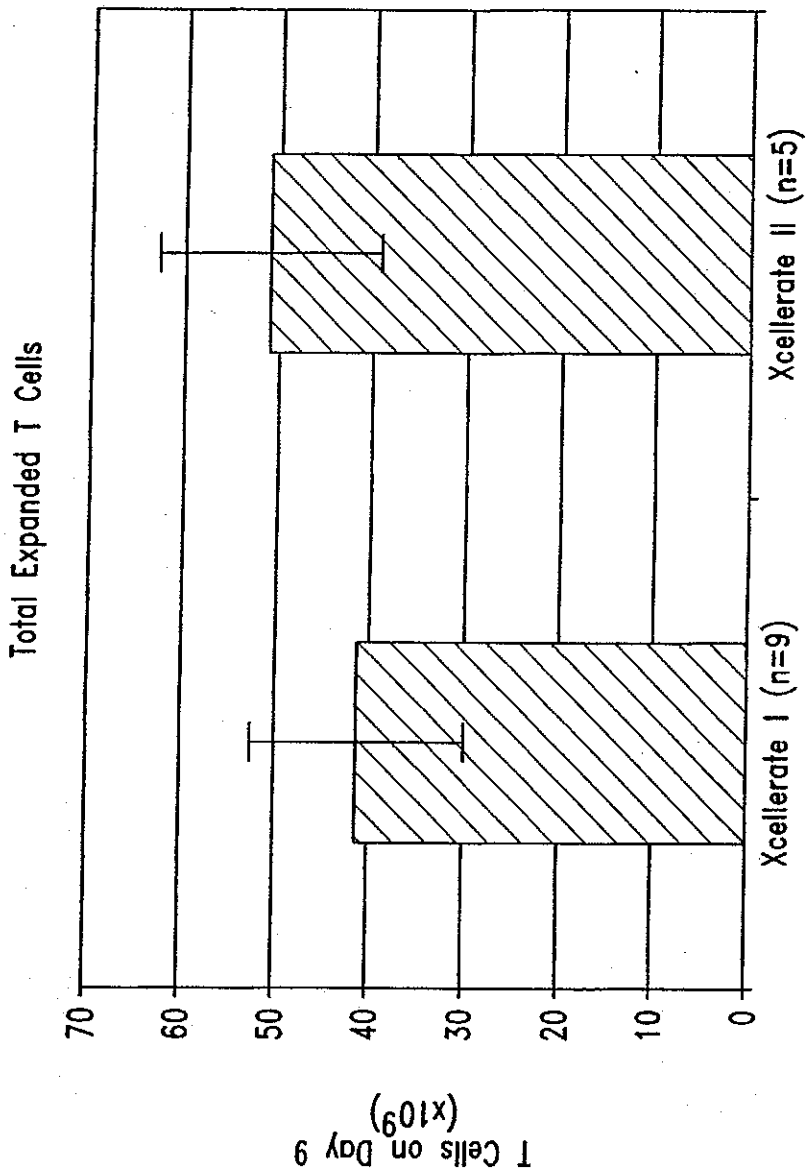

Total Expanded T Cells
T Cells on Day 9 (x10⁹)
70
60
50
40
30
20
10
0
Xcellerate I (n=9)
Xcellerate II (n=5)

Fig. 1

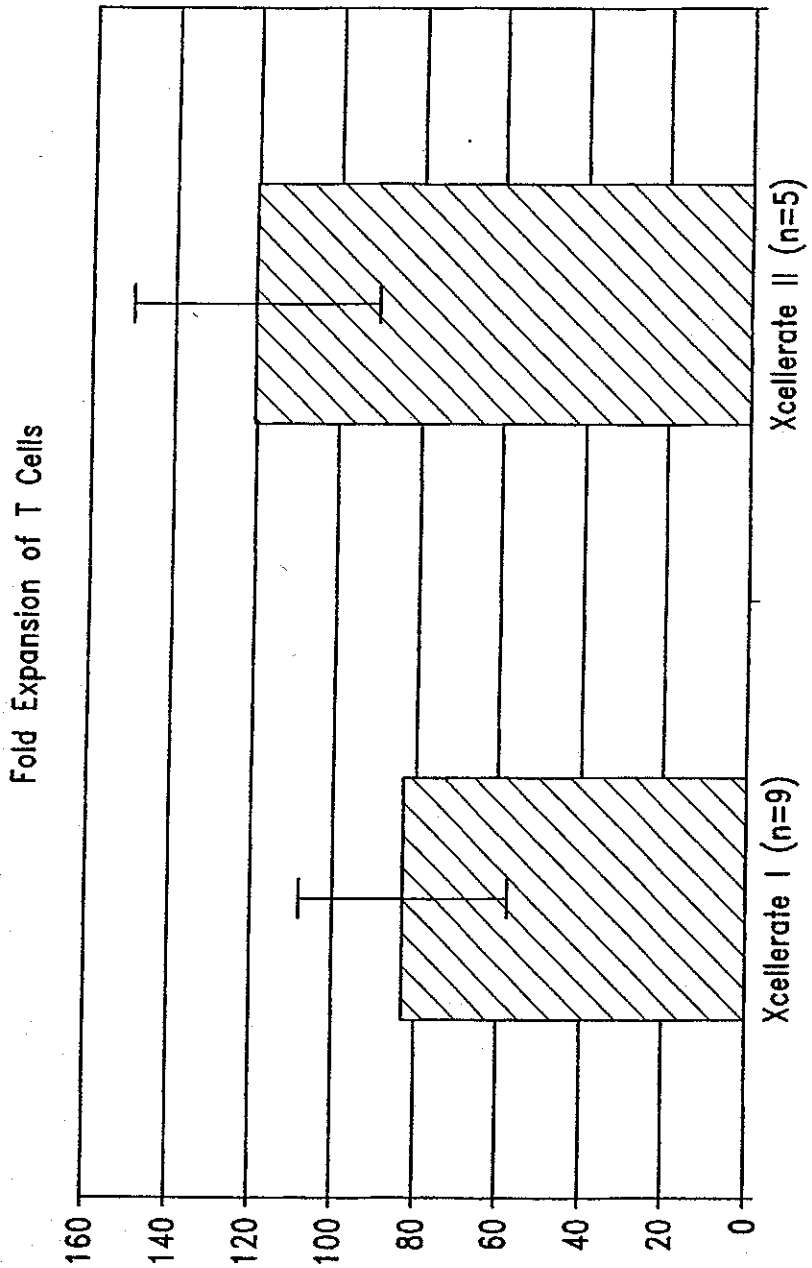

Fold Expansion of T Cells
160
140
120
100
80
60
40
20
0
Xcellerate I (n=9)
Xcellerate II (n=5)

Fig. 2

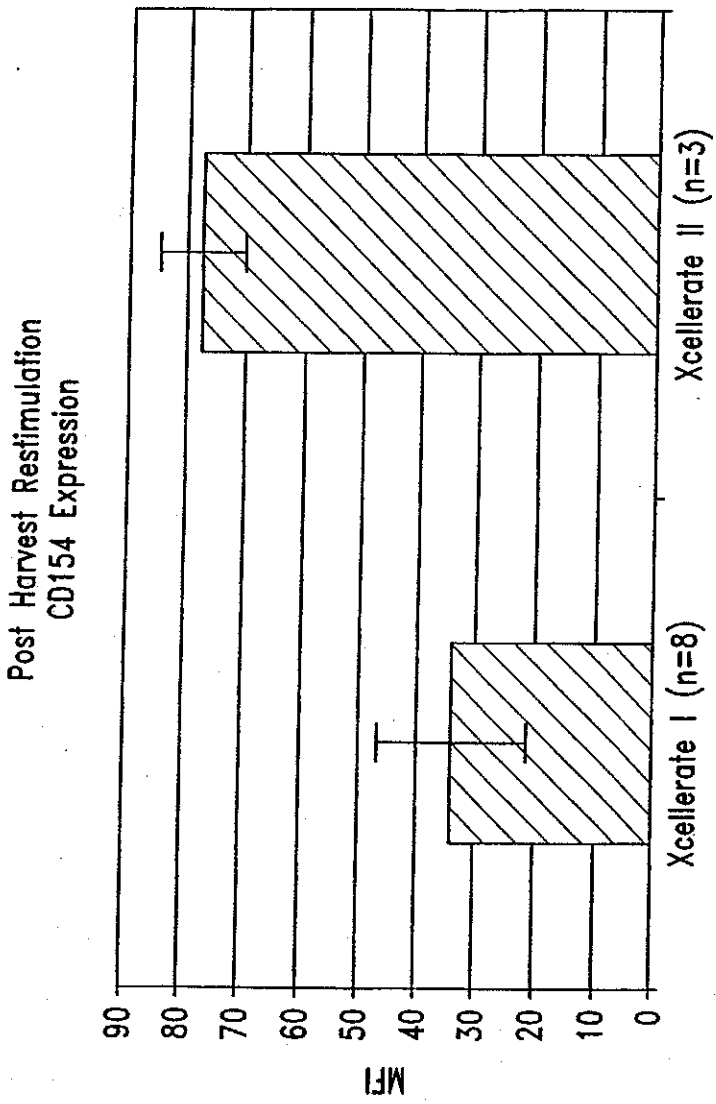

Post Harvest Restimulation
CD154 Expression
MFI
90
80
70
60
50
40
30
20
10
0
Xcellerate I (n=8)
Xcellerate II (n=3)

Fig. 3

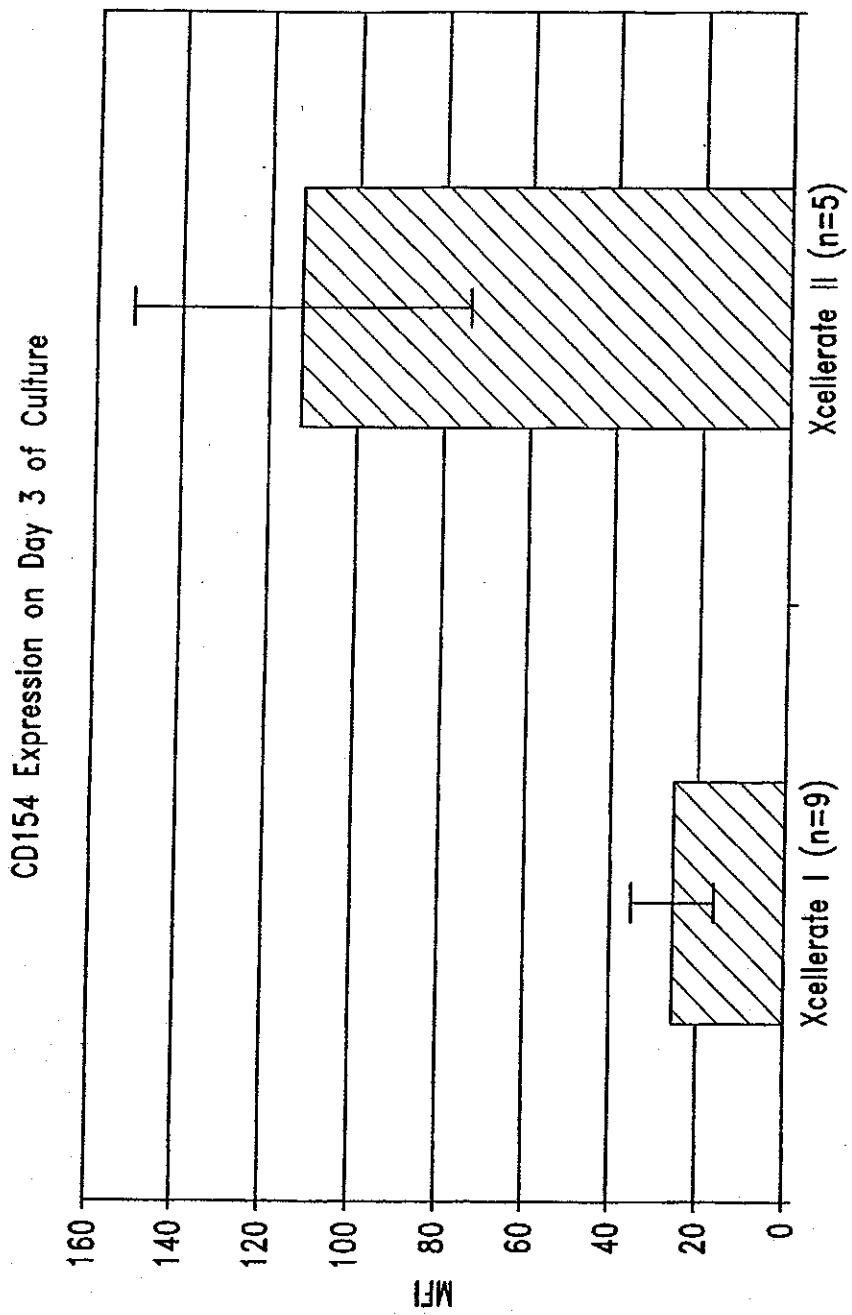

CD154 Expression on Day 3 of Culture
MFI
160
140
120
100
80
60
40
20
0
Xcellerate II (n=5)
Xcellerate I (n=9)

Fig. 4

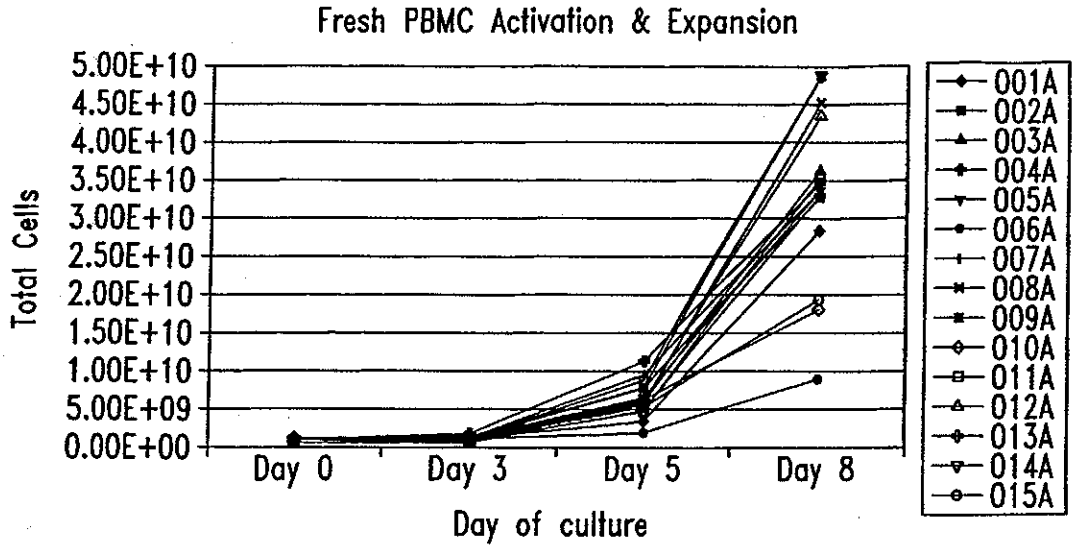

Fresh PBMC Activation & Expansion
Total Cells
5.00E+10
4.50E+10
4.00E+10
3.50E+10
3.00E+10
2.50E+10
2.00E+10
1.50E+10
1.00E+10
5.00E+09
0.00E+00
Day 0
Day 3
Day 5
Day 8
Day of culture
001A
002A
003A
004A
005A
006A
007A
008A
009A
010A
011A
012A
013A
014A
015A

Fig. 5A

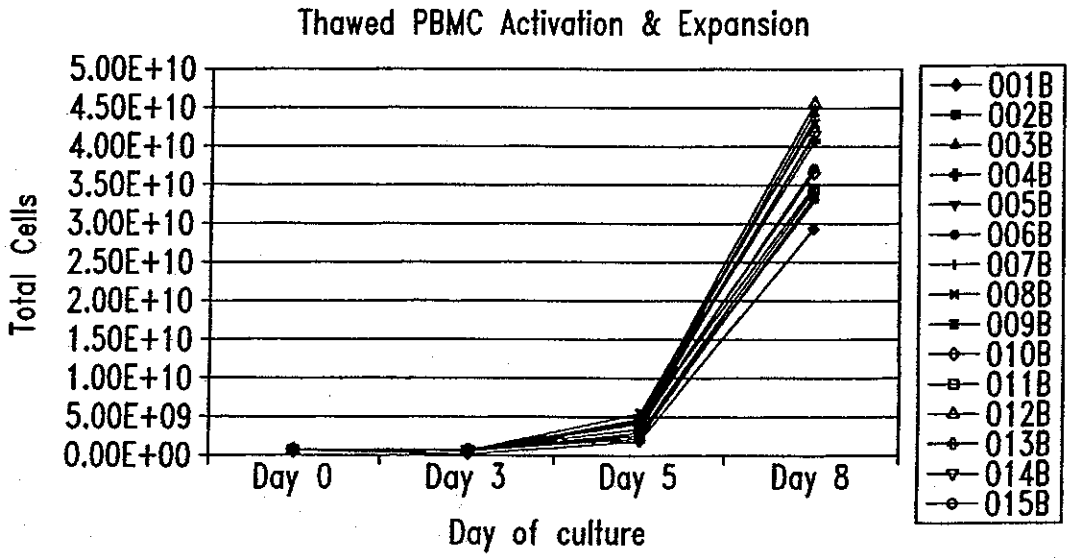

Thawed PBMC Activation & Expansion
Total Cells
5.00E+10
4.50E+10
4.00E+10
3.50E+10
3.00E+10
2.50E+10
2.00E+10
1.50E+10
1.00E+10
5.00E+09
0.00E+00
Day 0
Day 3
Day 5
Day 8
Day of culture
001B
002B
003B
004B
005B
006B
007B
008B
009B
010B
011B
012B
013B
014B
015B

Fig. 5B

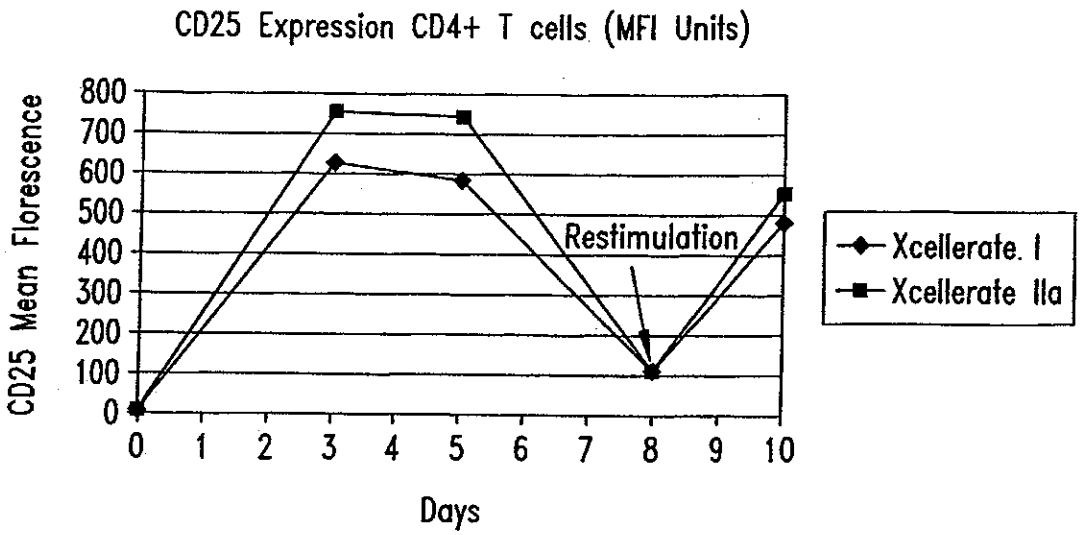

CD25 Expression CD4+ T cells (MFI Units)
CD25 Mean Florescence
800
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10
Days
Restimulation
Xcellerate. I
Xcellerate IIa

Fig. 6A

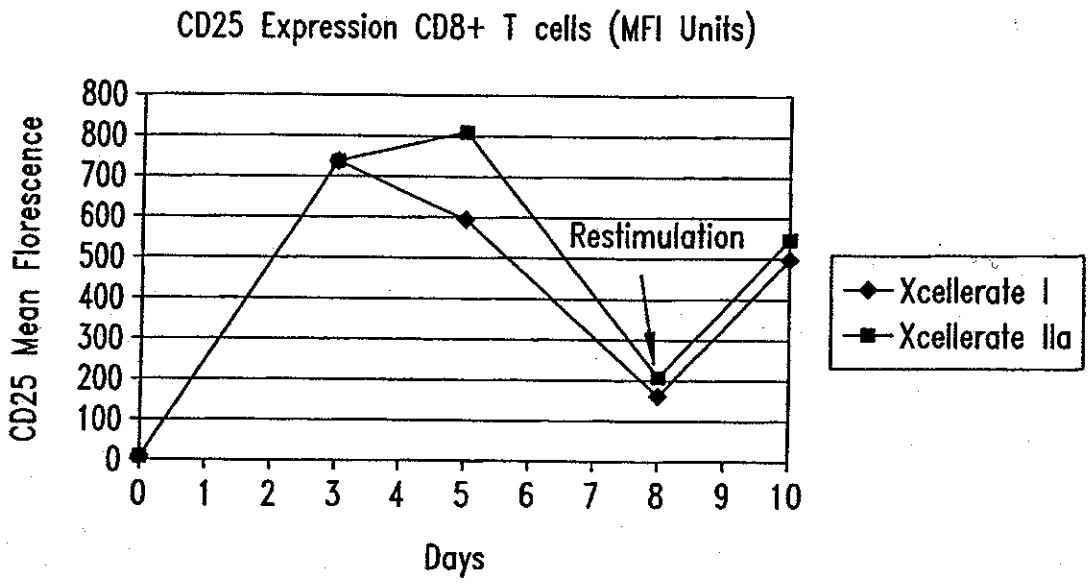

CD25 Expression CD8+ T cells (MFI Units)
CD25 Mean Florescence
800
800
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10
Days
Restimulation
Xcellerate I
Xcellerate IIa

