VH sequence:

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAAACTCTCC
TGTGCAGCCTCTGGATTCAAGTTTAGTAGATATGCCATGTCTTGGGTTCGCCAGGCTCCGGGGA
AGAGGCTGGAGTGGGTCGCAACCATTAGTAGTGGTGGTAGTTACATCTACTATCCAGACAGTG
TGAAGGGTCGATTCACCATCTCCAGAGACAATGTCAAGAACACCCTGTATCTGCAAATGAGCA
GTCTGAGGTCTGAGGACACGGCCATGTATTACTGTGCAAGACGAGATTACGACCTGGACTACT
TTGACTCCTGGGGCCAAGGCACCCTTGTCACCGTCTCCTCA

Vk sequence:

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCGGTGGGAGACAGAGTCACTATC
ACTTGCAAGGCGAGTCGGGACATTAGAAGCTATTTAACCTGGTACCAGCAGAAACCAGGGAA
AGCTCCTAAGACCCTGATCTATTATGCAACAAGCTTGGCAGATGGGGTCCCGTCGAGATTCAG
TGGCAGTGGATCTGGGCAAGATTATTCTCTCACCATCAGCAGCCTGGAGTCTGACGATACAGC
AACTTACTACTGTCTACAACATGGTGAGAGTCCATTCACGCTCGGCTCGGGGACCAAGCTGGA
AATCAAA

Figure 1a

VH sequence:

EVQLVESGGGLVKPGGSLKLSCAASGFKFSRYAMSWVRQAPGKRLEW
VATISSGGSYIYYPDSVKGRFTISRDNVKNTLYLQMSSLRSEDTAMYY
CARRDYDLDYFDSWGQGTLVTVSS

VK sequence:

