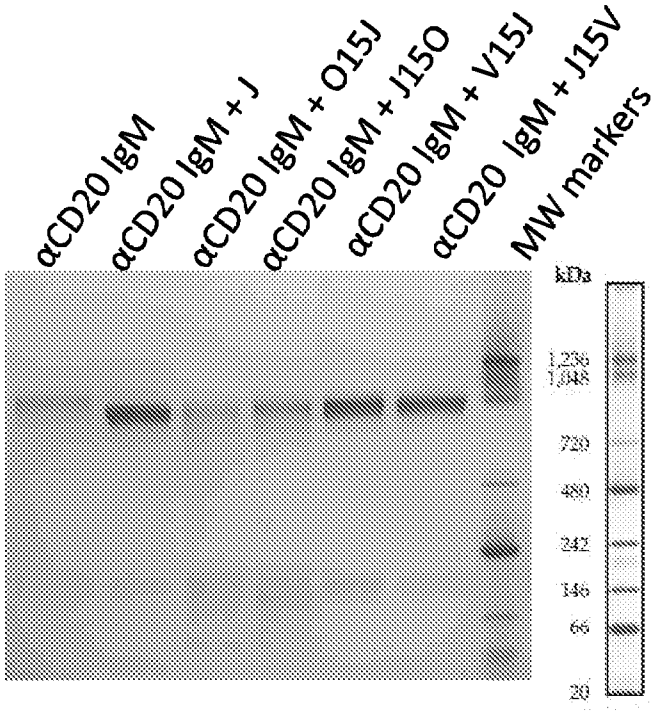
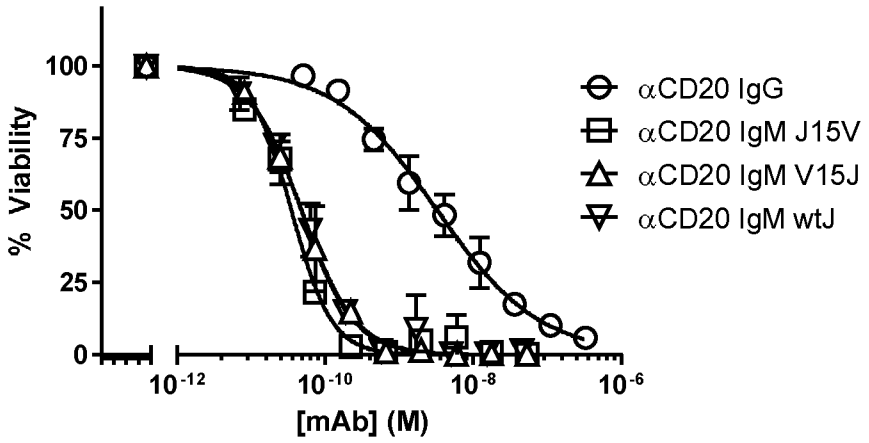


FIG. 13

V15J/J15V variants *retain CDC activity*



EC50(pM)			
αCD20 IgG	αCD20 IgM wt J	αCD20 IgM V15J	αCD20 IgM J15V
3292	48.4	47.9	35.2

FIG. 14

Bispecific IgM: active T cell killing of B-cells
$\alpha CD20_{IgM}$ x $\alpha CD3_{J\ chain}$ highly potent at B-cell depletion