Fig. 6B

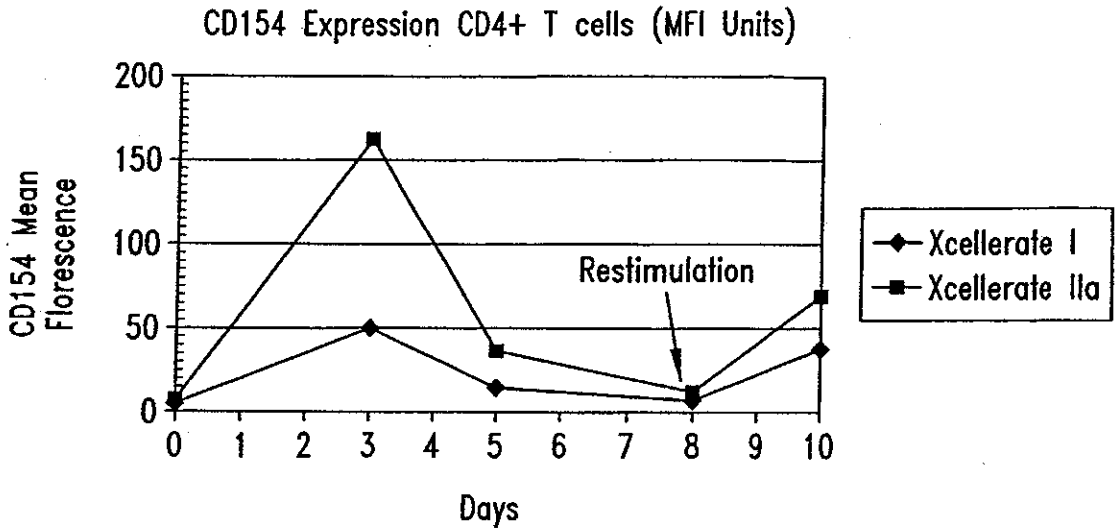

CD154 Expression CD4+ T cells (MFI Units)
CD154 Mean Florescence
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10
Days
Restimulation
Xcellerate I
Xcellerate IIa

Fig. 7A

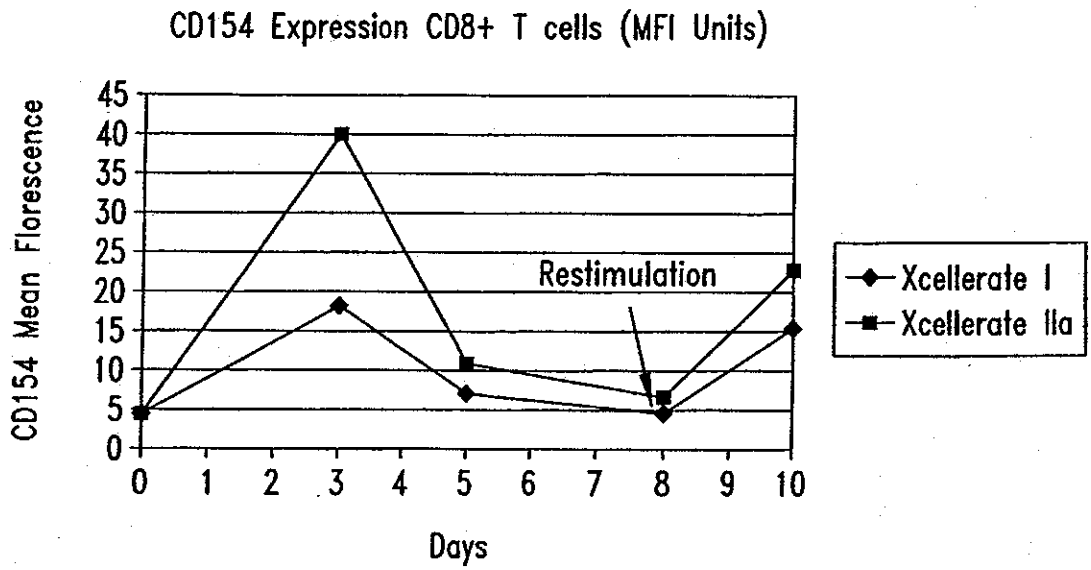

CD154 Expression CD8+ T cells (MFI Units)
CD154 Mean Florescence
45
40
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
Days
Restimulation
Xcellerate I
Xcellerate IIa

Fig. 7B

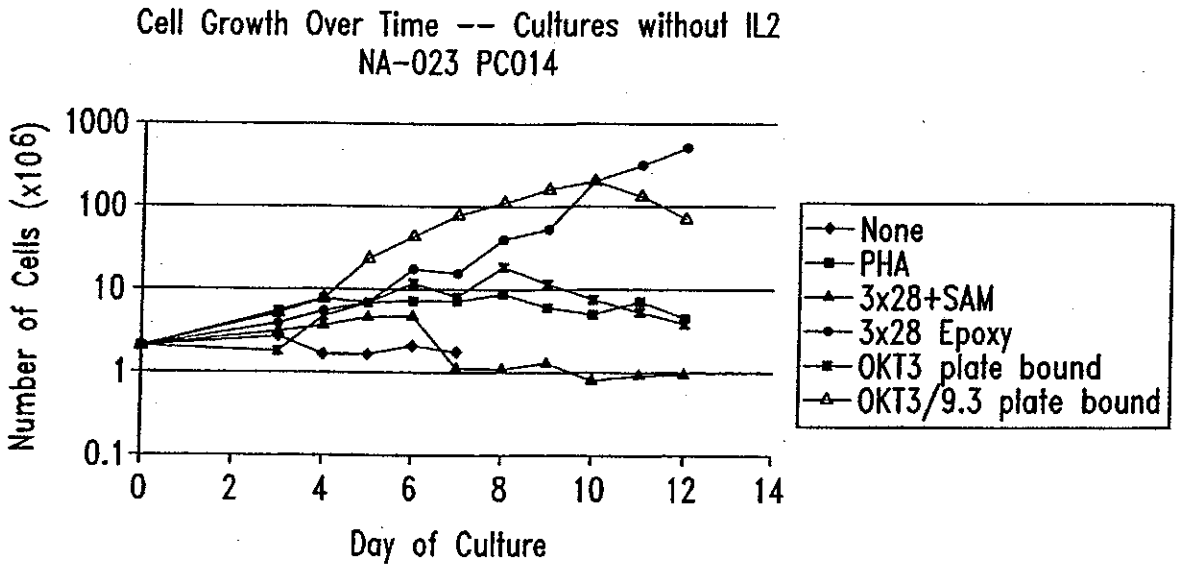

Cell Growth Over Time -- Cultures without IL2
NA-023 PC014
Number of Cells (x106)
1000
100
10
1
0.1
0
2
4
6
8
10
12
14
Day of Culture
None
PHA
3x28+SAM
3x28 Epoxy
OKT3 plate bound
OKT3/9.3 plate bound

Fig. 8A

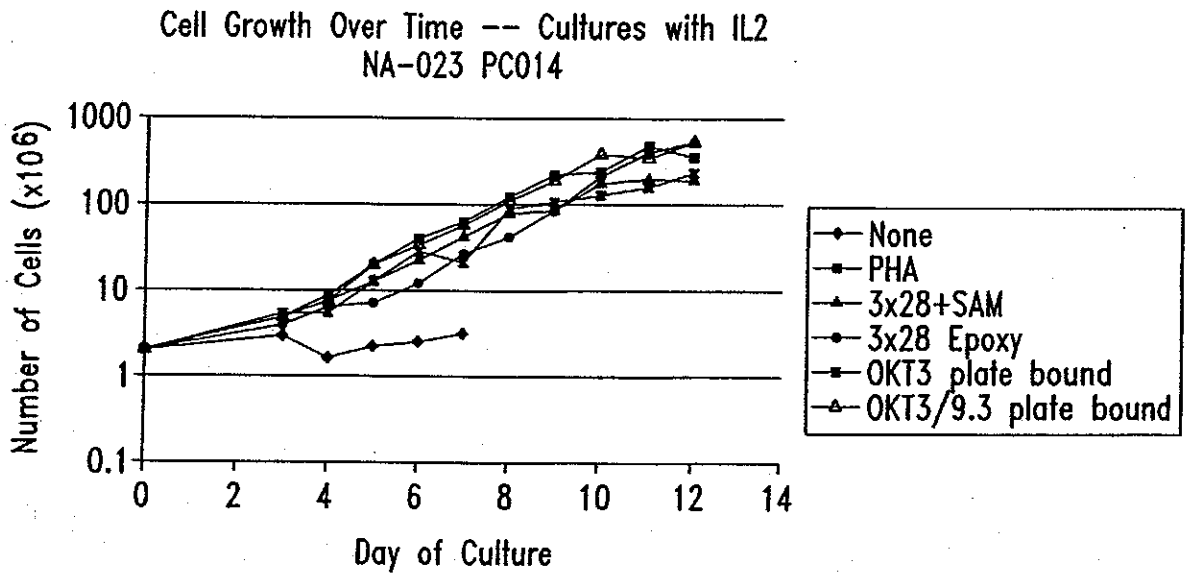

Cell Growth Over Time -- Cultures with IL2
NA-023 PC014
Number of Cells (x106)
1000
100
10
1
0.1
0
2
4
6
8
10
12
14
Day of Culture
None
PHA
3x28+SAM
3x28 Epoxy
OKT3 plate bound
OKT3/9.3 plate bound

Fig. 8B

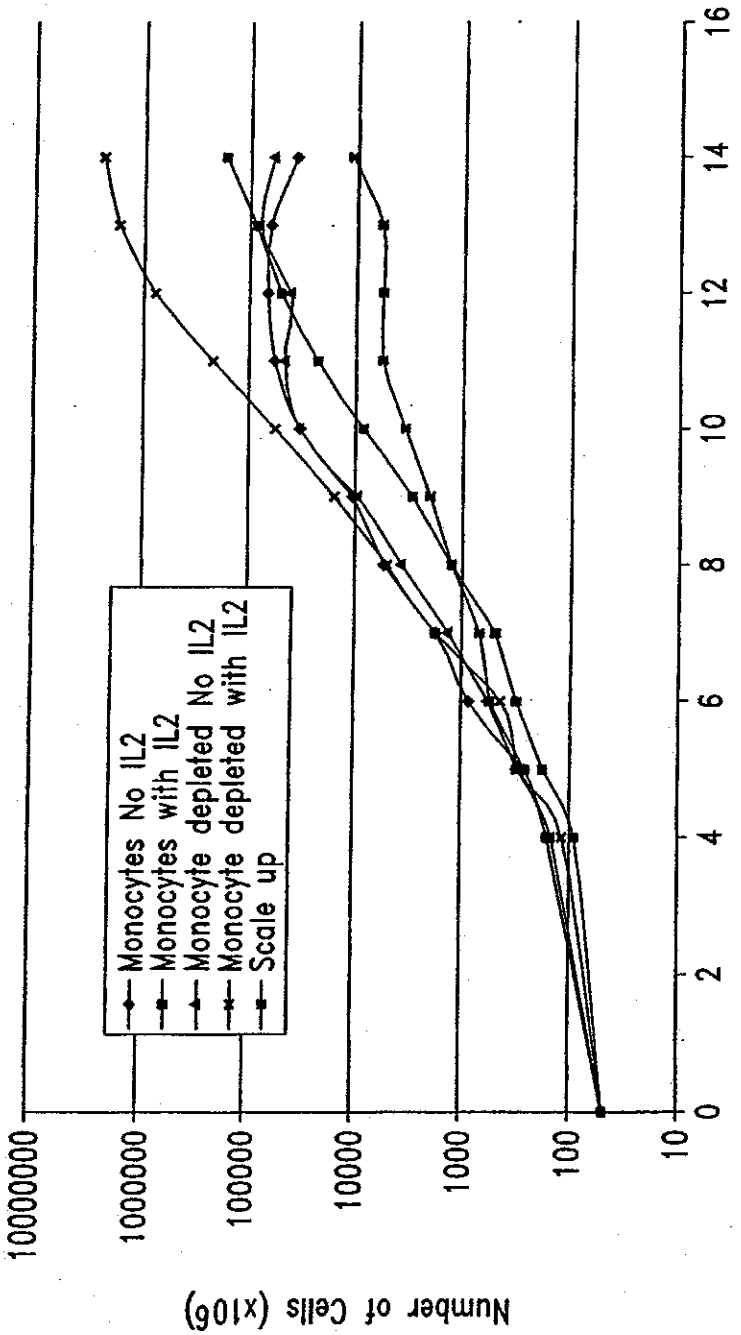

Number of Cells (x10⁶)
10000000
1000000
100000
10000
1000
100
10
0
2
4
6
8
10
12
14
16
Day of Culture
Monocytes No IL2
Monocytes with IL2
Monocyte depleted No IL2
Monocyte depleted with IL2
Scale up

Fig. 9

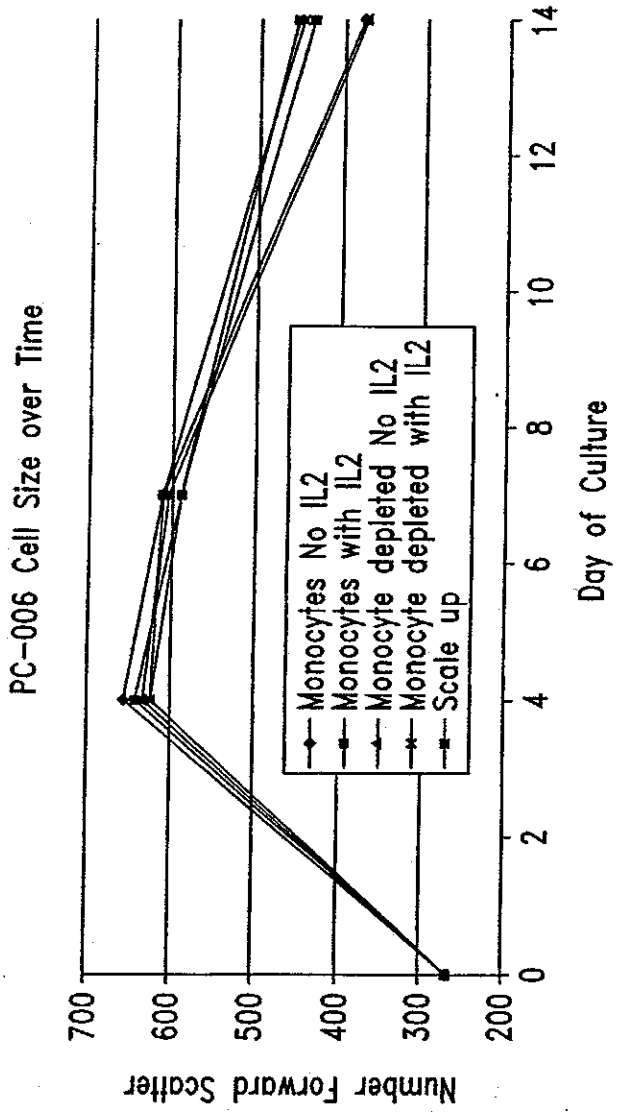

PC-006 Cell Size over Time
Number Forward Scatter
Day of Culture
Monocytes No IL2
Monocytes with IL2
Monocyte depleted No IL2
Monocyte depleted with IL2
Scale up
700
600
500
400
300
200
0
2
4
6
8
10
12
14