DIQMTQSPSSLSASVGDRVTITCKASRDIRSYLTWYQQKPGKAPKTLI
YYATSLADGVPSRFSGSGSGQDYSLTISSLESDDTATYYCLQHGESPFT
LGSGTKLEIK

Figure 1b

DIQMTQSPSSLSASVGDRVTITCKASRDIRSYLTWYQQKP

DIQMTQSPSSLSASVGDRVTITCKASRDIRSYLTWYQQKP

DIQMTQSPSSLSASVGDRVTITCKASRDIRSYLTWYQQKP

GKAPKTLIYYATSLADGVPSRFSGSGSGQDYSLTISSLESD

GKAPKTLIYYATSLADGVPSRFSGSGSGQDYSLTISSLESD

GKAPKTLIYYATSLADGVPSRFSGSGSGQDYSLTISSLESD

DTATYYCLQHGESPFTFGSGTKLEIKRA EP0807125 B1,

DTATYYCLQHGESPFTLGSGTKLEIK-- Translated Nucleotide sequence from

DTATYYCLQHGESPFTLGSGTKLEIK-- Amino acid sequence

Figure 1c

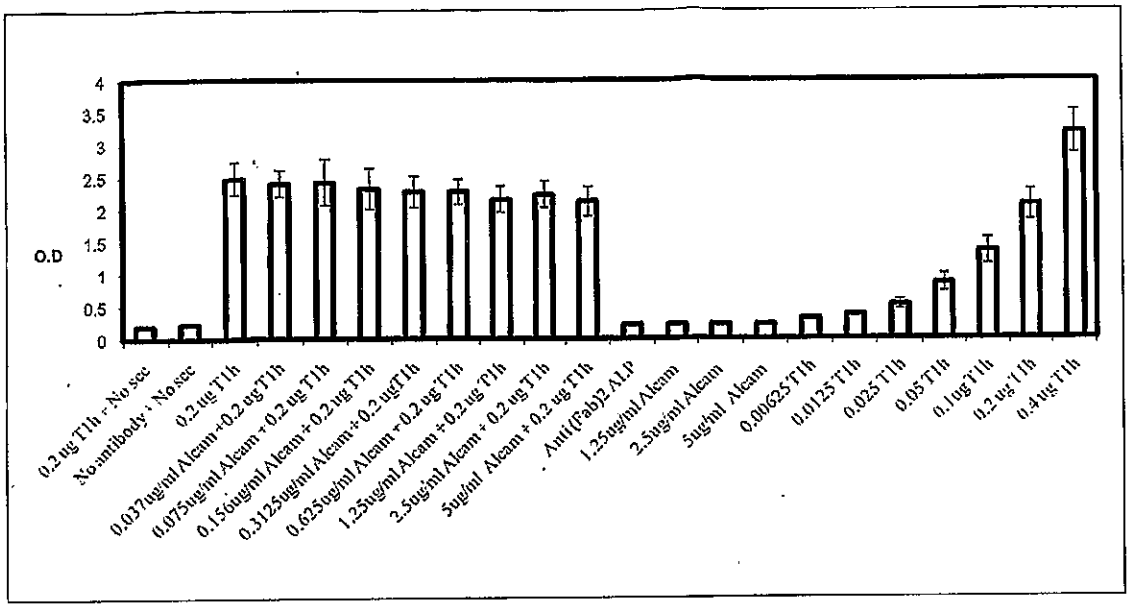

O.D
4
3.5
3
2.5
2
1.5
1
0.5
0
0.2 ug T1h + No sec
No antibody + No sec
0.2 ug T1h
0.037ug/ml Alcam + 0.2 ug T1h
0.075ug/ml Alcam + 0.2 ug T1h
0.156ug/ml Alcam + 0.2 ug T1h
0.3125ug/ml Alcam + 0.2 ug T1h
0.625ug/ml Alcam + 0.2 ug T1h
1.25ug/ml Alcam + 0.2 ug T1h
2.5ug/ml Alcam + 0.2 ug T1h
5ug/ml Alcam + 0.2 ug T1h
Anti(Fab)2 ALP
1.25ug/ml Alcam
2.5ug/ml Alcam
5ug/ml Alcam
0.00625 T1h
0.0125 T1h
0.025 T1h
0.05 T1h
0.1ug T1h
0.2 ug T1h
0.4 ug T1h

Figure 2

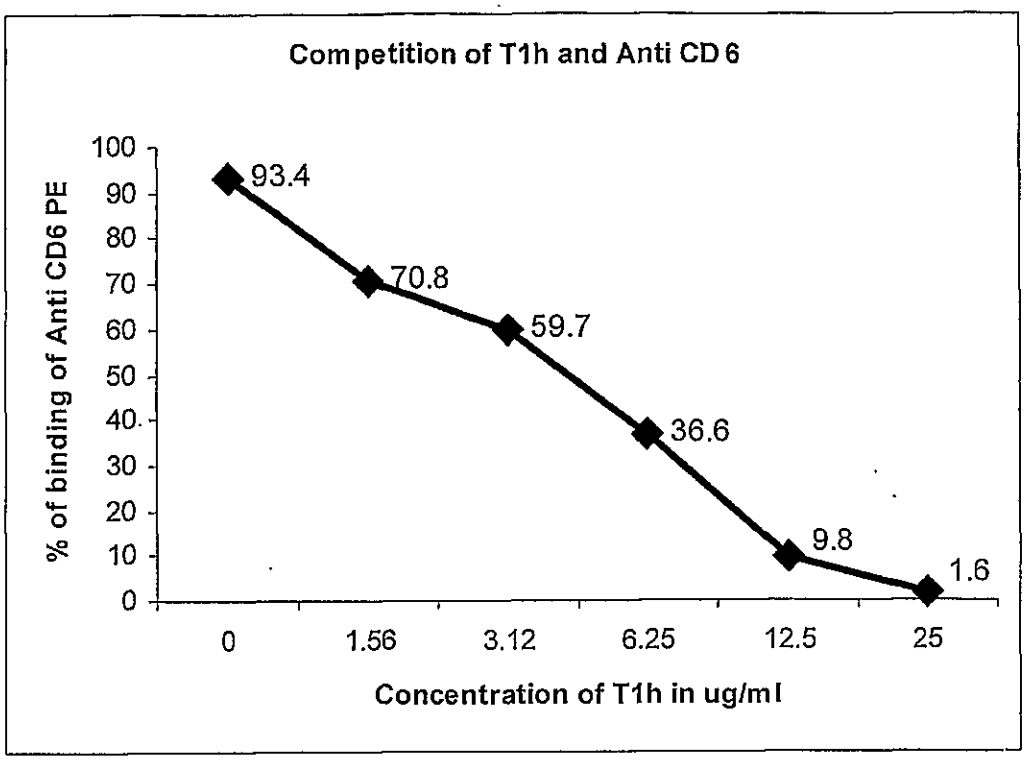
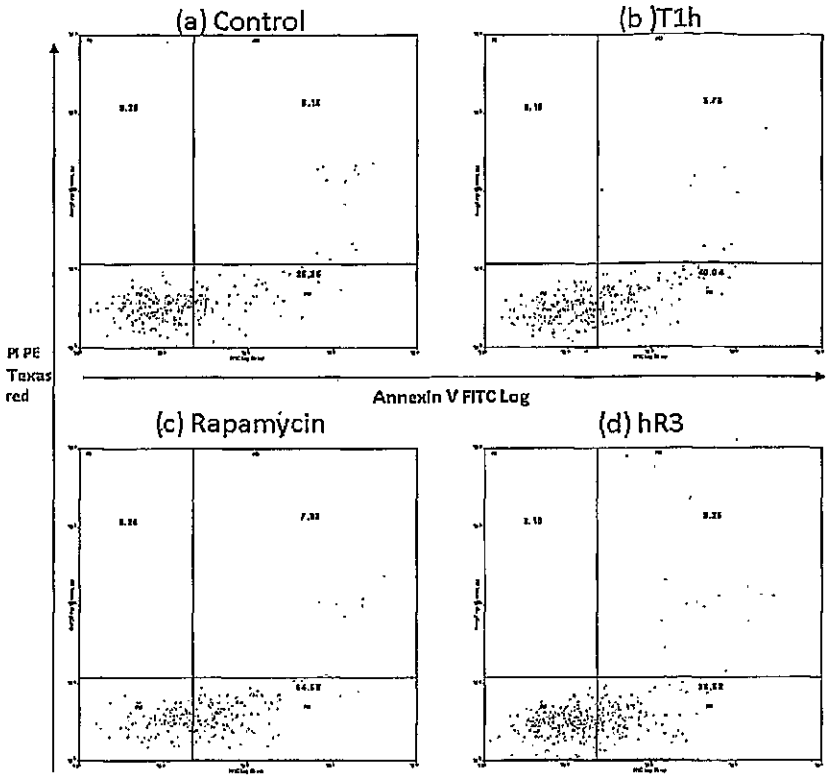