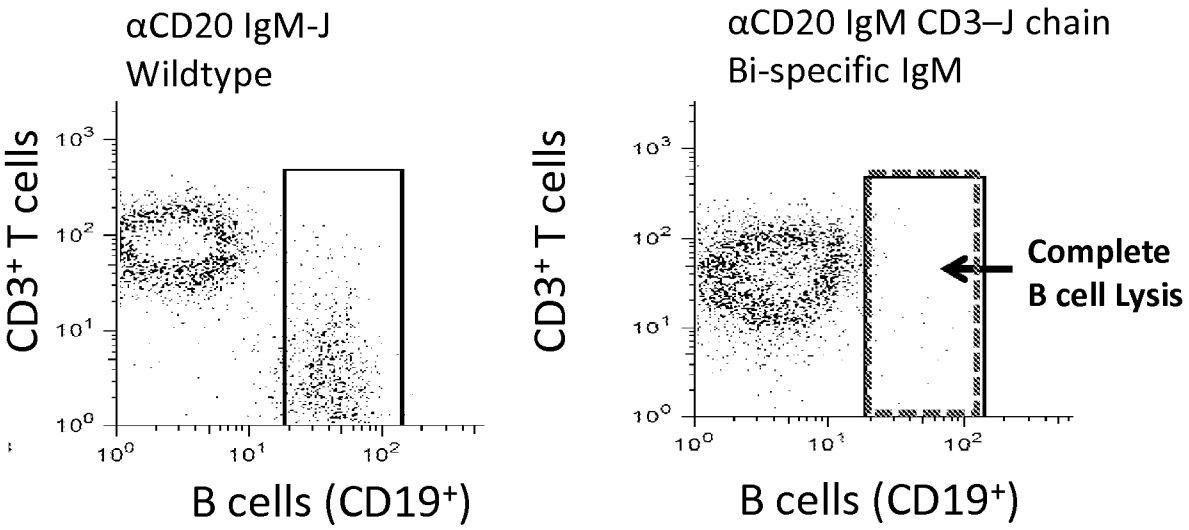


FIG. 15

Bi-specific IgM: αCD20 very active
Raji cells (B cells) killing by T cells with αCD20 x αCD3 IgM

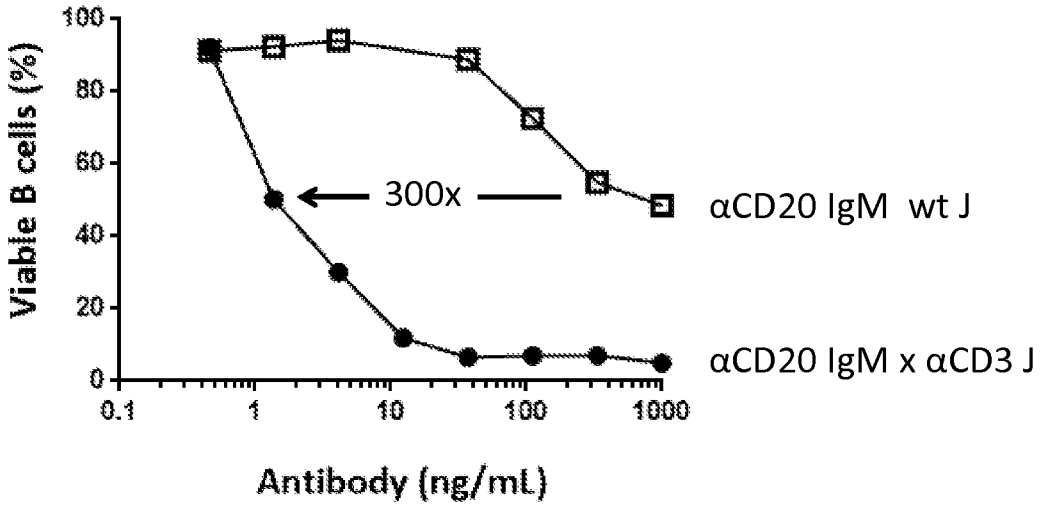


FIG. 16

In vivo B cell Depletion with Anti-CD20
IgG, IgM and bi-specific IgM are very active