Fig. 10

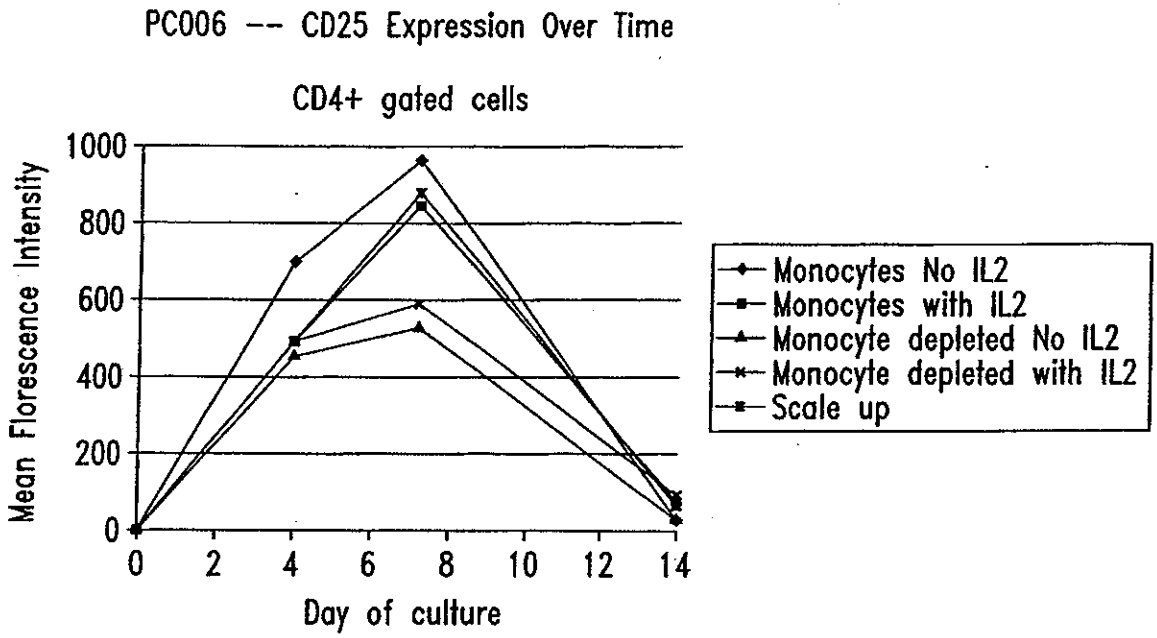


Fig. 11A

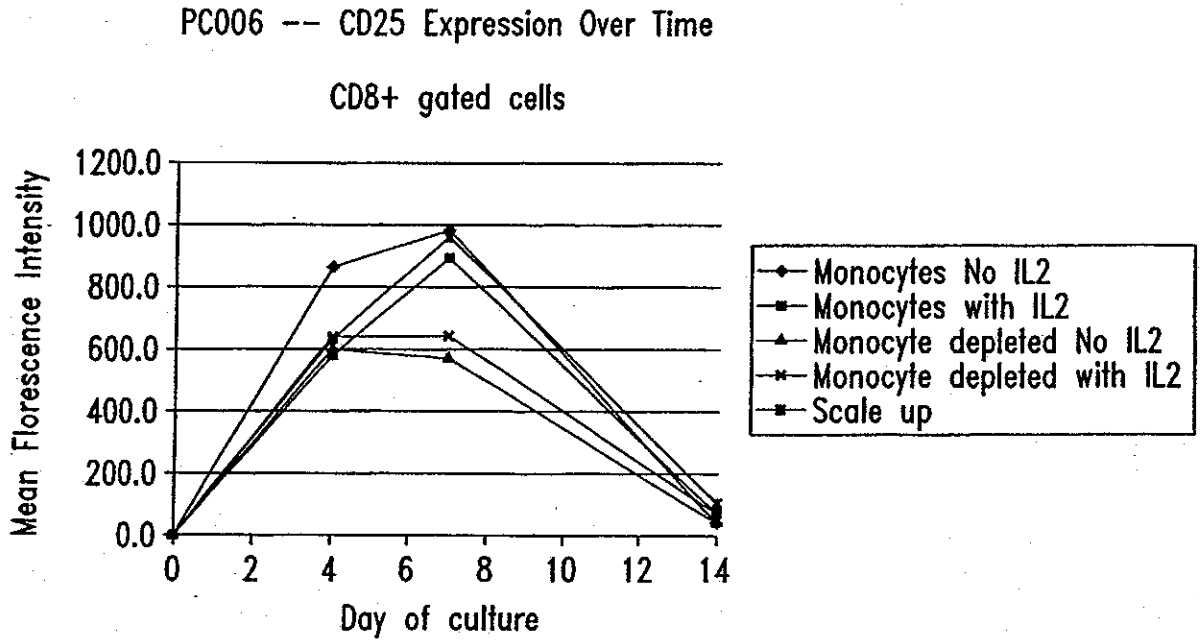


Fig. 11B

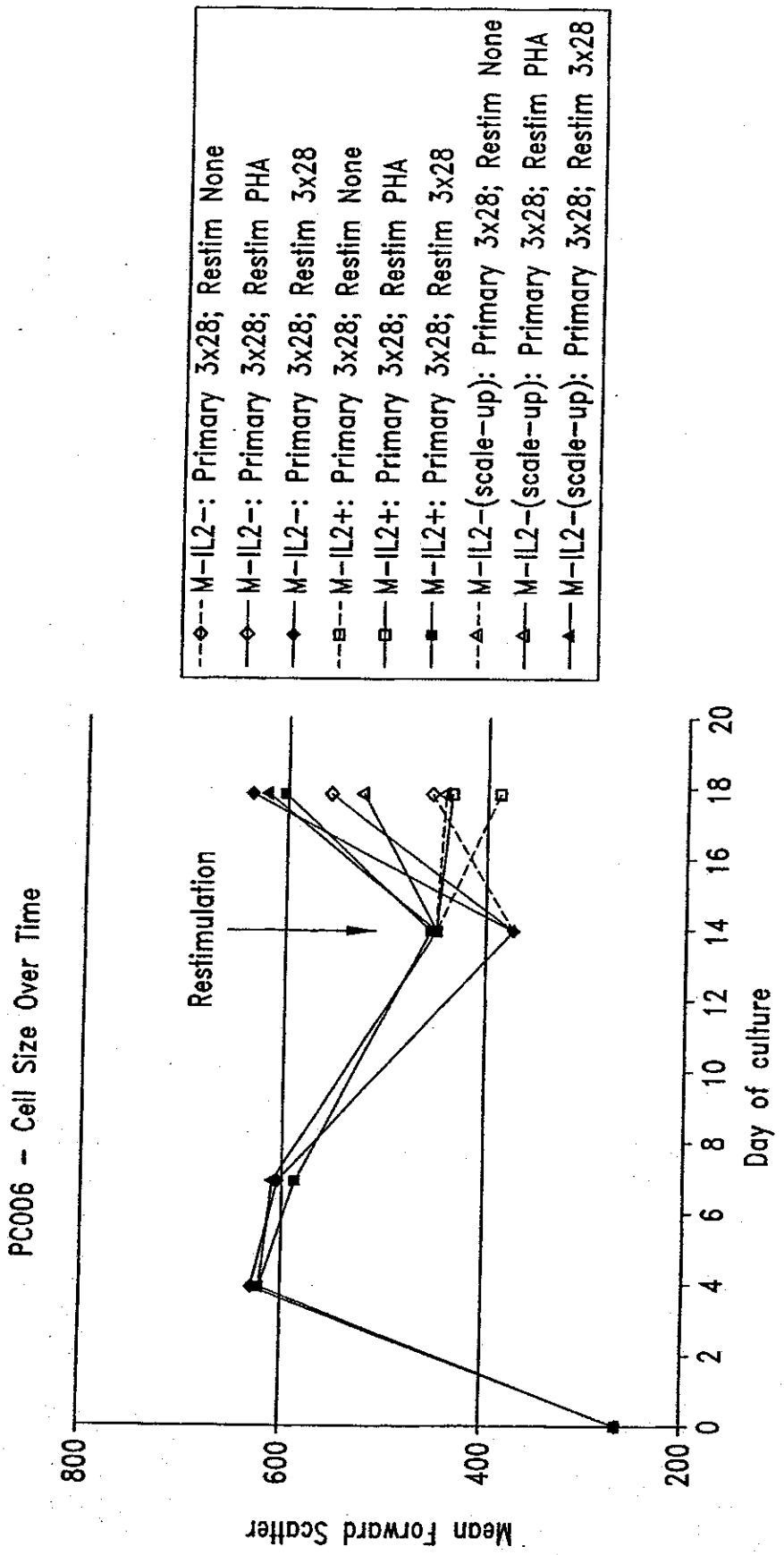

PC006 - Cell Size Over Time
Restimulation
Mean Forward Scatter
Day of culture
800
600
400
200
0
2
4
6
8
10
12
14
16
18
20
M-IL2-: Primary 3x28; Restim None
M-IL2-: Primary 3x28; Restim PHA
M-IL2-: Primary 3x28; Restim 3x28
M-IL2+: Primary 3x28; Restim None
M-IL2+: Primary 3x28; Restim PHA
M-IL2+: Primary 3x28; Restim 3x28
M-IL2-(scale-up): Primary 3x28; Restim None
M-IL2-(scale-up): Primary 3x28; Restim PHA
M-IL2-(scale-up): Primary 3x28; Restim 3x28

Fig. 12

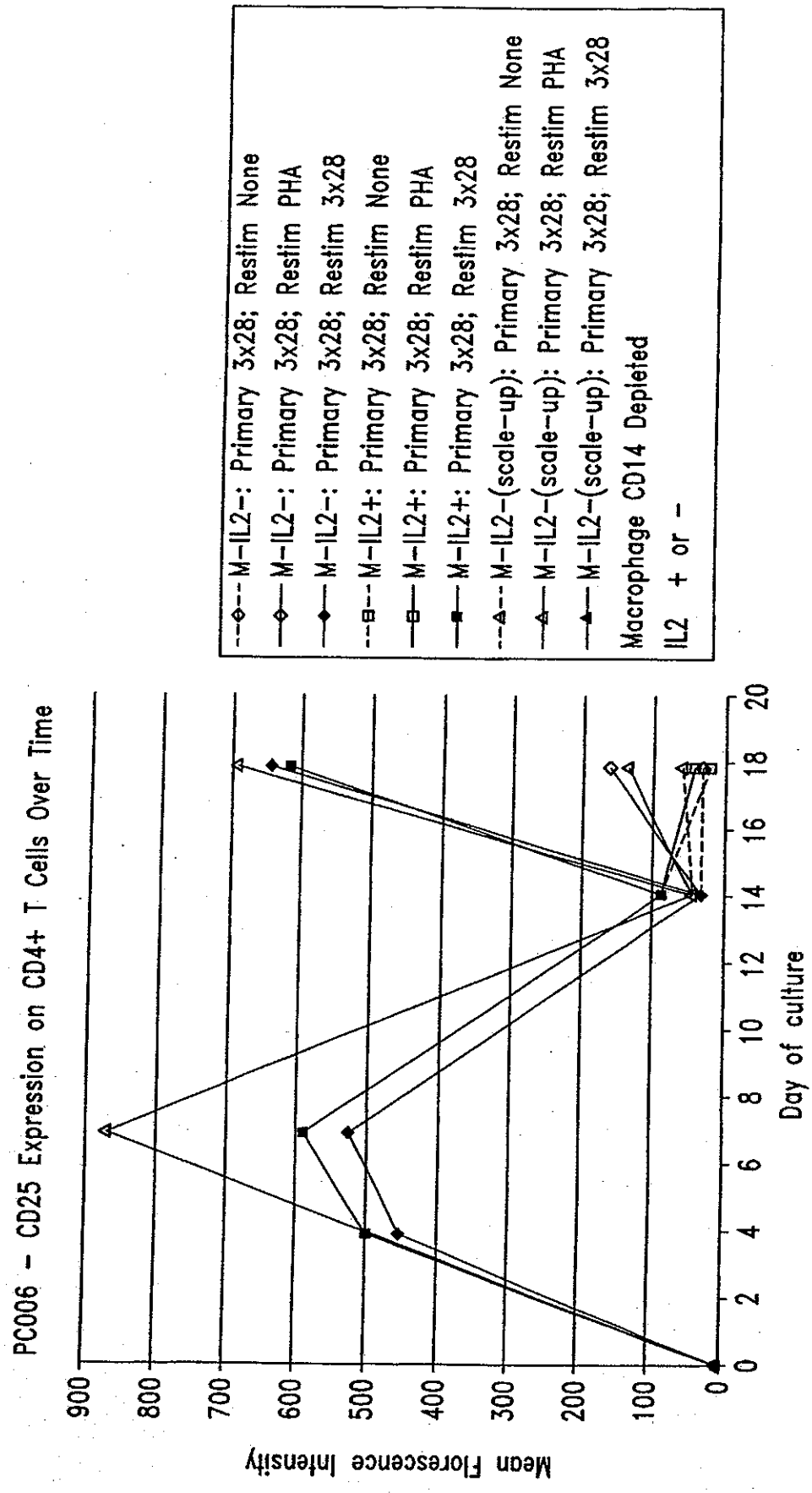

PC006 - CD25 Expression on CD4+ T Cells Over Time
Mean Florescence Intensity
Day of culture
0
100
200
300
400
500
600
700
800
900
0
2
4
6
8
10
12
14
16
18
20
M-IL2-: Primary 3x28; Restim None
M-IL2-: Primary 3x28; Restim PHA
M-IL2-: Primary 3x28; Restim 3x28
M-IL2+: Primary 3x28; Restim None
M-IL2+: Primary 3x28; Restim PHA
M-IL2+: Primary 3x28; Restim 3x28
M-IL2-(scale-up): Primary 3x28; Restim None
M-IL2-(scale-up): Primary 3x28; Restim PHA
M-IL2-(scale-up): Primary 3x28; Restim 3x28
Macrophage CD14 Depleted
IL2 + or -

Fig. 13A

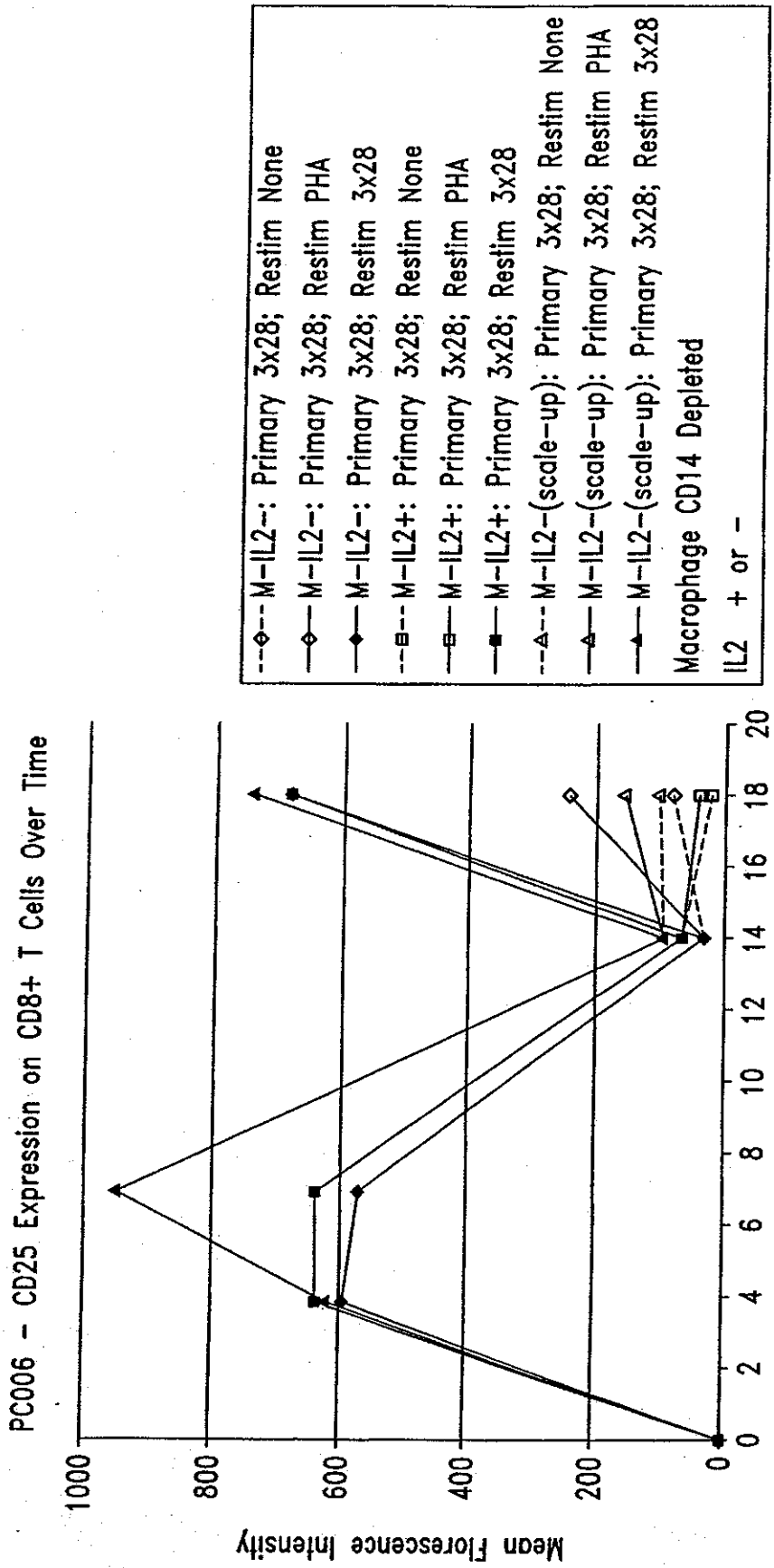

PC006 - CD25 Expression on CD8+ T Cells Over Time
Mean Florescence Intensity
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
18
20
Day of culture
M-IL2-: Primary 3x28; Restim None
M-IL2-: Primary 3x28; Restim PHA
M-IL2-: Primary 3x28; Restim 3x28
M-IL2+: Primary 3x28; Restim None
M-IL2+: Primary 3x28; Restim PHA
M-IL2+: Primary 3x28; Restim 3x28
M-IL2-(scale-up): Primary 3x28; Restim None
M-IL2-(scale-up): Primary 3x28; Restim PHA
M-IL2-(scale-up): Primary 3x28; Restim 3x28
Macrophage CD14 Depleted
IL2 + or -

Fig. 13B

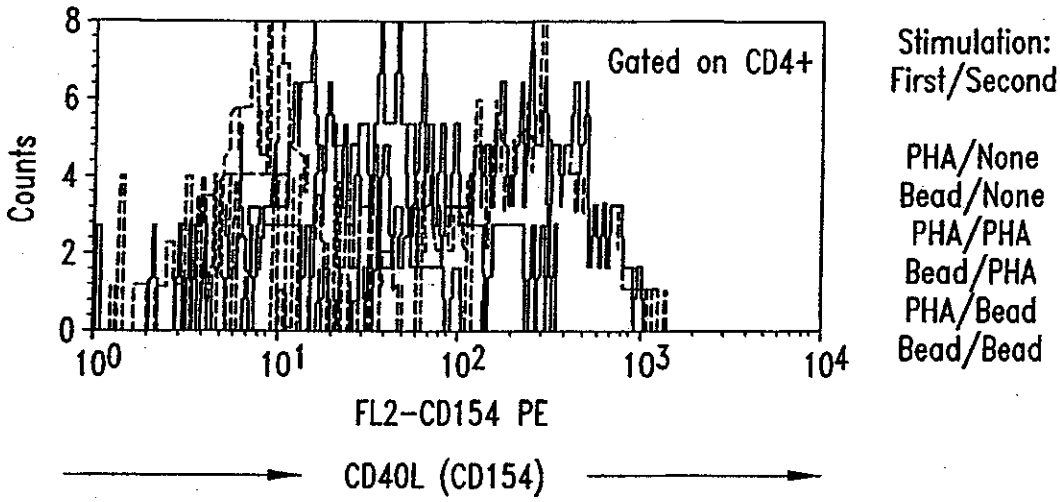


Fig. 14A

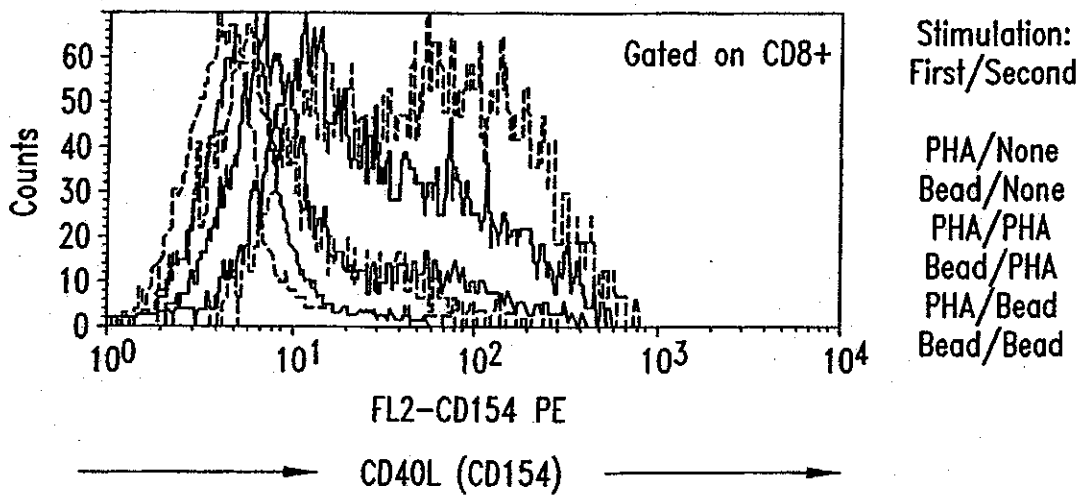


Fig. 14B

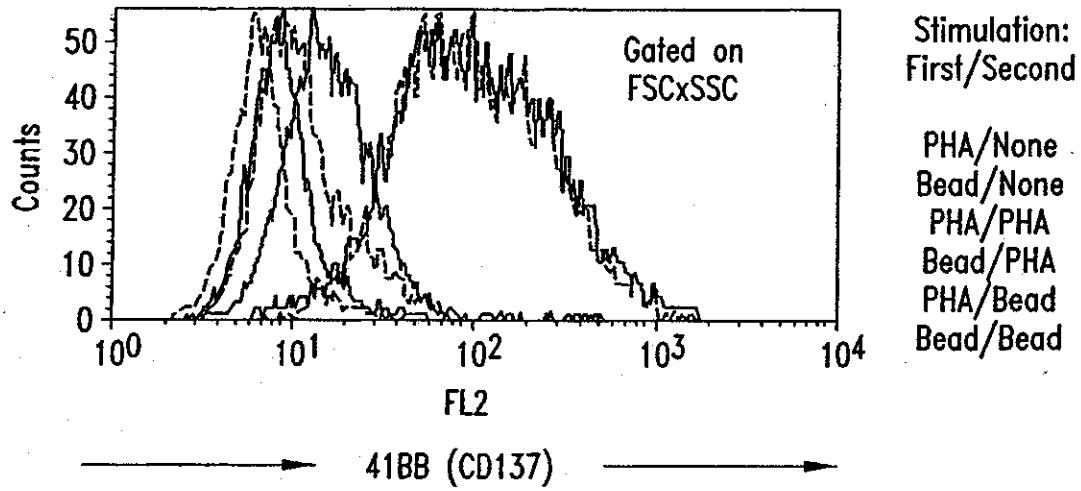

Counts
50
40
30
20
10
0
10^0
10^1
10^2
10^3
10^4
FL2
Gated on FSCxSSC
41BB (CD137)
Stimulation: First/Second
PHA/None
Bead/None
PHA/PHA
Bead/PHA
PHA/Bead
Bead/Bead