Competition of T1h and Anti CD 6
% of binding of Anti CD6 PE
100
90
80
70
60
50
40
30
20
10
0
93.4
70.8
59.7
36.6
9.8
1.6
0
1.56
3.12
6.25
12.5
25
Concentration of T1h in ug/ml

Figure 3



	Annexin v positive
Control	35.3%
T1h (5ug/ml)	40.0%
Rapamycin (1.2ug/ml)	54.3%
hR3 (5ug/ml)	36.5%

Figure 4

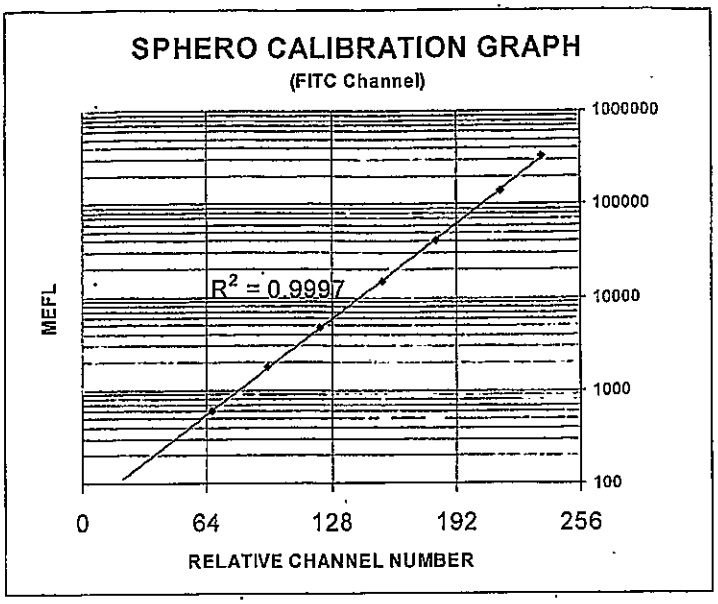
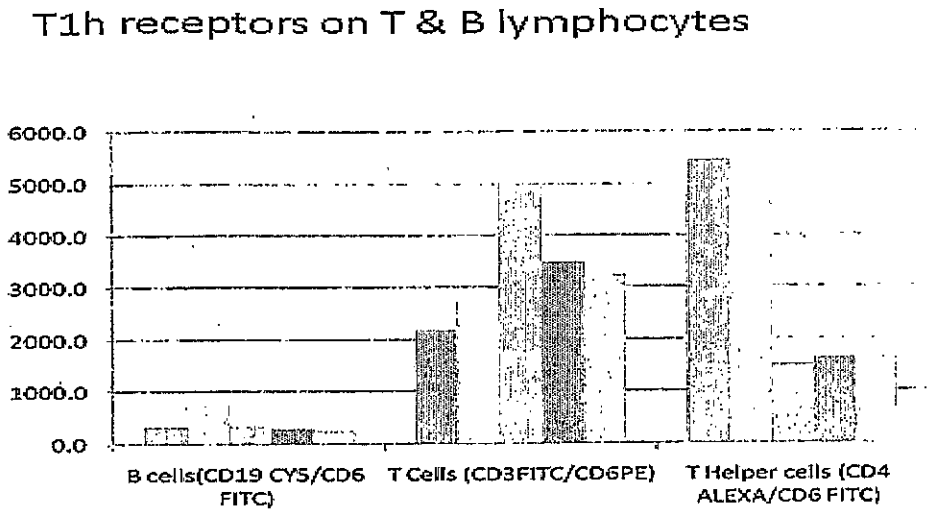

SPHERO CALIBRATION GRAPH
(FITC Channel)
MEFL
1000000
100000
10000
1000
100
R^2 = 0.9997
0
64
128
192
256
RELATIVE CHANNEL NUMBER

Figure 5



	B cells(CD19 CY5/CD6 FITC)	T Cells (CD3FITC/CD6PE)	T Helper cells (CD4 ALEXA/CD6 FITC)
v1	320.0	2174.0	5439.8
v2	762.1	2739.0	4910.0
v3	340.0	4962.2	1505.4
v4	280.6	3483.4	1631.7
v5	257.9	3230.4	1686.3