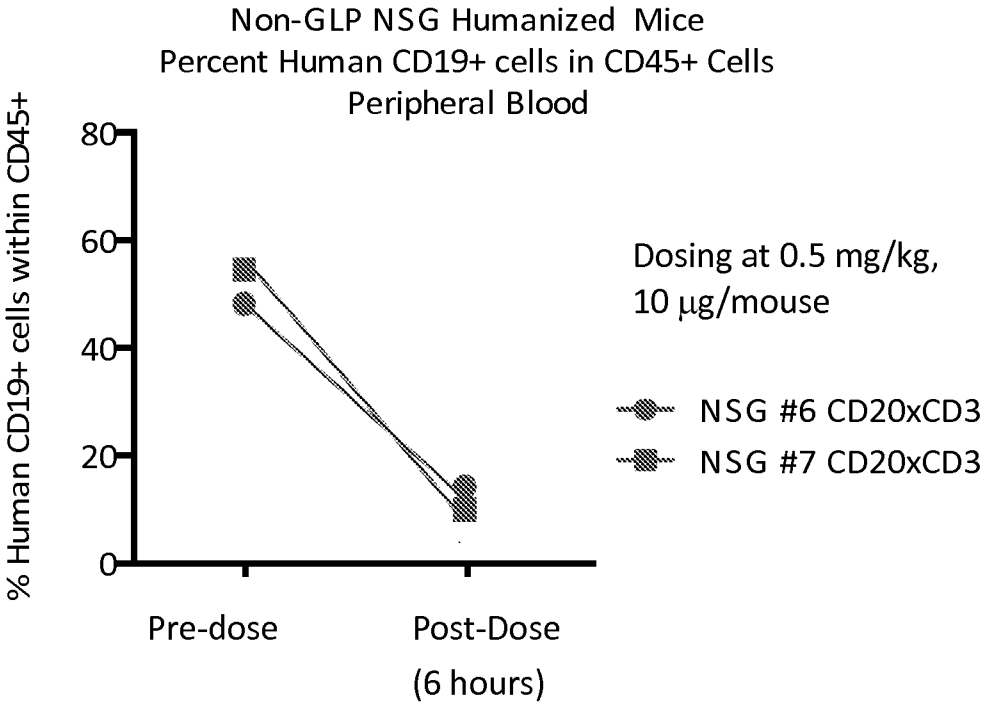


FIG. 17

CD16 specific Vh camelid-J chain

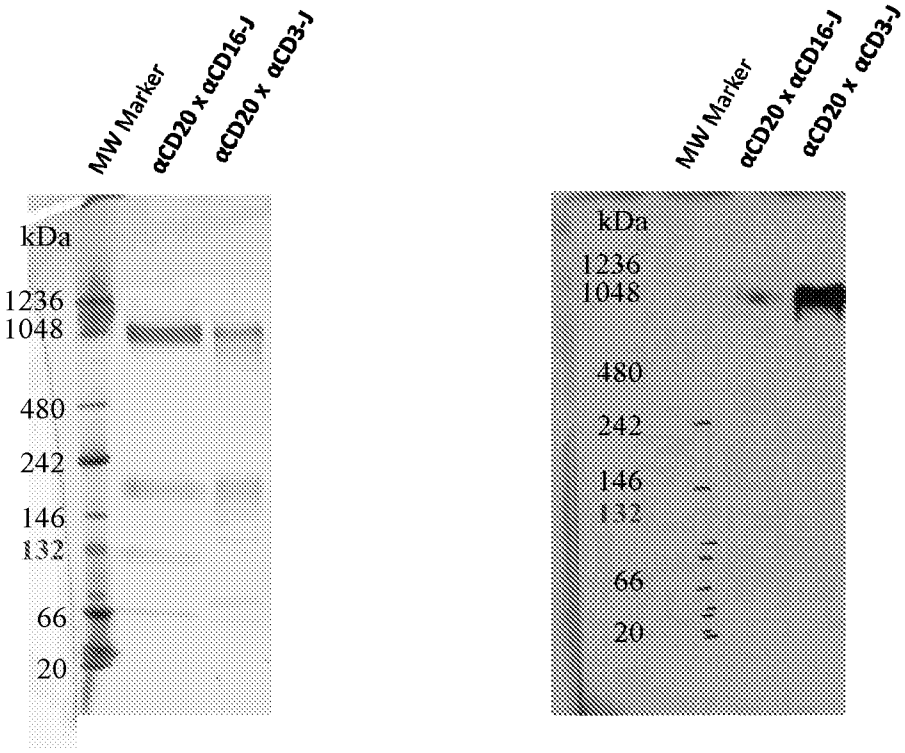


FIG. 18

Menu driven approach to Bi-specific Abs
Target cells X Effector cells: for Oncology Applications

Target Associated Antigen	Immune Effector Cell
Hematologic cancer CDIM, CD19, CD20, CD22, CD33, CD70, CD56, CD138 **Epithelial tumor** EpCAM, Her2, Eph-A2 PSMA, MCSP, AGS-5 GPNMB **Carbohydrate antigens** CEA, CA-125, TADG78 Sialyl Lewis-X (CD15) **Viral Antigens** PreS1 (HBV), GP120 (HIV)	**T Cells**: CD3 epsilon **NK Cells,** CD16, CD64 NKG2D **Macrophages** CD14 **Neutrophils** CD16b, CD177

FIG. 19