Fig. 15

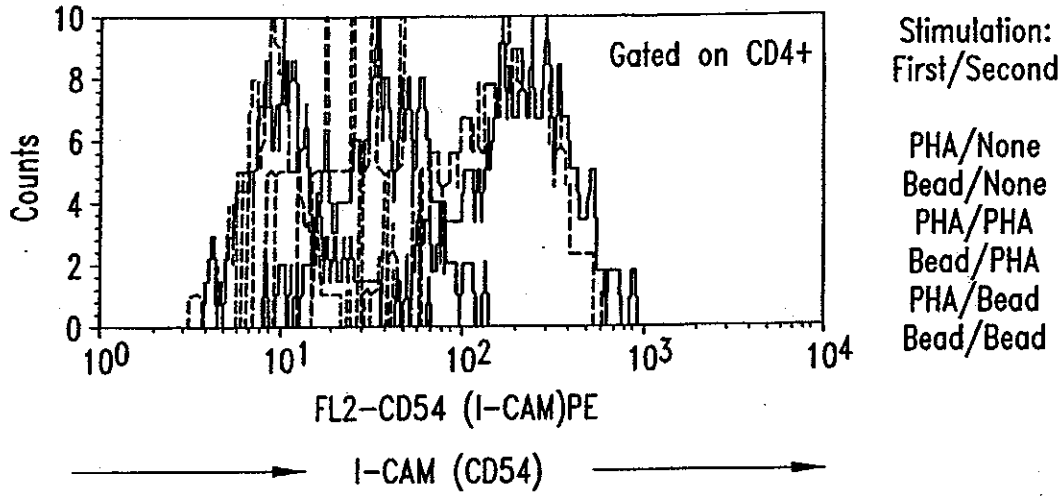


Fig. 16A

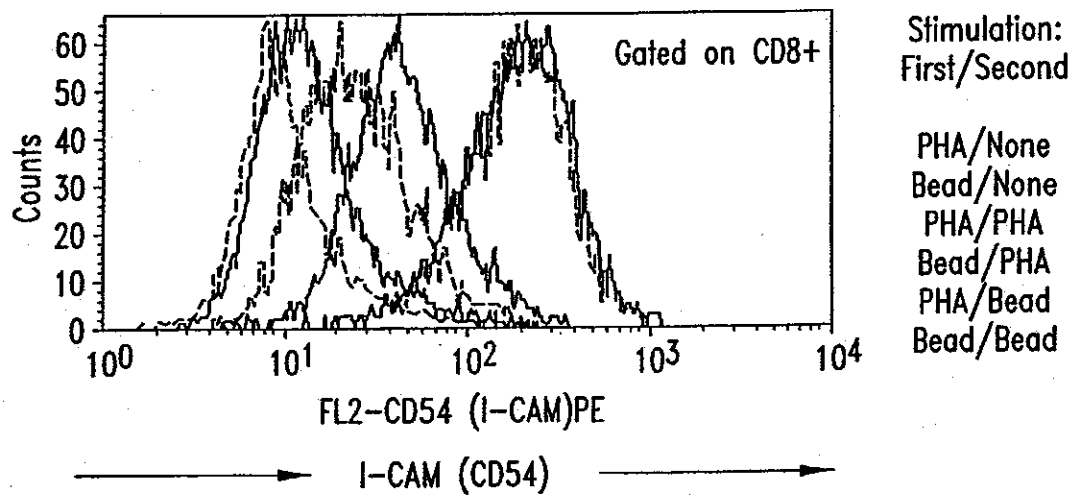


Fig. 16B

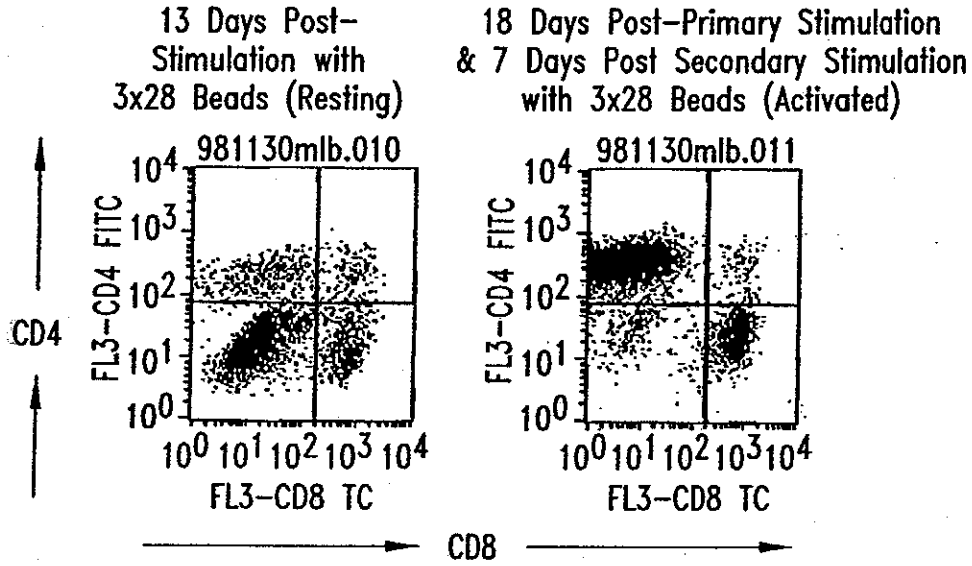


Fig. 17A *Fig. 17B*

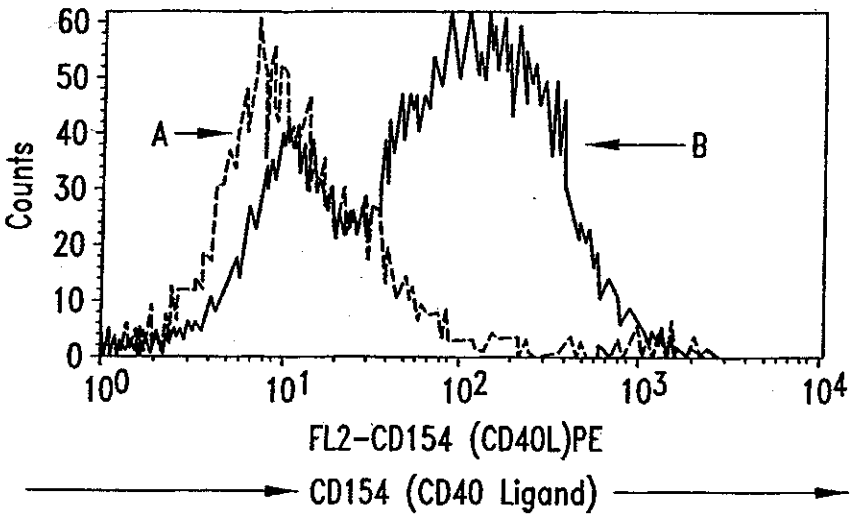


Fig. 17C

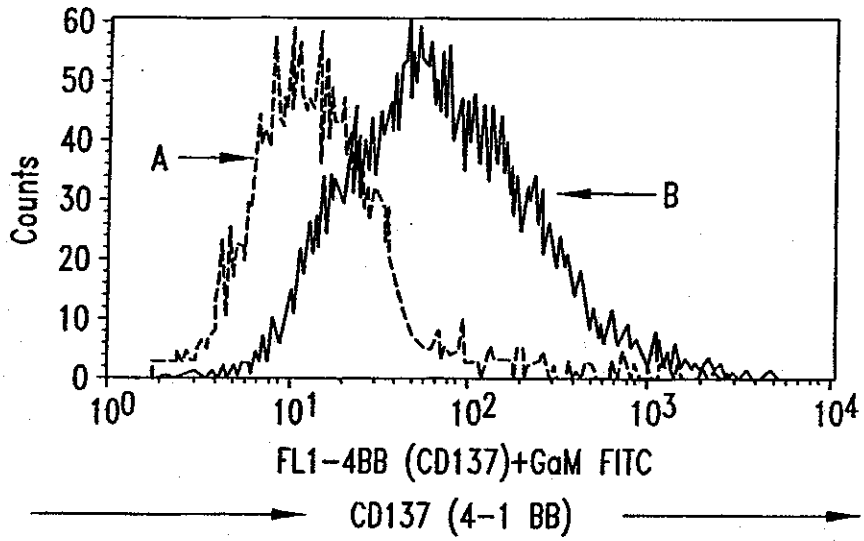


Fig. 17D

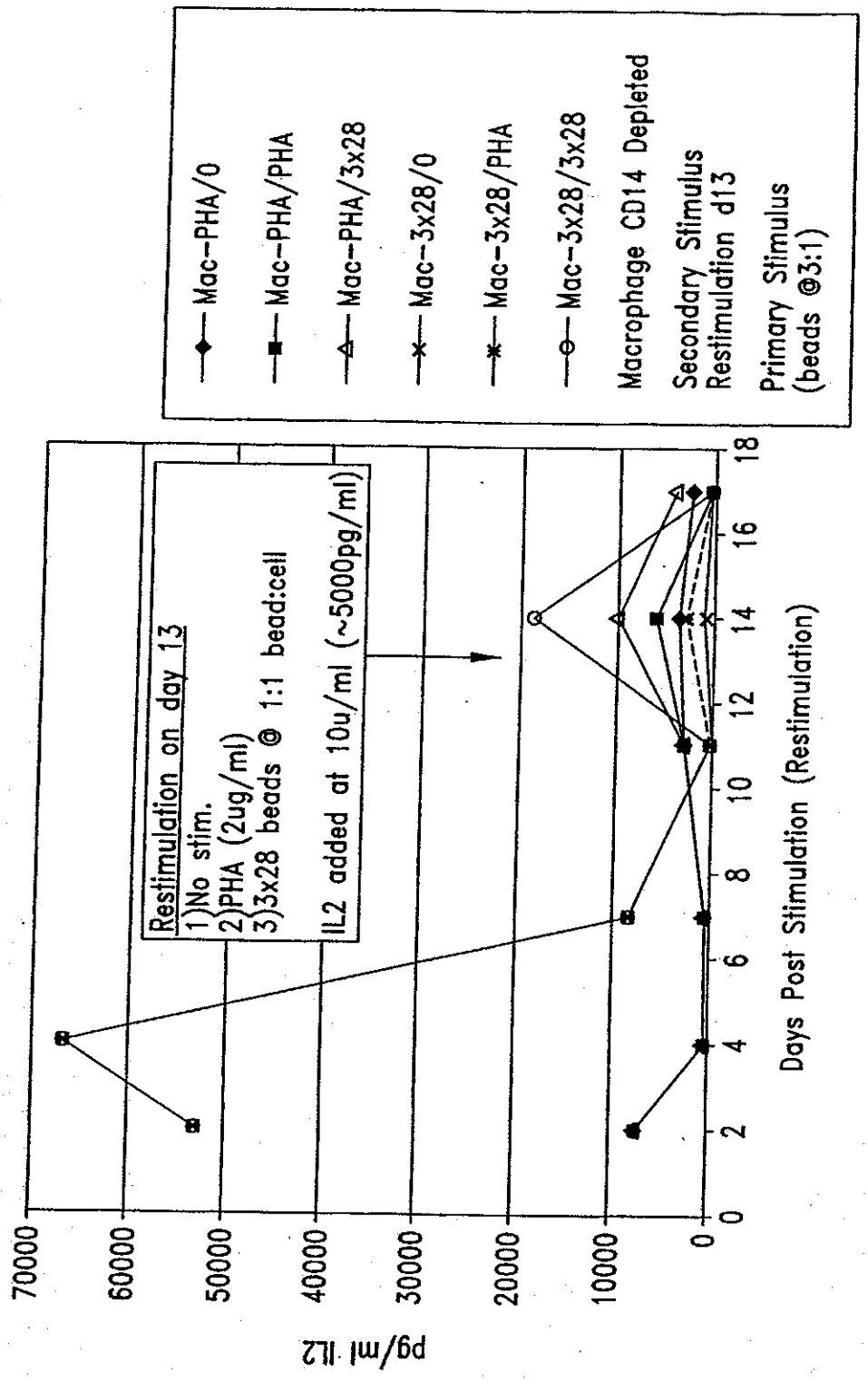

Mac-PHA/0
Mac-PHA/PHA
Mac-PHA/3x28
Mac-3x28/0
Mac-3x28/PHA
Mac-3x28/3x28
Macrophage CD14 Depleted
Secondary Stimulus
Restimulation d13
Primary Stimulus
(beads @3:1)
Restimulation on day 13
1)No stim.
2)PHA (2ug/ml)
3)3x28 beads @ 1:1 bead:cell
IL2 added at 10u/ml (~5000pg/ml)
pg/ml IL2
70000
60000
50000
40000
30000
20000
10000
0
0
2
4
6
8
10
12
14
16
18
Days Post Stimulation (Restimulation)

Fig. 18A

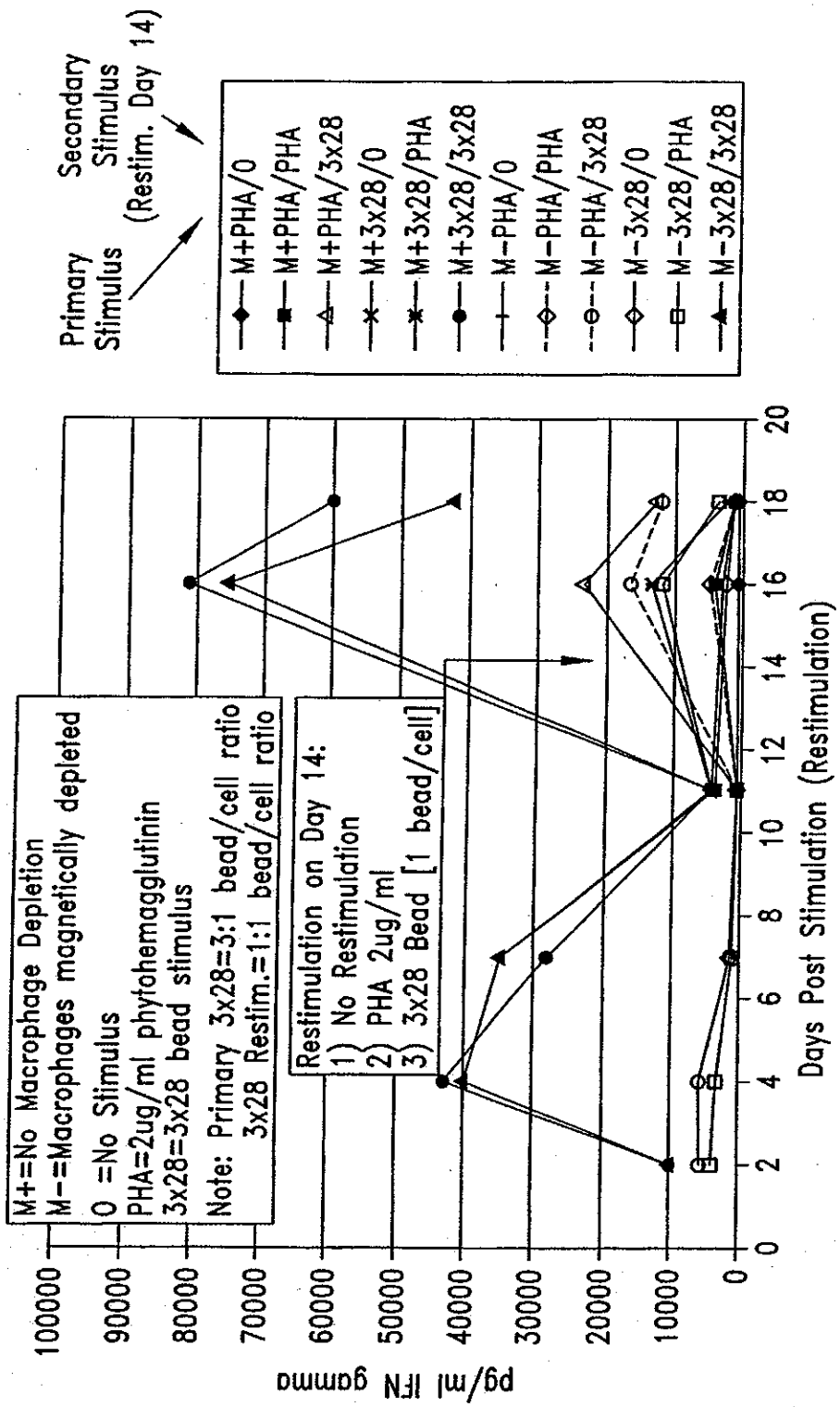

Primary Stimulus
Secondary Stimulus (Restim. Day 14)
M+PHA/0
M+PHA/PHA
M+PHA/3x28
M+3x28/0
M+3x28/PHA
M+3x28/3x28
M-PHA/0
M-PHA/PHA
M-PHA/3x28
M-3x28/0
M-3x28/PHA
M-3x28/3x28
M+=No Macrophage Depletion
M-=Macrophages magnetically depleted
0 =No Stimulus
PHA=2ug/ml phytohemagglutinin
3x28=3x28 bead stimulus
Note: Primary 3x28=3:1 bead/cell ratio
3x28 Restim.=1:1 bead/cell ratio
Restimulation on Day 14:
1) No Restimulation
2) PHA 2ug/ml
3) 3x28 Bead [1 bead/cell]
pg/ml IFN gamma
Days Post Stimulation (Restimulation)

Fig. 18B

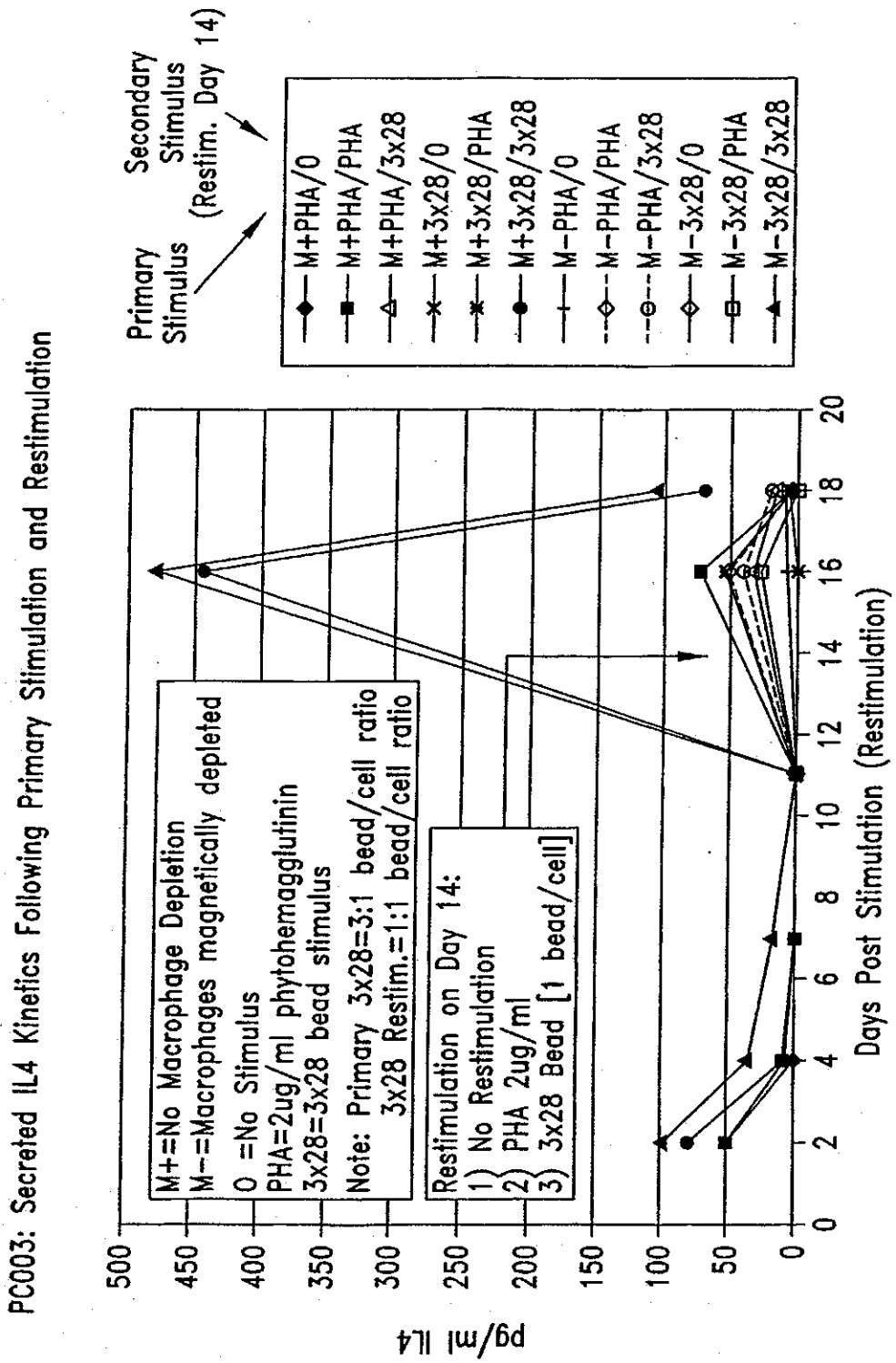

PC003: Secreted IL4 Kinetics Following Primary Stimulation and Restimulation
pg/ml IL4
Days Post Stimulation (Restimulation)
Primary Stimulus
Secondary Stimulus (Restim. Day 14)
M+PHA/0
M+PHA/PHA
M+PHA/3x28
M+3x28/0
M+3x28/PHA
M+3x28/3x28
M-PHA/0
M-PHA/PHA
M-PHA/3x28
M-3x28/0
M-3x28/PHA
M-3x28/3x28
M+=No Macrophage Depletion
M-=Macrophages magnetically depleted
0 =No Stimulus
PHA=2ug/ml phytohemagglutinin
3x28=3x28 bead stimulus
Note: Primary 3x28=3:1 bead/cell ratio
3x28 Restim.=1:1 bead/cell ratio
Restimulation on Day 14:
1) No Restimulation
2) PHA 2ug/ml
3) 3x28 Bead [1 bead/cell]

Fig. 18C

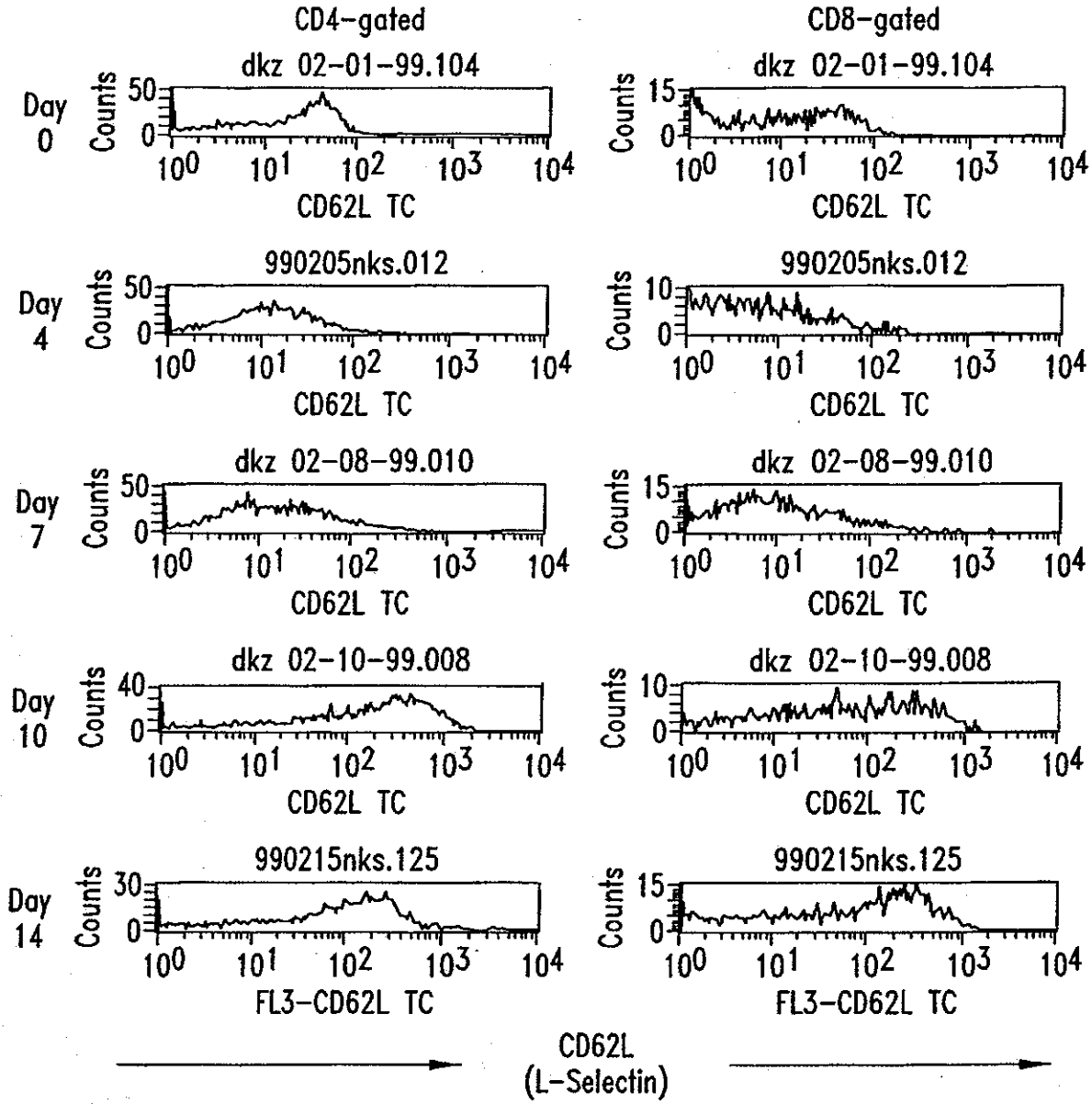

CD4-gated
CD8-gated
dkz 02-01-99.104
990205nks.012
dkz 02-08-99.010
dkz 02-10-99.008
990215nks.125
Day 0
Day 4
Day 7
Day 10
Day 14
Counts
CD62L TC
FL3-CD62L TC
CD62L
(L-Selectin)

Fig. 19A

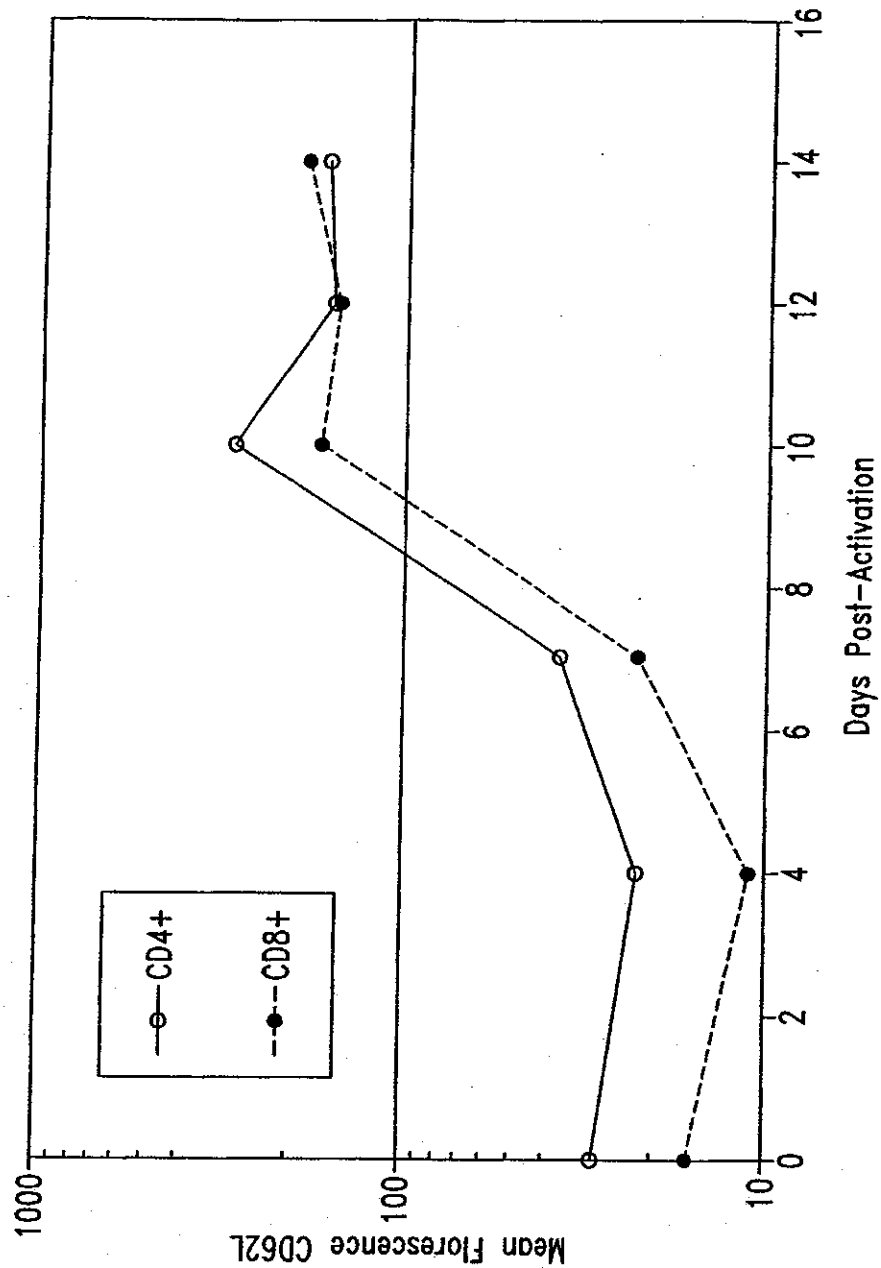

Mean Florescence CD62L
1000
100
10
0
2
4
6
8
10
12
14
16
Days Post-Activation
CD4+
CD8+

Fig. 19B

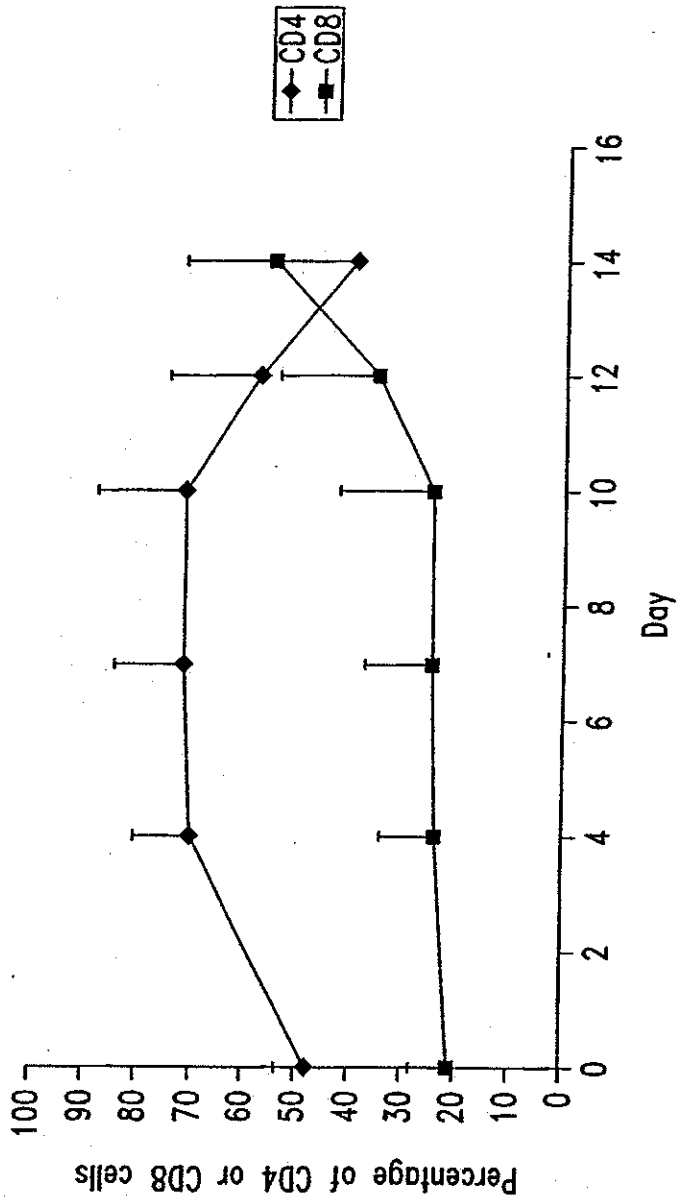

CD4
CD8
Percentage of CD4 or CD8 cells
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
Day

Fig. 20

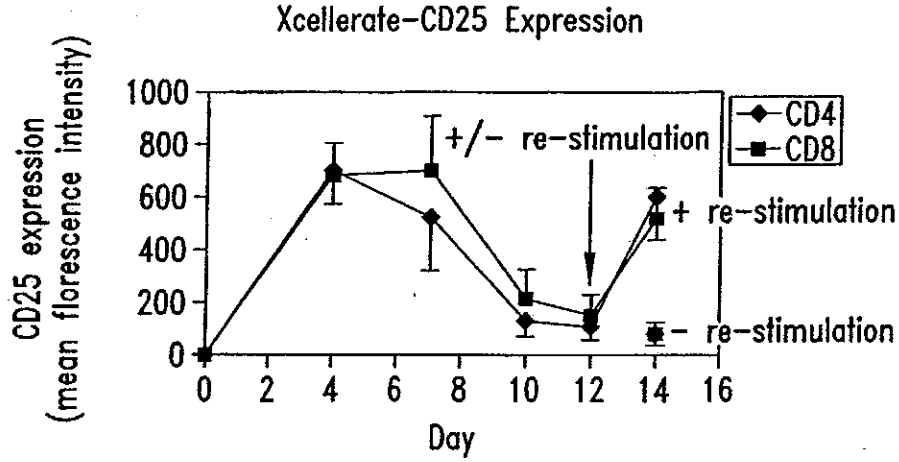


Fig. 21A

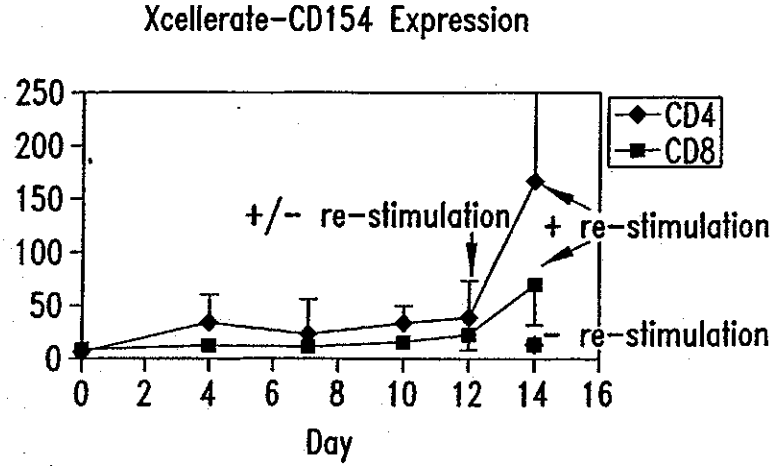


Fig. 21B

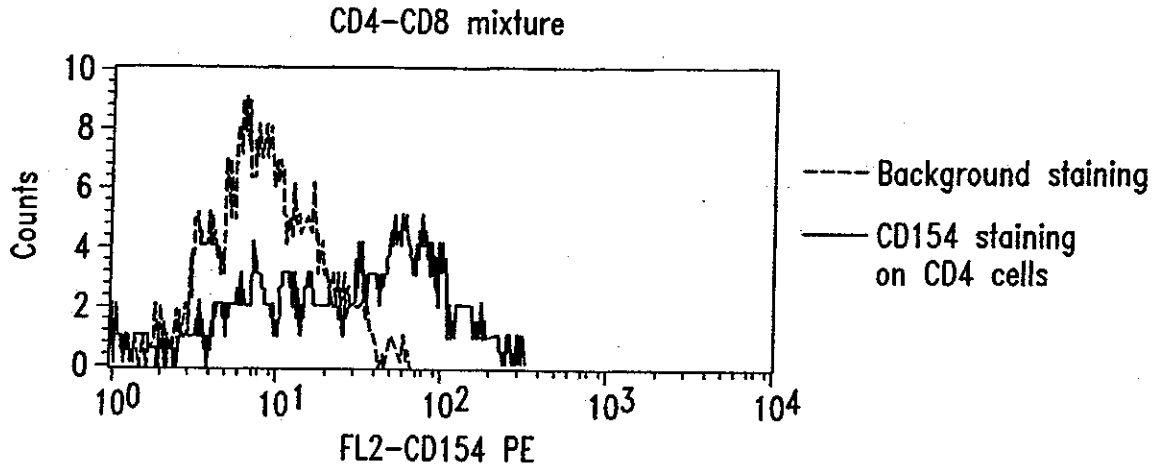


Fig. 22A

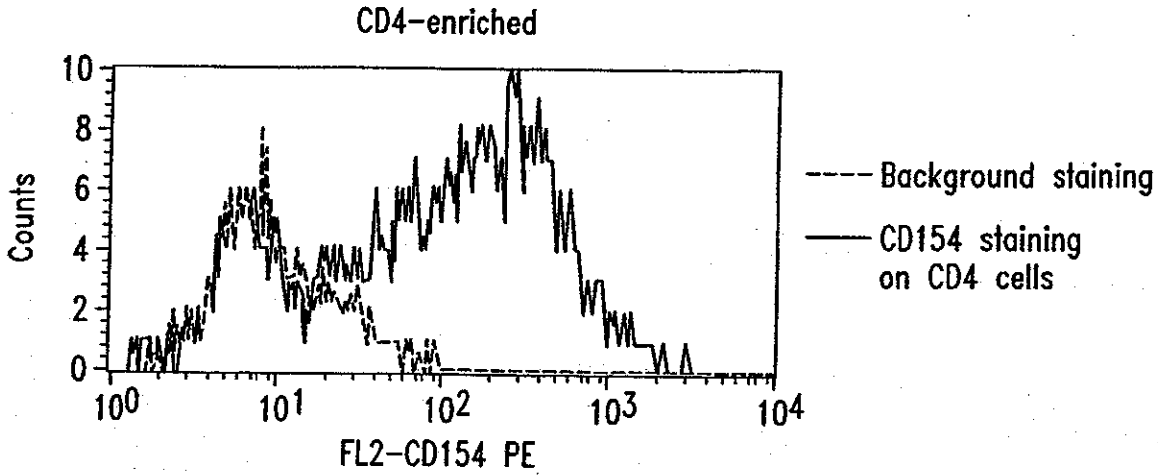


Fig. 22B

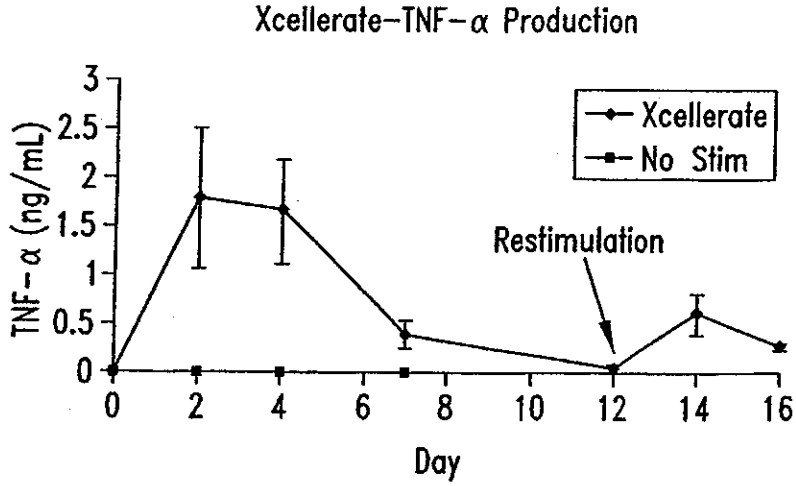


Fig. 23A

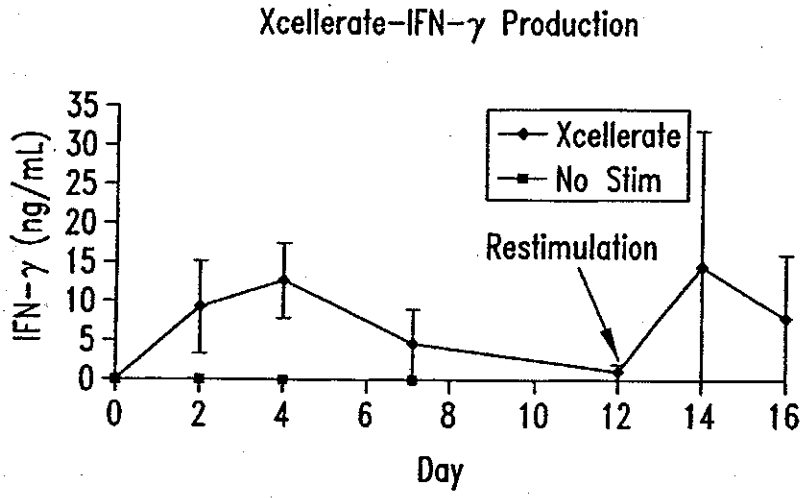


Fig. 23B

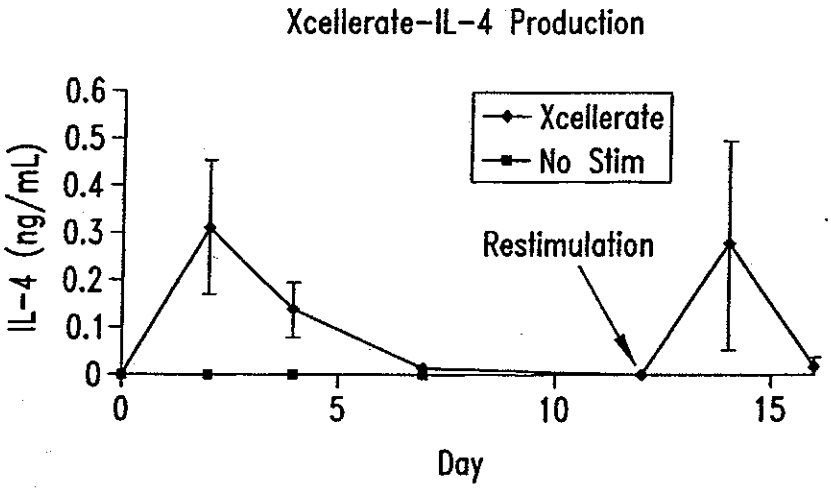


Fig. 24A

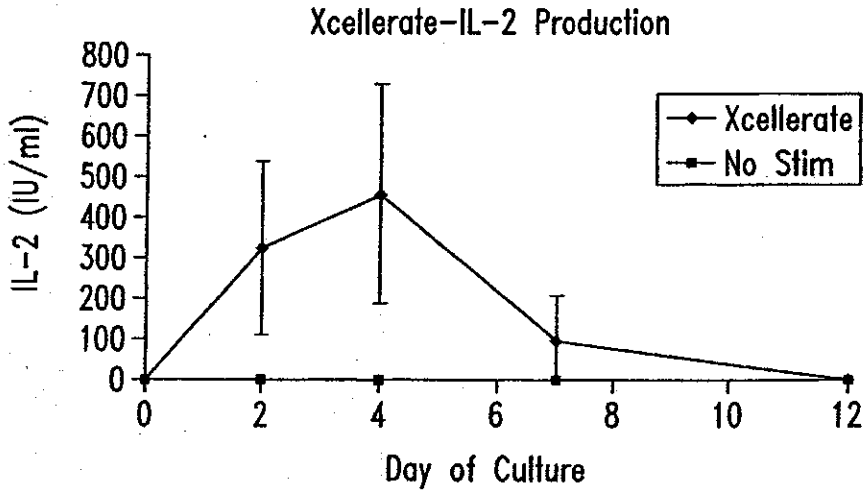


Fig. 24B

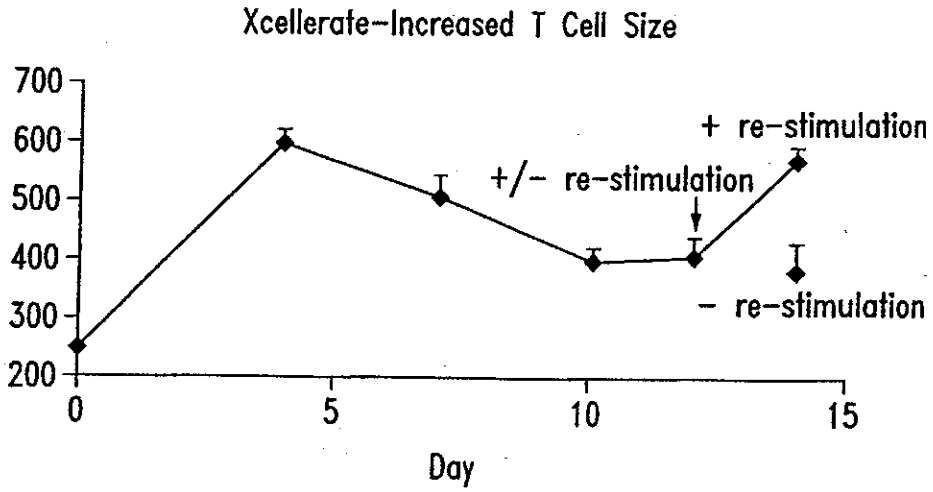
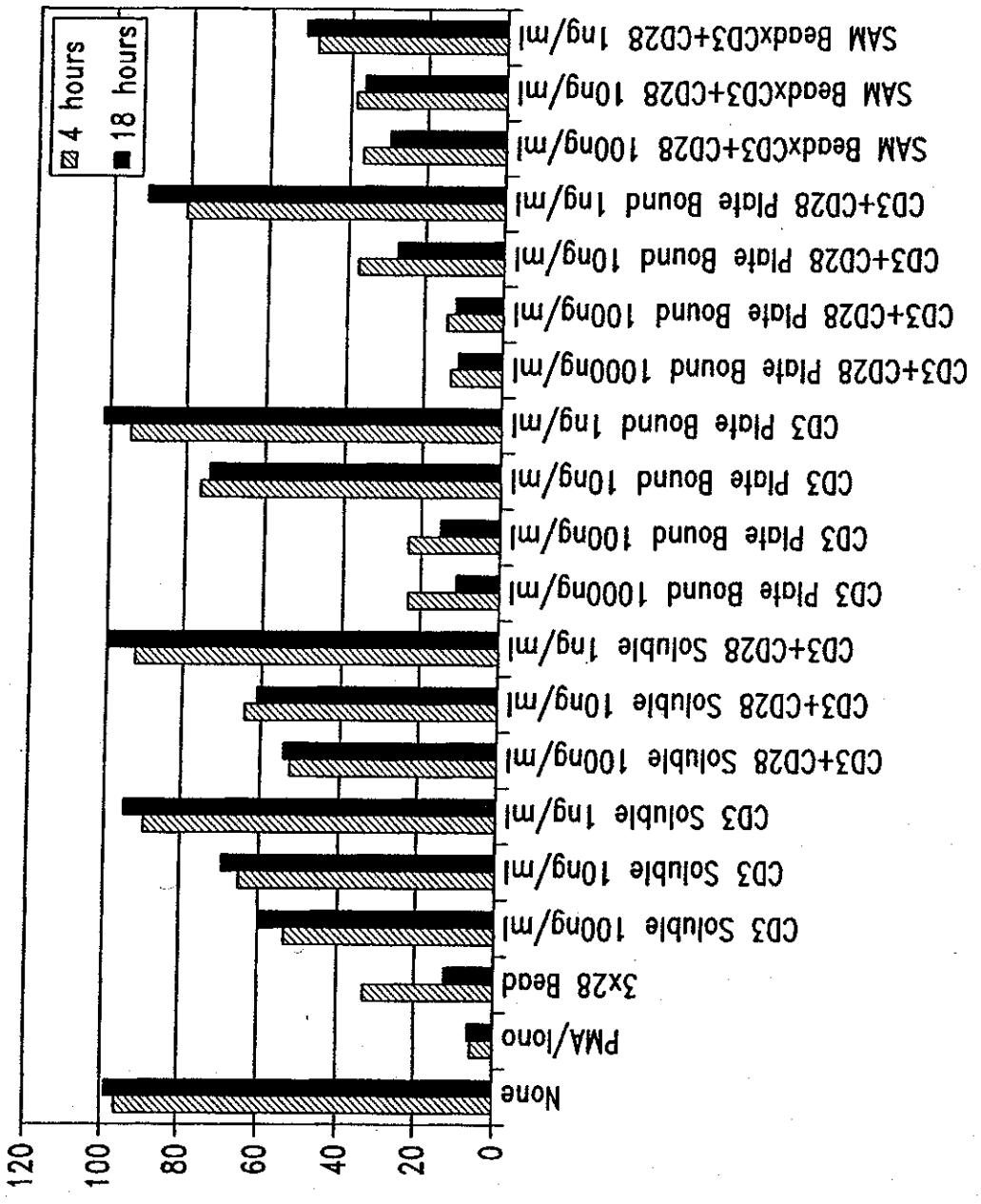
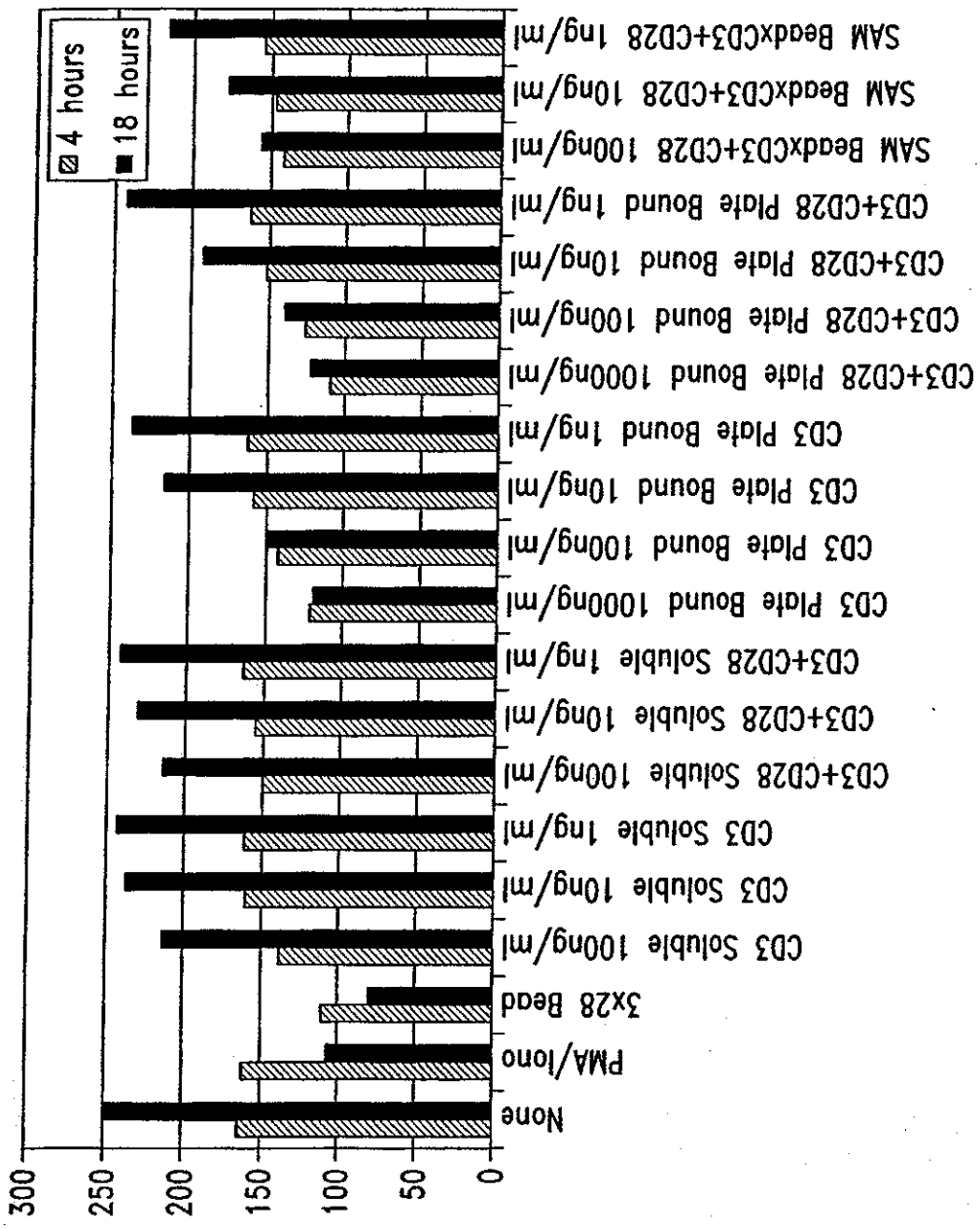

Xcellerate-Increased T Cell Size
700
600
500
400
300
200
0
5
10
15
Day
+ re-stimulation
+/- re-stimulation
- re-stimulation

Fig. 25



Restimulation of NDa055 (Day8):
CD62L (L-Selectin) Expression on CD4+ T Cells

Fig. 26A



Restimulation of NDa055 (Day8):
CD49d (VLA4) Expression on CD4+ T Cells

Fig. 26B

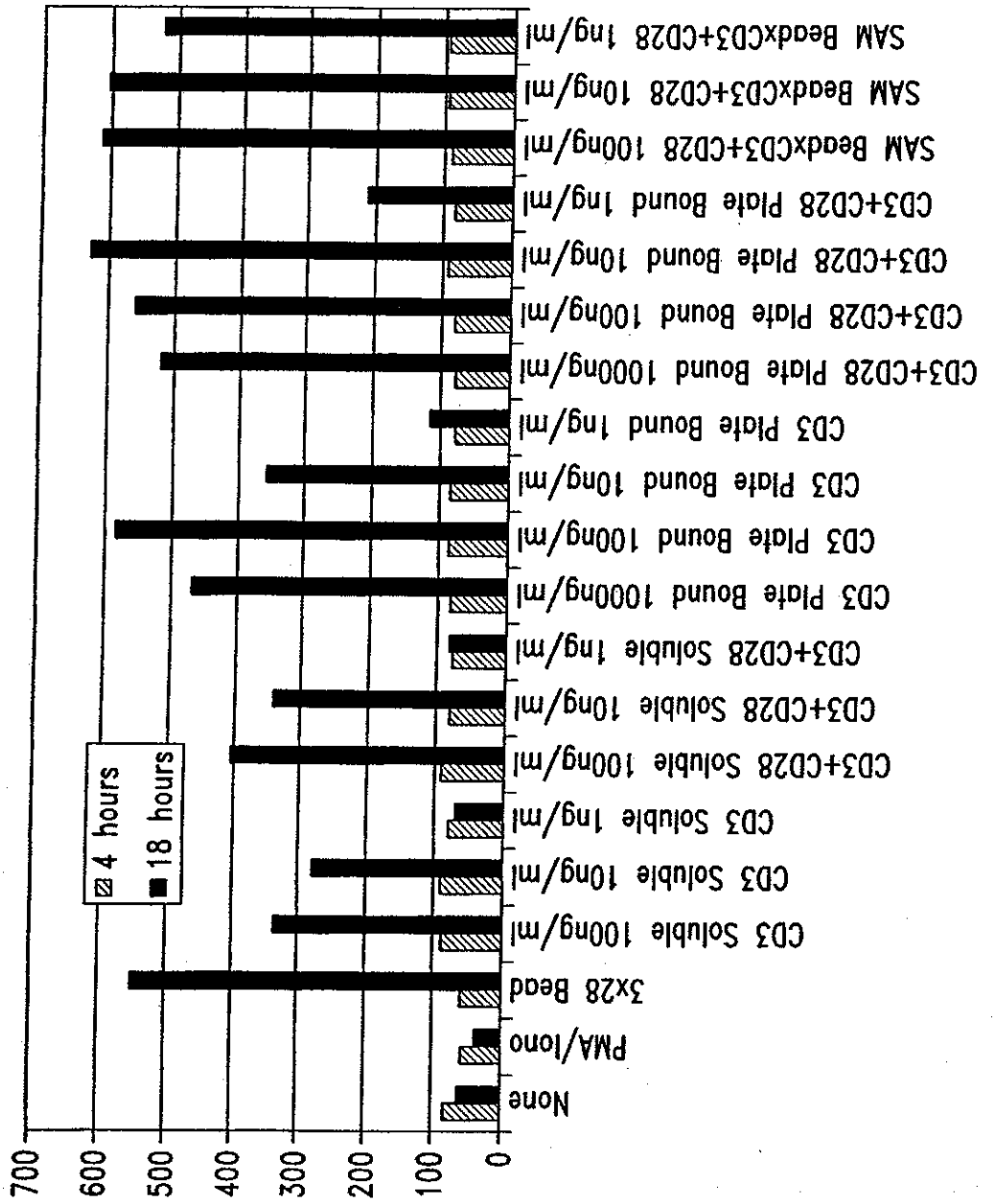
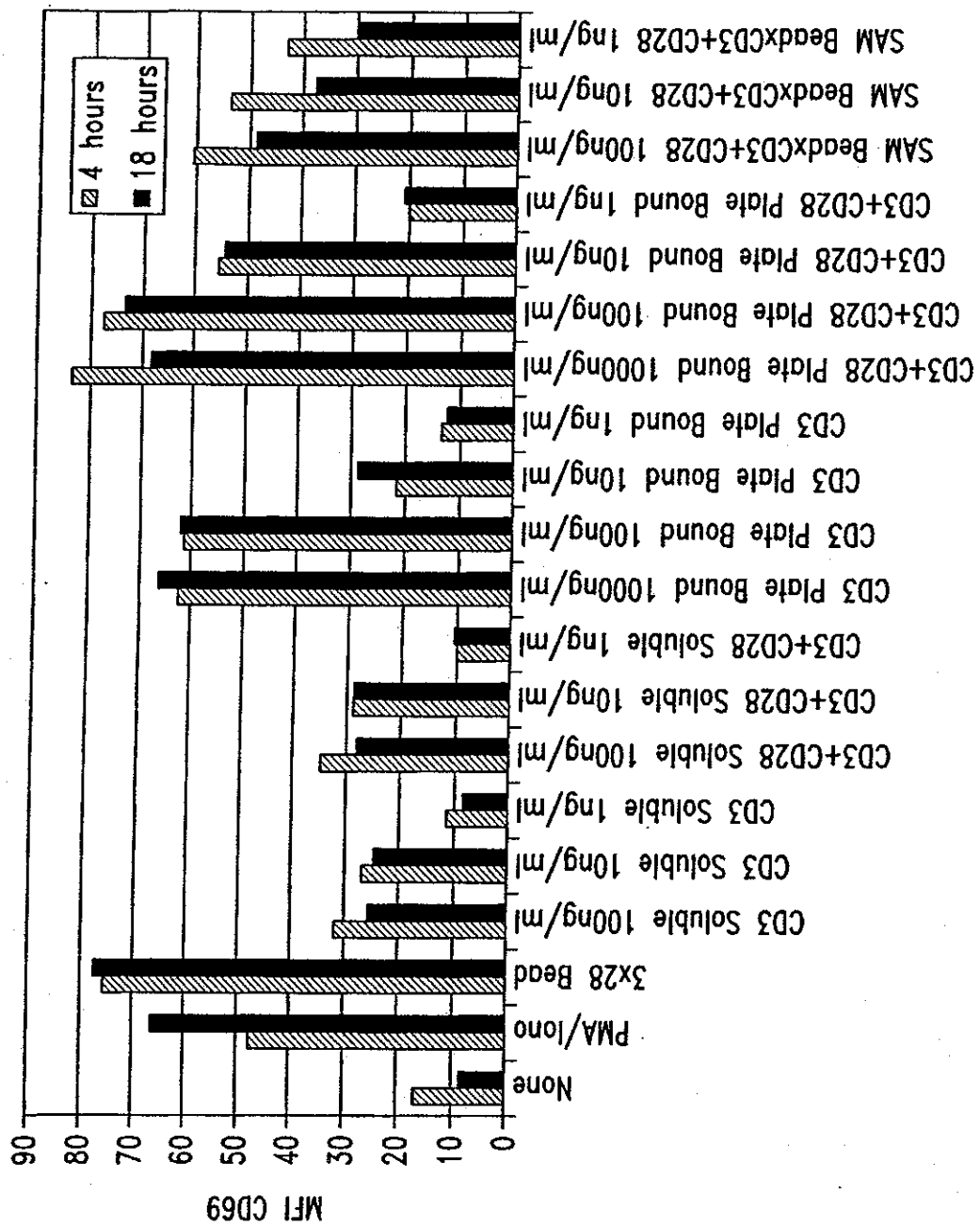
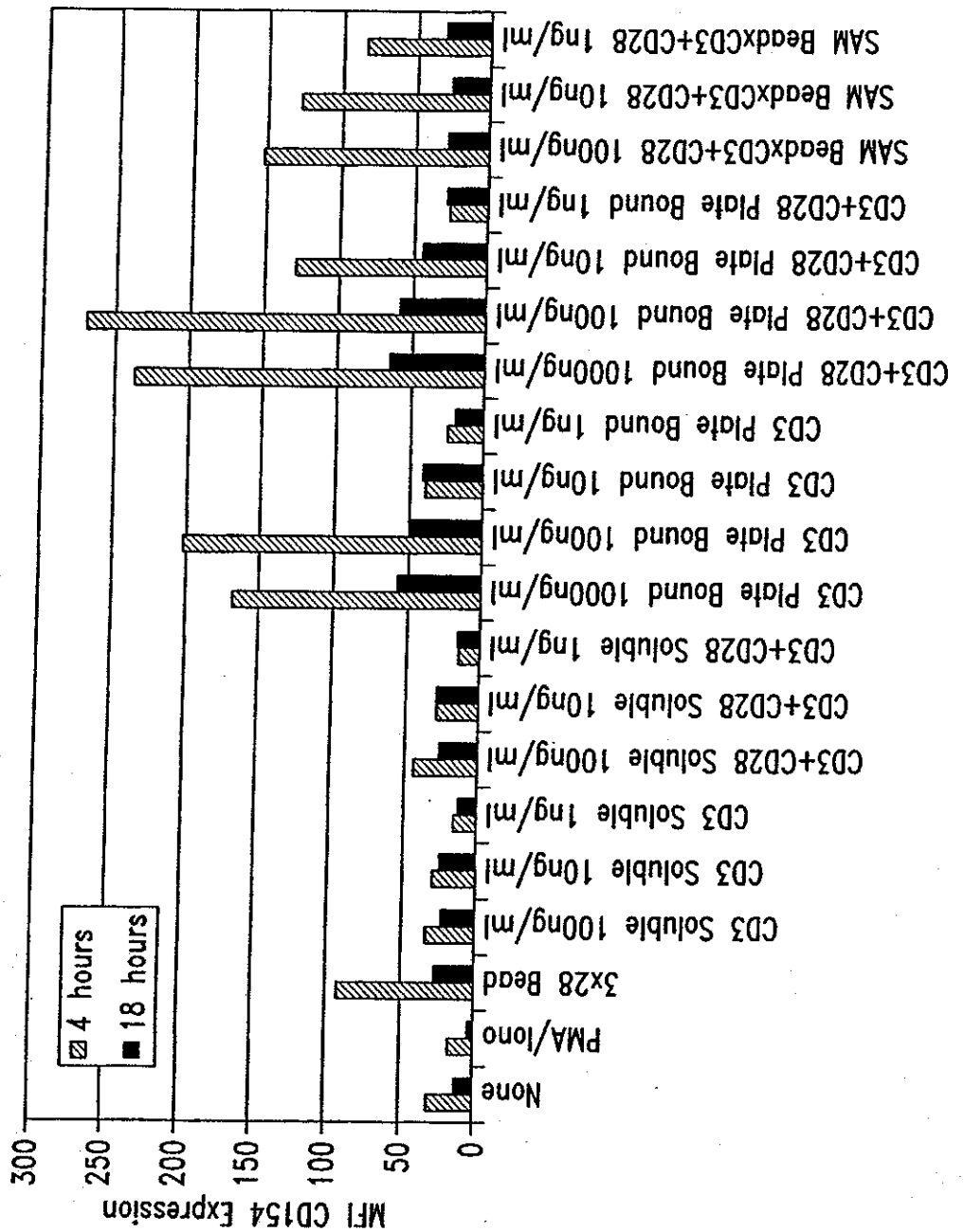

Restimulation of NDa055 (Day8):
CD25 (IL2-R) Expression on CD4+ T Cells
4 hours
18 hours
700
600
500
400
300
200
100
0
None
PMA/Iono
3x28 Bead
CD3 Soluble 100ng/ml
CD3 Soluble 10ng/ml
CD3 Soluble 1ng/ml
CD3+CD28 Soluble 100ng/ml
CD3+CD28 Soluble 10ng/ml
CD3+CD28 Soluble 1ng/ml
CD3 Plate Bound 1000ng/ml
CD3 Plate Bound 100ng/ml
CD3 Plate Bound 10ng/ml
CD3 Plate Bound 1ng/ml
CD3+CD28 Plate Bound 1000ng/ml
CD3+CD28 Plate Bound 100ng/ml
CD3+CD28 Plate Bound 10ng/ml
CD3+CD28 Plate Bound 1ng/ml
SAM BeadxCD3+CD28 100ng/ml
SAM BeadxCD3+CD28 10ng/ml
SAM BeadxCD3+CD28 1ng/ml

Fig. 26C



Restimulation of NDa055 (Day8):
CD69 Expression on CD4+ T Cells

Fig. 26D



Restimulation of NDa055 (Day8):
CD154 (CD40L) Expression on CD4+ T Cells

Fig. 26E

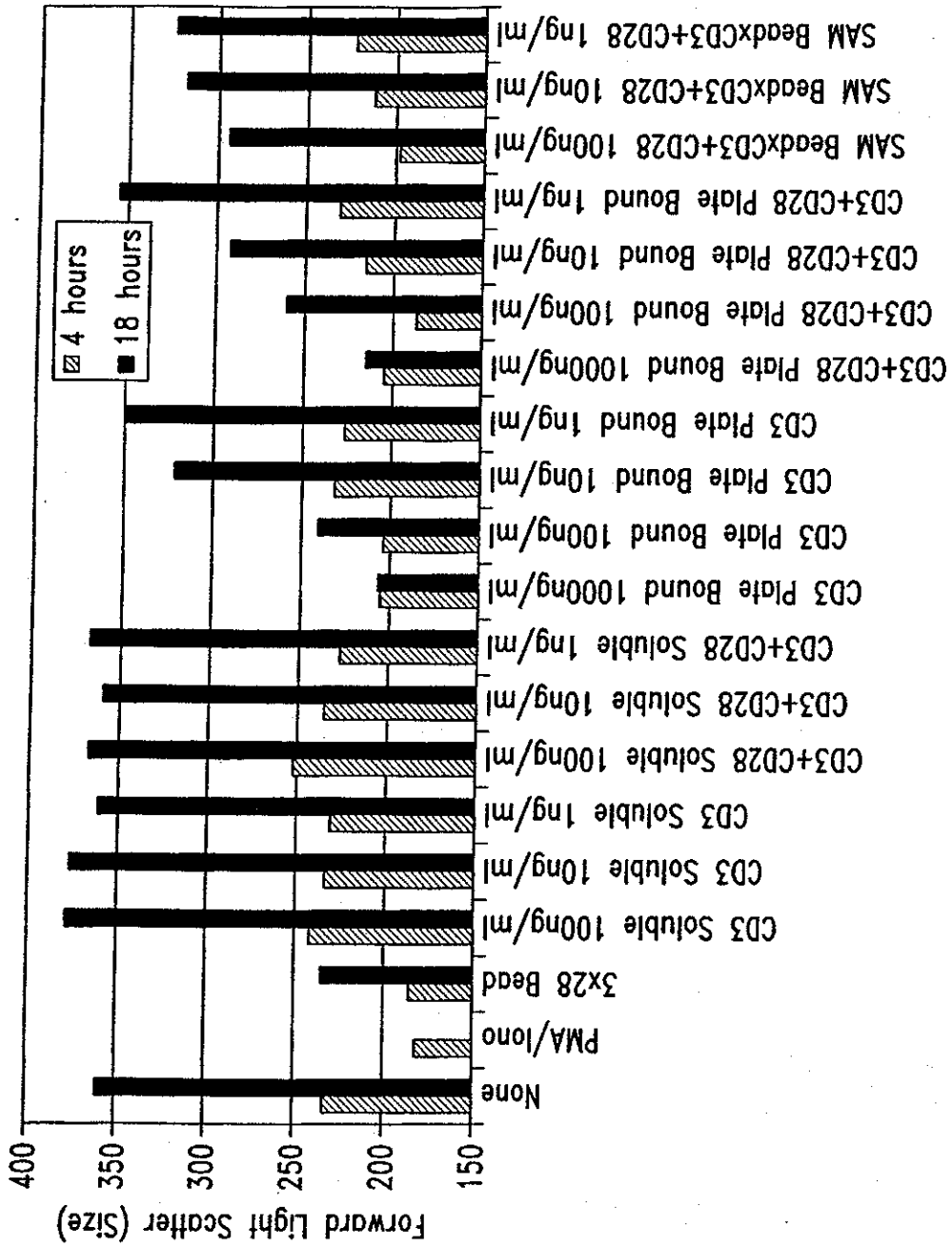

Restimulation of NDa055 (Day8):
Forward Light Scatter (Size)
Forward Light Scatter (Size)
400
350
300
250
200
150
4 hours
18 hours
None
PMA/Iono
3x28 Bead
CD3 Soluble 100ng/ml
CD3 Soluble 10ng/ml
CD3 Soluble 1ng/ml
CD3+CD28 Soluble 100ng/ml
CD3+CD28 Soluble 10ng/ml
CD3+CD28 Soluble 1ng/ml
CD3 Plate Bound 1000ng/ml
CD3 Plate Bound 100ng/ml
CD3 Plate Bound 10ng/ml
CD3 Plate Bound 1ng/ml
CD3+CD28 Plate Bound 1000ng/ml
CD3+CD28 Plate Bound 100ng/ml
CD3+CD28 Plate Bound 10ng/ml
CD3+CD28 Plate Bound 1ng/ml
SAM BeadxCD3+CD28 100ng/ml
SAM BeadxCD3+CD28 10ng/ml
SAM BeadxCD3+CD28 1ng/ml

Fig. 26F

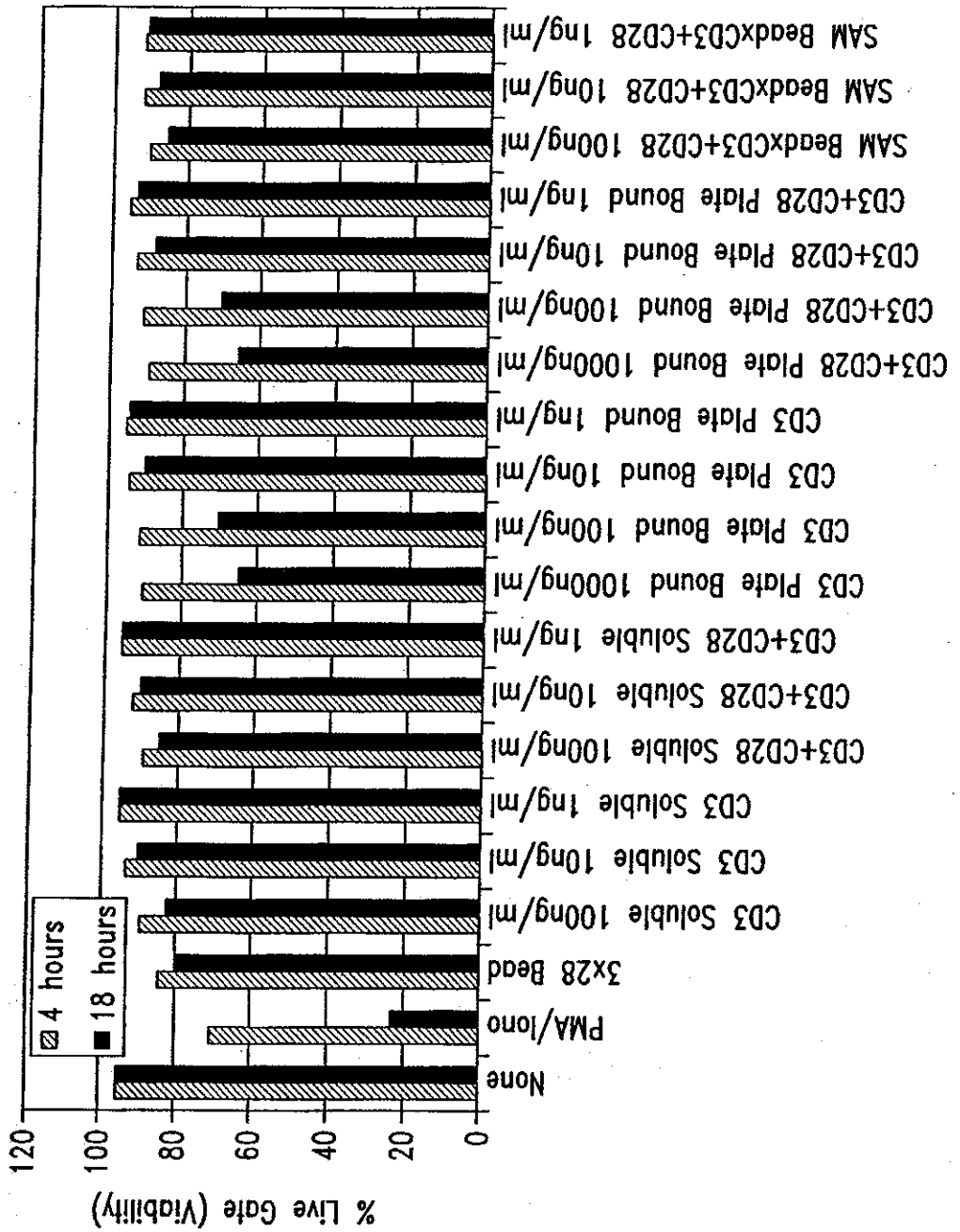

Restimulation of NDa055 (Day8):
Live Gate (Viability)
% Live Gate (Viability)
120
100
80
60
40
20
0
4 hours
18 hours
None
PMA/Iono
3x28 Bead
CD3 Soluble 100ng/ml
CD3 Soluble 10ng/ml
CD3 Soluble 1ng/ml
CD3+CD28 Soluble 100ng/ml
CD3+CD28 Soluble 10ng/ml
CD3+CD28 Soluble 1ng/ml
CD3 Plate Bound 1000ng/ml
CD3 Plate Bound 100ng/ml
CD3 Plate Bound 10ng/ml
CD3 Plate Bound 1ng/ml
CD3+CD28 Plate Bound 1000ng/ml
CD3+CD28 Plate Bound 100ng/ml
CD3+CD28 Plate Bound 10ng/ml
CD3+CD28 Plate Bound 1ng/ml
SAM BeadxCD3+CD28 100ng/ml
SAM BeadxCD3+CD28 10ng/ml
SAM BeadxCD3+CD28 1ng/ml

Fig. 26G

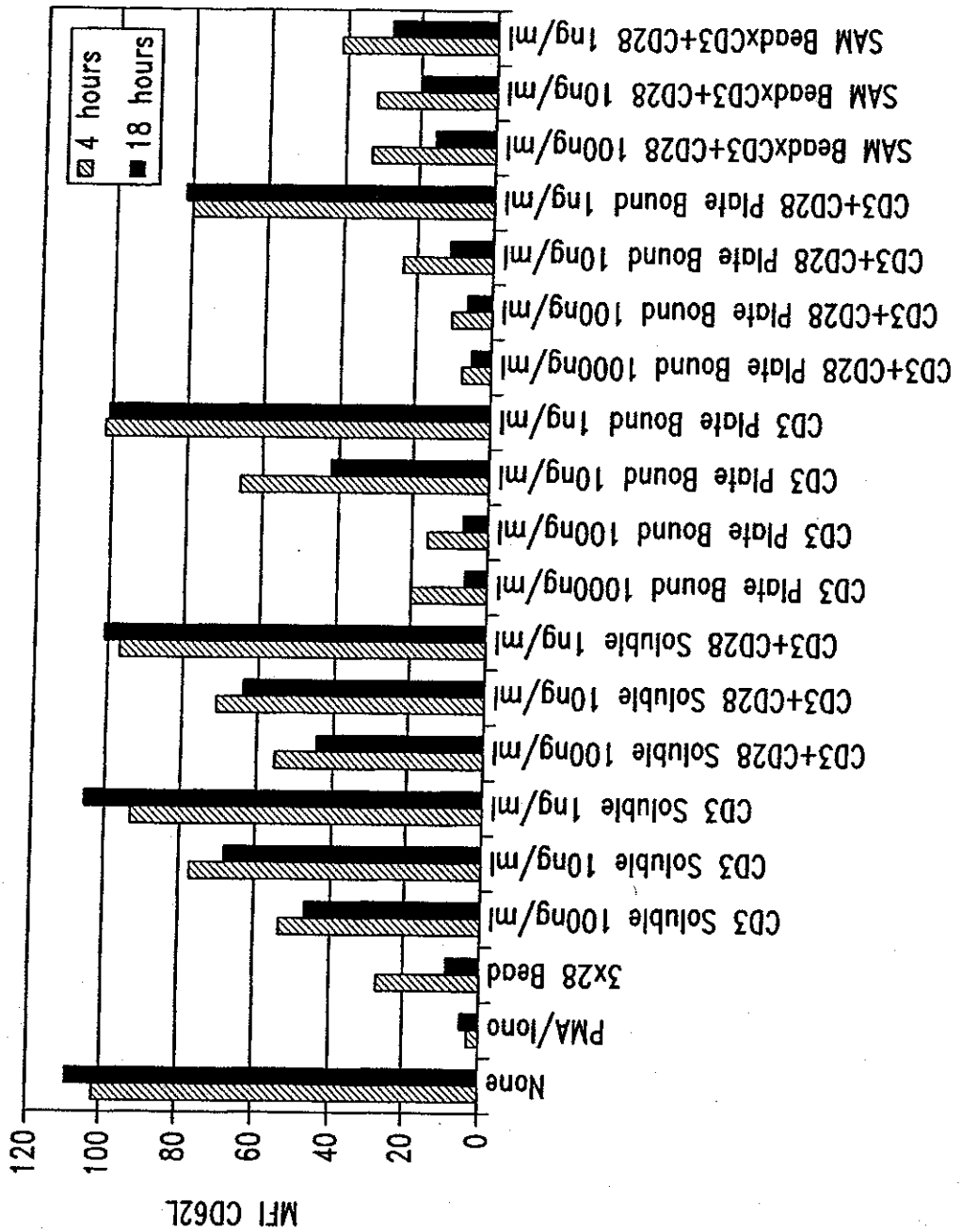

MFI CD62L
120
100
80
60
40
20
0
4 hours
18 hours
None
PMA/Iono
3x28 Bead
CD3 Soluble 100ng/ml
CD3 Soluble 10ng/ml
CD3 Soluble 1ng/ml
CD3+CD28 Soluble 100ng/ml
CD3+CD28 Soluble 10ng/ml
CD3+CD28 Soluble 1ng/ml
CD3 Plate Bound 1000ng/ml
CD3 Plate Bound 100ng/ml
CD3 Plate Bound 10ng/ml
CD3 Plate Bound 1ng/ml
CD3+CD28 Plate Bound 1000ng/ml
CD3+CD28 Plate Bound 100ng/ml
CD3+CD28 Plate Bound 10ng/ml
CD3+CD28 Plate Bound 1ng/ml
SAM BeadxCD3+CD28 100ng/ml
SAM BeadxCD3+CD28 10ng/ml
SAM BeadxCD3+CD28 1ng/ml

Fig. 26H

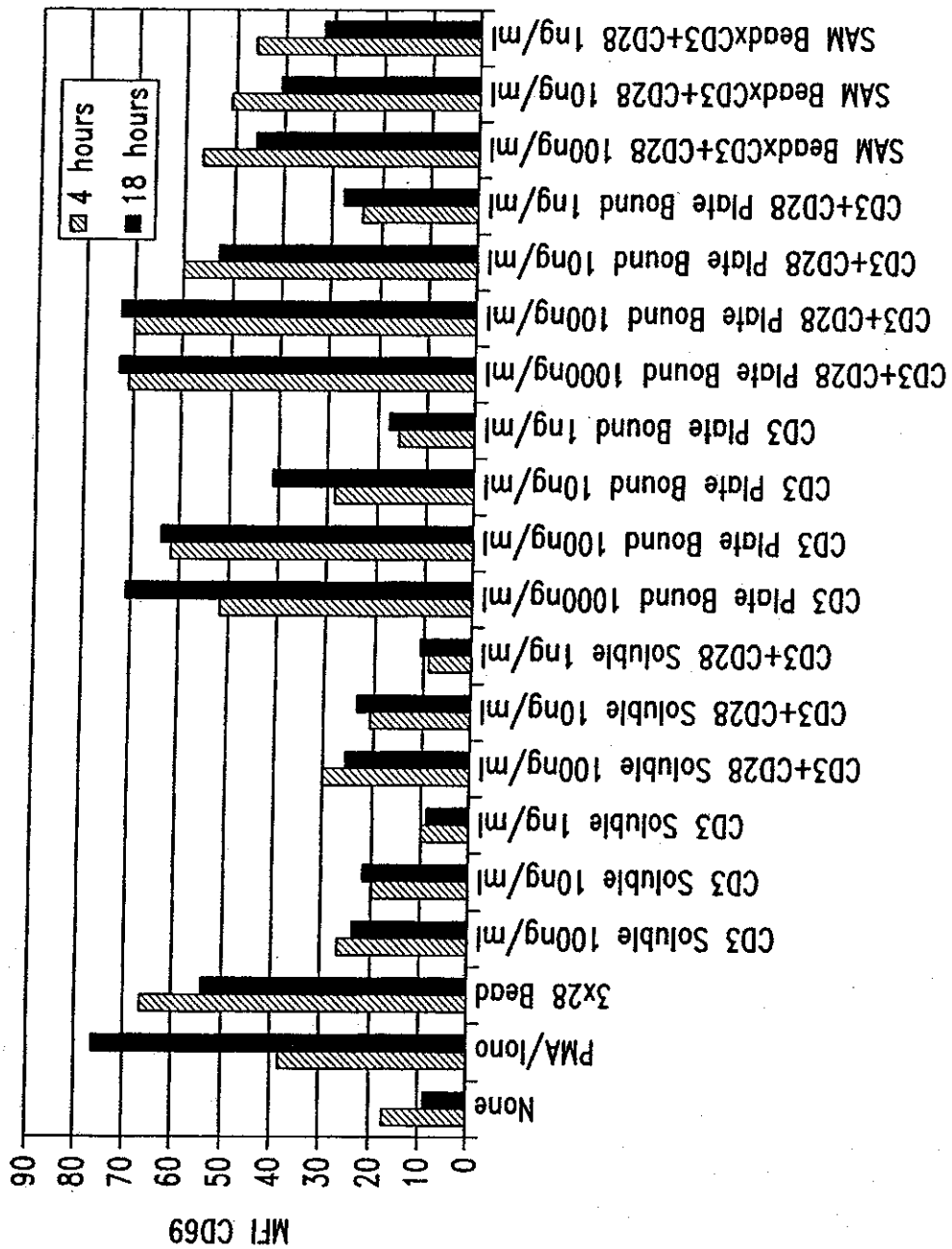

MFI CD69
0
10
20
30
40
50
60
70
80
90
4 hours
18 hours
None
PMA/Iono
3x28 Bead
CD3 Soluble 100ng/ml
CD3 Soluble 10ng/ml
CD3 Soluble 1ng/ml
CD3+CD28 Soluble 100ng/ml
CD3+CD28 Soluble 10ng/ml
CD3+CD28 Soluble 1ng/ml
CD3 Plate Bound 1000ng/ml
CD3 Plate Bound 100ng/ml
CD3 Plate Bound 10ng/ml
CD3 Plate Bound 1ng/ml
CD3+CD28 Plate Bound 1000ng/ml
CD3+CD28 Plate Bound 100ng/ml
CD3+CD28 Plate Bound 10ng/ml
CD3+CD28 Plate Bound 1ng/ml
SAM BeadxCD3+CD28 100ng/ml
SAM BeadxCD3+CD28 10ng/ml
SAM BeadxCD3+CD28 1ng/ml

Fig. 26I

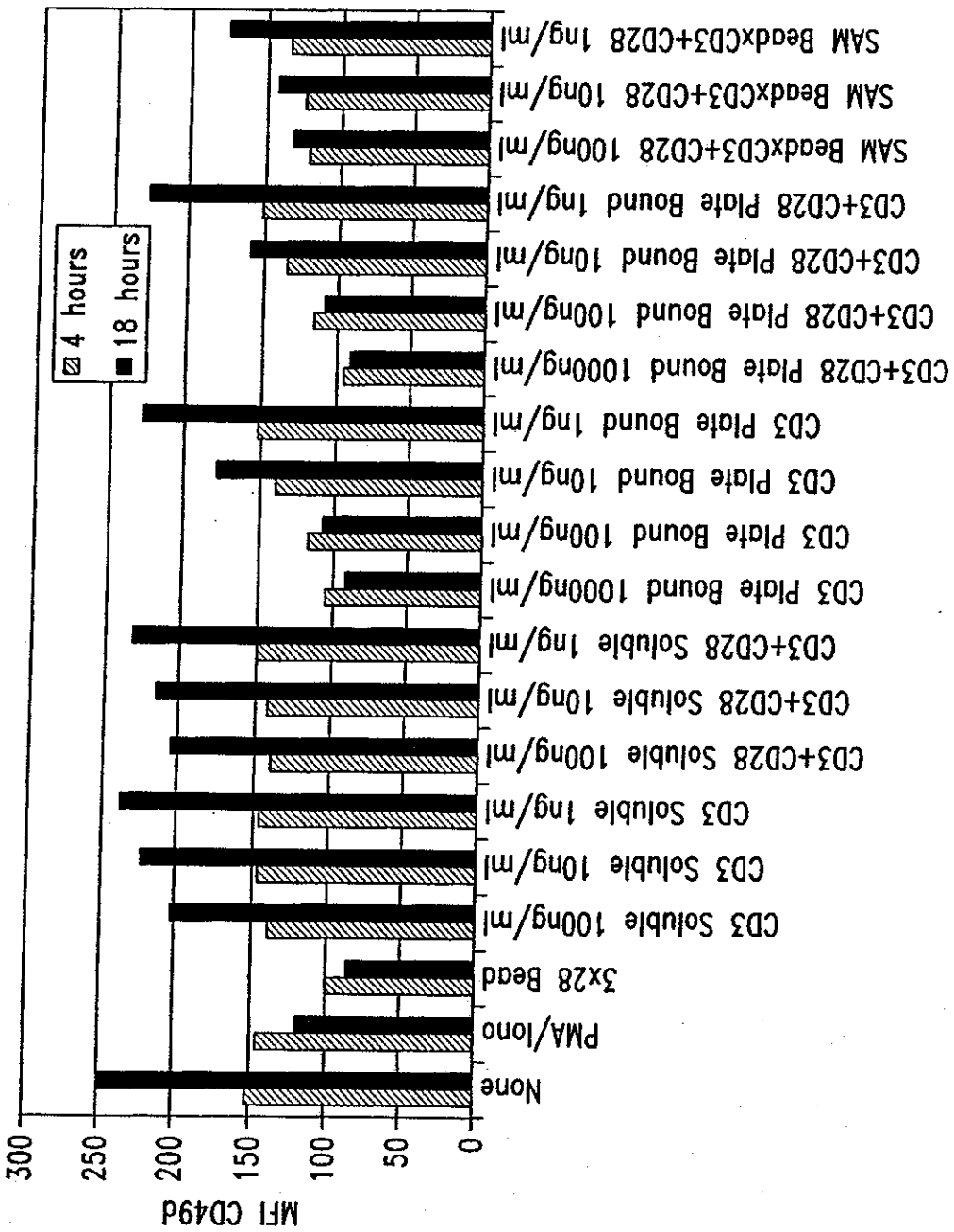

MFI CD49d
300
250
200
150
100
50
0
4 hours
18 hours
None
PMA/Iono
3x28 Bead
CD3 Soluble 100ng/ml
CD3 Soluble 10ng/ml
CD3 Soluble 1ng/ml
CD3+CD28 Soluble 100ng/ml
CD3+CD28 Soluble 10ng/ml
CD3+CD28 Soluble 1ng/ml
CD3 Plate Bound 1000ng/ml
CD3 Plate Bound 100ng/ml
CD3 Plate Bound 10ng/ml
CD3 Plate Bound 1ng/ml
CD3+CD28 Plate Bound 1000ng/ml
CD3+CD28 Plate Bound 100ng/ml
CD3+CD28 Plate Bound 10ng/ml
CD3+CD28 Plate Bound 1ng/ml
SAM BeadxCD3+CD28 100ng/ml
SAM BeadxCD3+CD28 10ng/ml
SAM BeadxCD3+CD28 1ng/ml

Fig. 26J

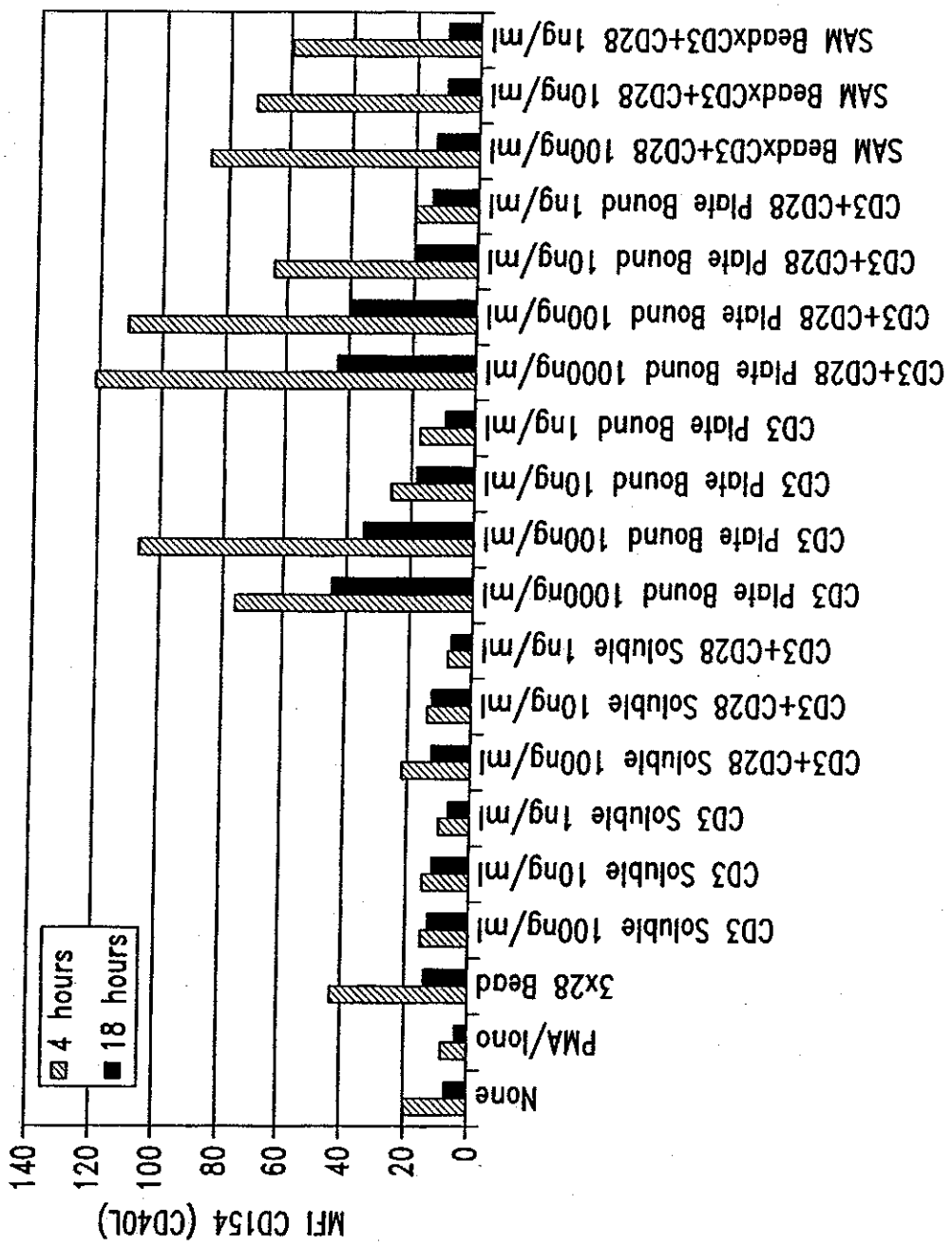

MFI CD154 (CD40L)
0
20
40
60
80
100
120
140
4 hours
18 hours
None
PMA/Iono
3x28 Bead
CD3 Soluble 100ng/ml
CD3 Soluble 10ng/ml
CD3 Soluble 1ng/ml
CD3+CD28 Soluble 100ng/ml
CD3+CD28 Soluble 10ng/ml
CD3+CD28 Soluble 1ng/ml
CD3 Plate Bound 1000ng/ml
CD3 Plate Bound 100ng/ml
CD3 Plate Bound 10ng/ml
CD3 Plate Bound 1ng/ml
CD3+CD28 Plate Bound 1000ng/ml
CD3+CD28 Plate Bound 100ng/ml
CD3+CD28 Plate Bound 10ng/ml
CD3+CD28 Plate Bound 1ng/ml
SAM BeadxCD3+CD28 100ng/ml
SAM BeadxCD3+CD28 10ng/ml
SAM BeadxCD3+CD28 1ng/ml

Fig. 26K

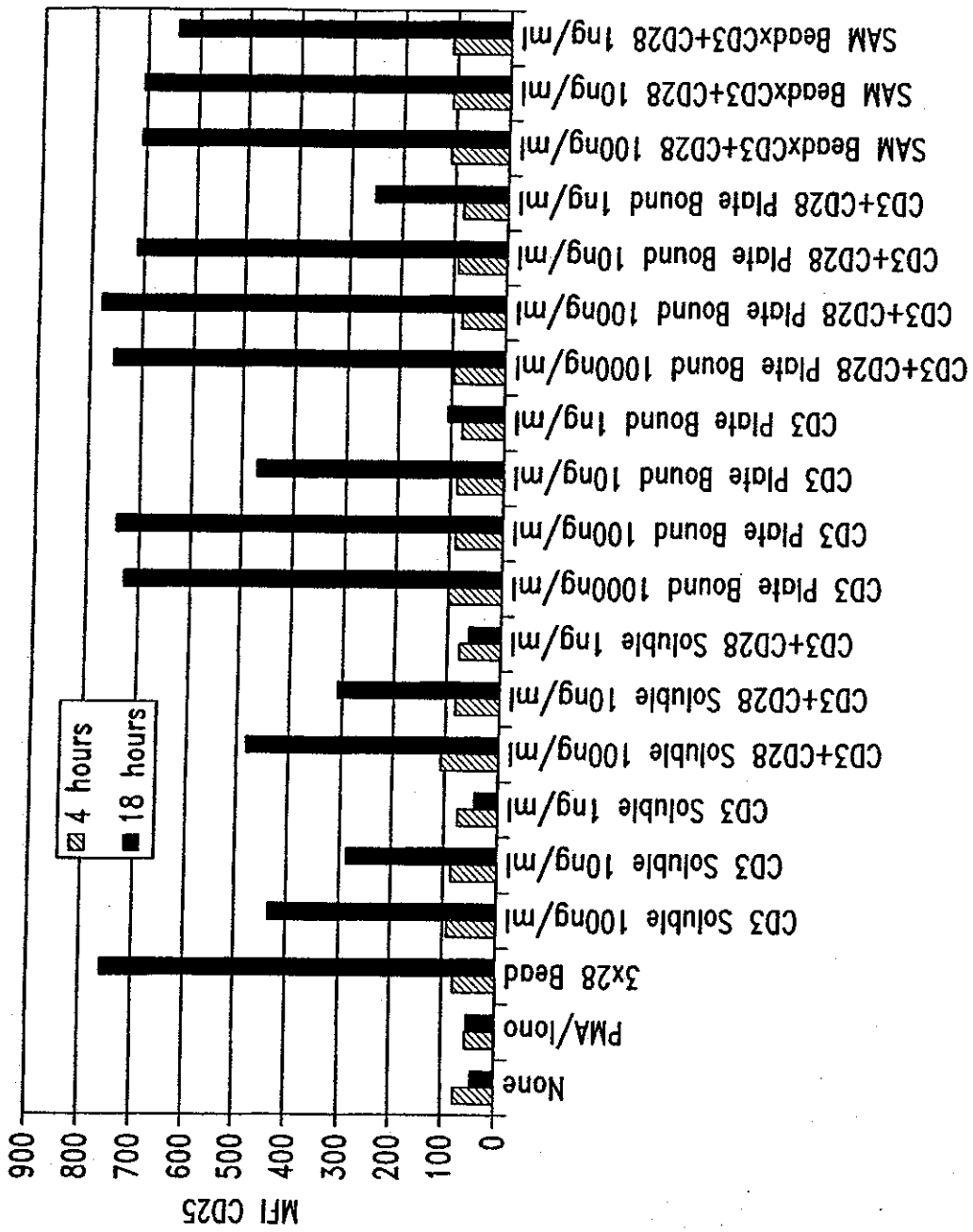

MFI CD25
0
100
200
300
400
500
600
700
800
900
4 hours
18 hours
None
PMA/Iono
3x28 Bead
CD3 Soluble 100ng/ml
CD3 Soluble 10ng/ml
CD3 Soluble 1ng/ml
CD3+CD28 Soluble 100ng/ml
CD3+CD28 Soluble 10ng/ml
CD3+CD28 Soluble 1ng/ml
CD3 Plate Bound 1000ng/ml
CD3 Plate Bound 100ng/ml
CD3 Plate Bound 10ng/ml
CD3 Plate Bound 1ng/ml
CD3+CD28 Plate Bound 1000ng/ml
CD3+CD28 Plate Bound 100ng/ml
CD3+CD28 Plate Bound 10ng/ml
CD3+CD28 Plate Bound 1ng/ml
SAM BeadxCD3+CD28 100ng/ml
SAM BeadxCD3+CD28 10ng/ml
SAM BeadxCD3+CD28 1ng/ml

Fig. 26L

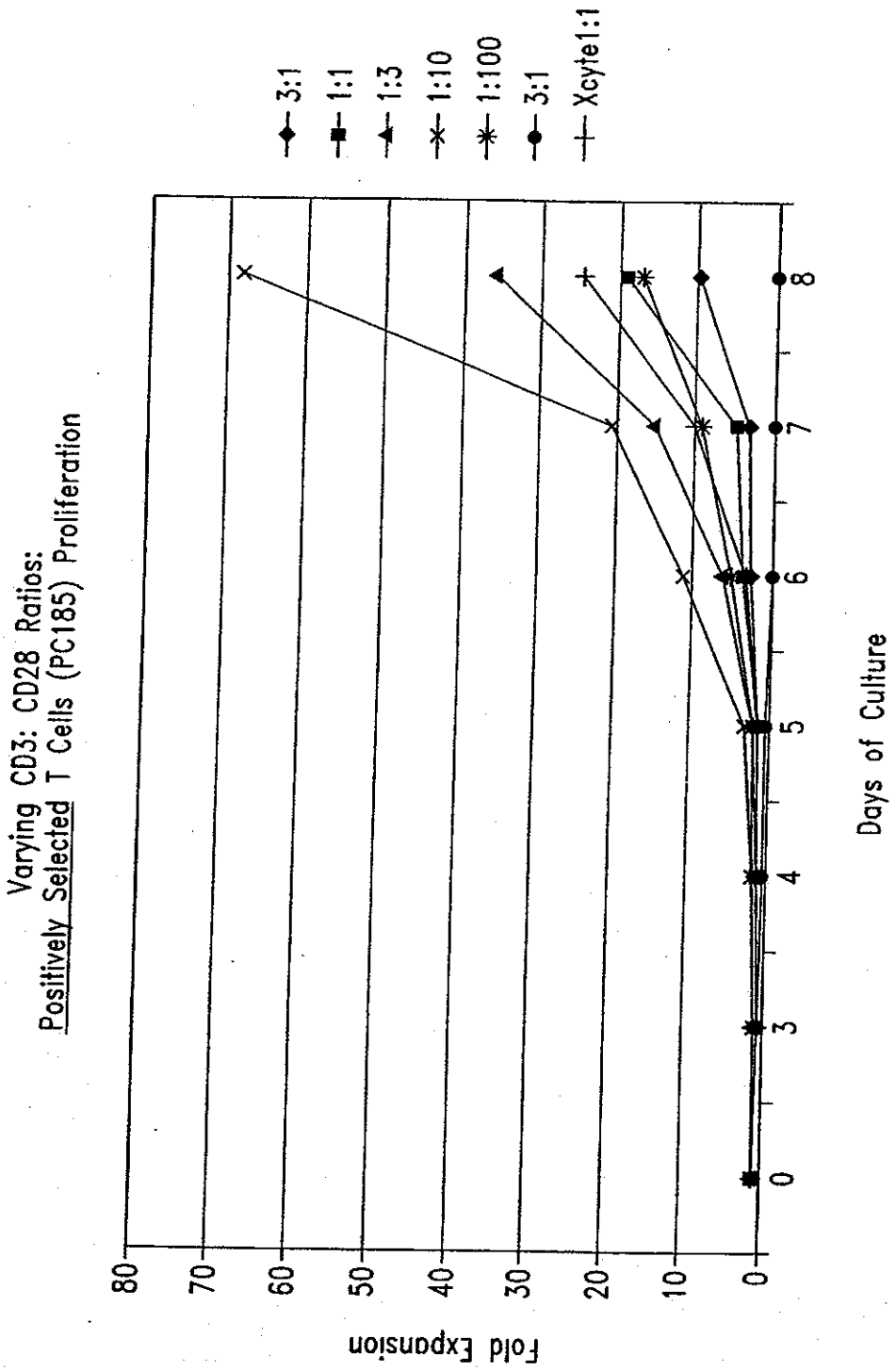

Varying CD3: CD28 Ratios:
Positively Selected T Cells (PC185) Proliferation
3:1
1:1
1:3
1:10
1:100
3:1
Xcyte1:1
Fold Expansion
0
10
20
30
40
50
60
70
80
Days of Culture
0
3
4
5
6
7
8

Fig. 27

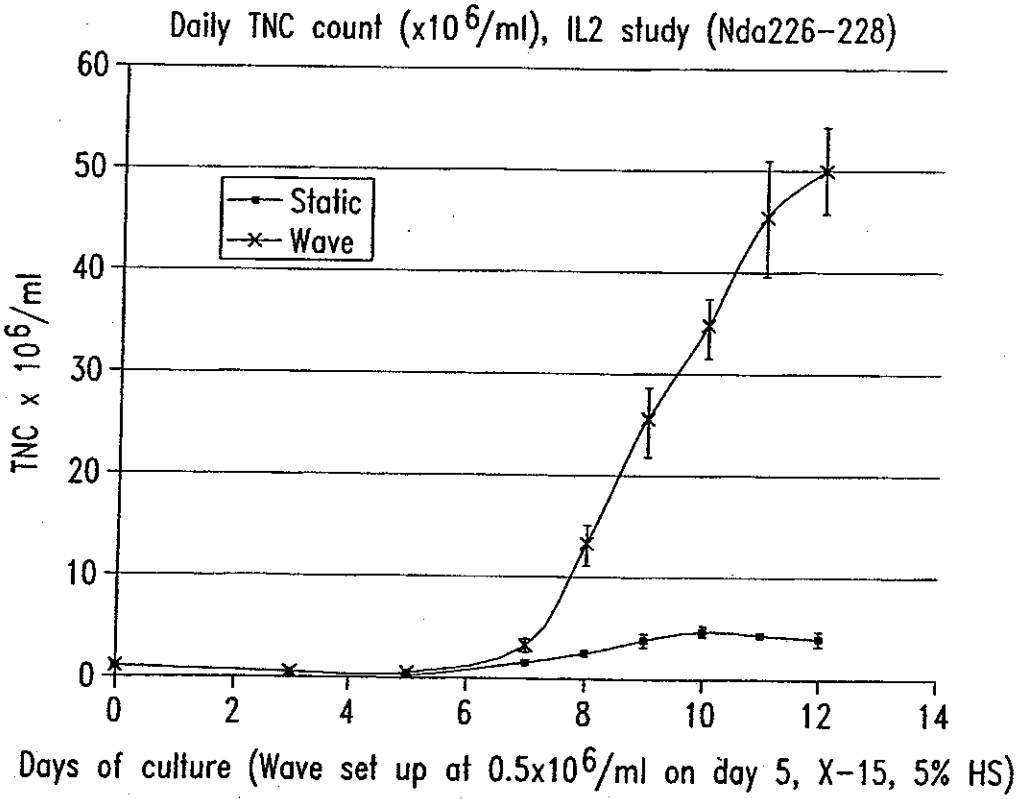

Daily TNC count (x10 6/ml), IL2 study (Nda226-228)
TNC x 10 6/ml
60
50
40
30
20
10
0
Static
Wave
0
2
4
6
8
10
12
14
Days of culture (Wave set up at 0.5x10 6/ml on day 5, X-15, 5% HS)

Fig. 28