V1 –V5 ARE NORMAL DONORS

Figure 6

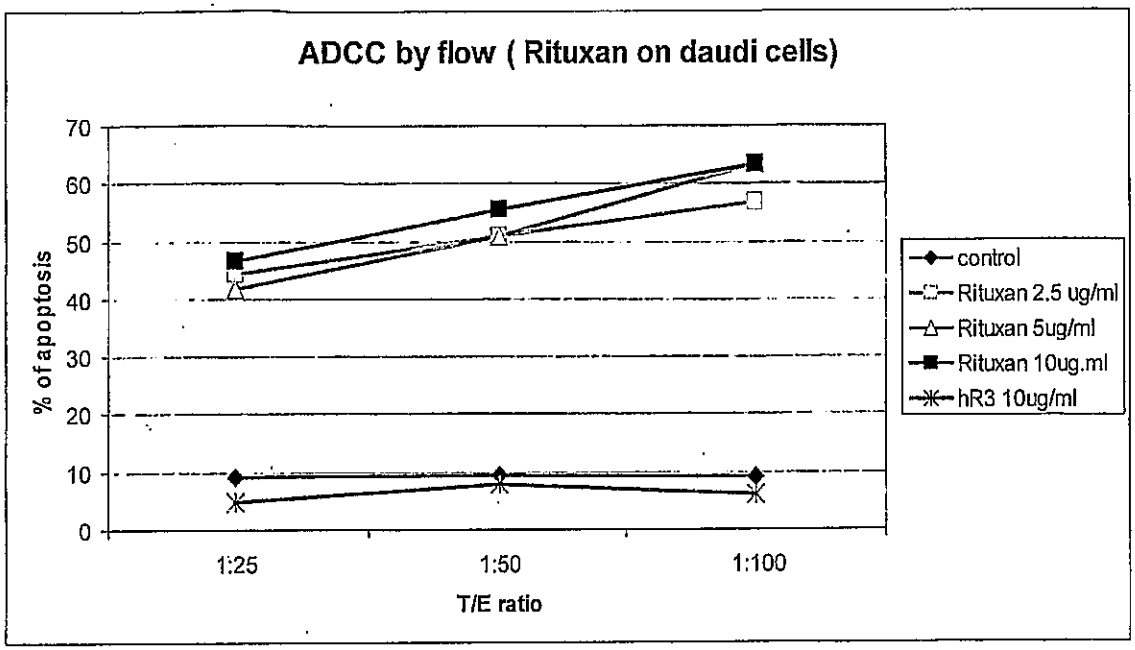

ADCC by flow (Rituxan on daudi cells)
% of apoptosis
70
60
50
40
30
20
10
0
1:25
1:50
1:100
T/E ratio
control
Rituxan 2.5 ug/ml
Rituxan 5ug/ml
Rituxan 10ug.ml
hR3 10ug/ml

Figure 7

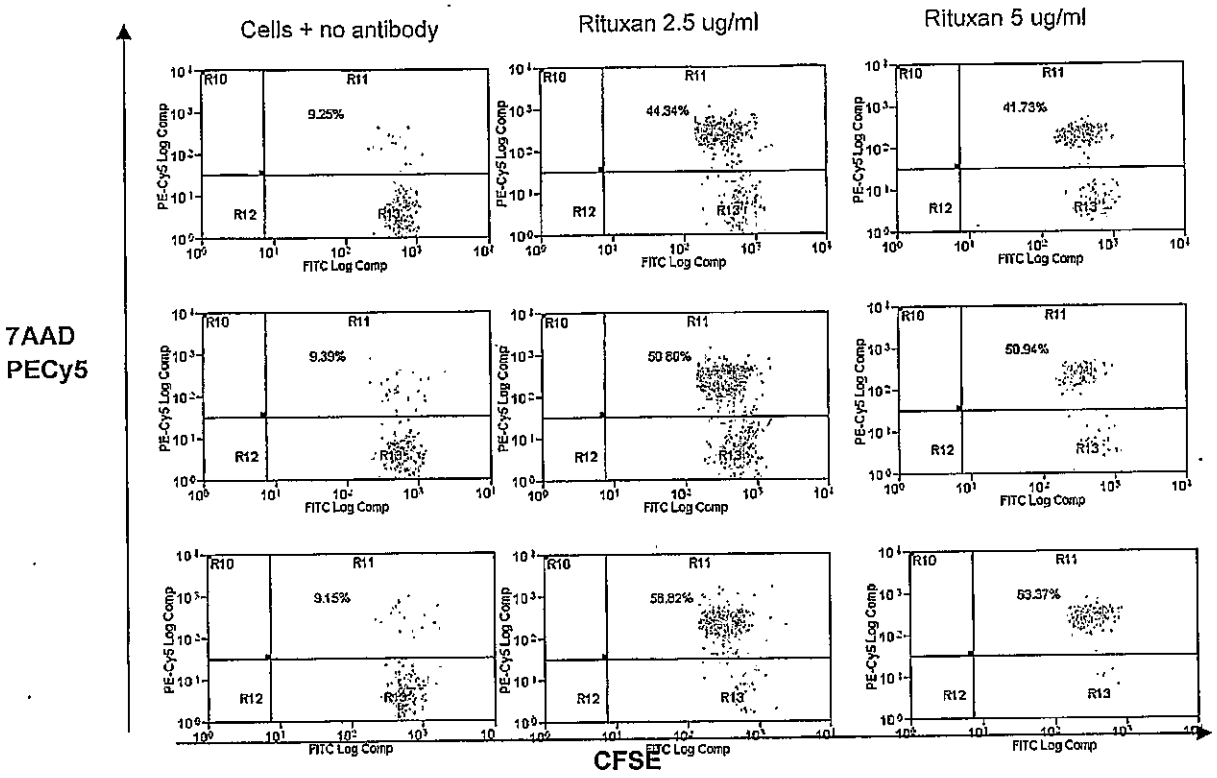

Cells + no antibody
Rituxan 2.5 ug/ml
Rituxan 5 ug/ml
7AAD
PECy5
CFSE
R10
R11
R12
R13
9.25%
44.34%
41.73%
9.39%
50.60%
50.94%
9.15%
58.82%
53.37%
PE-Cy5 Log Comp
FITC Log Comp

Figure 8

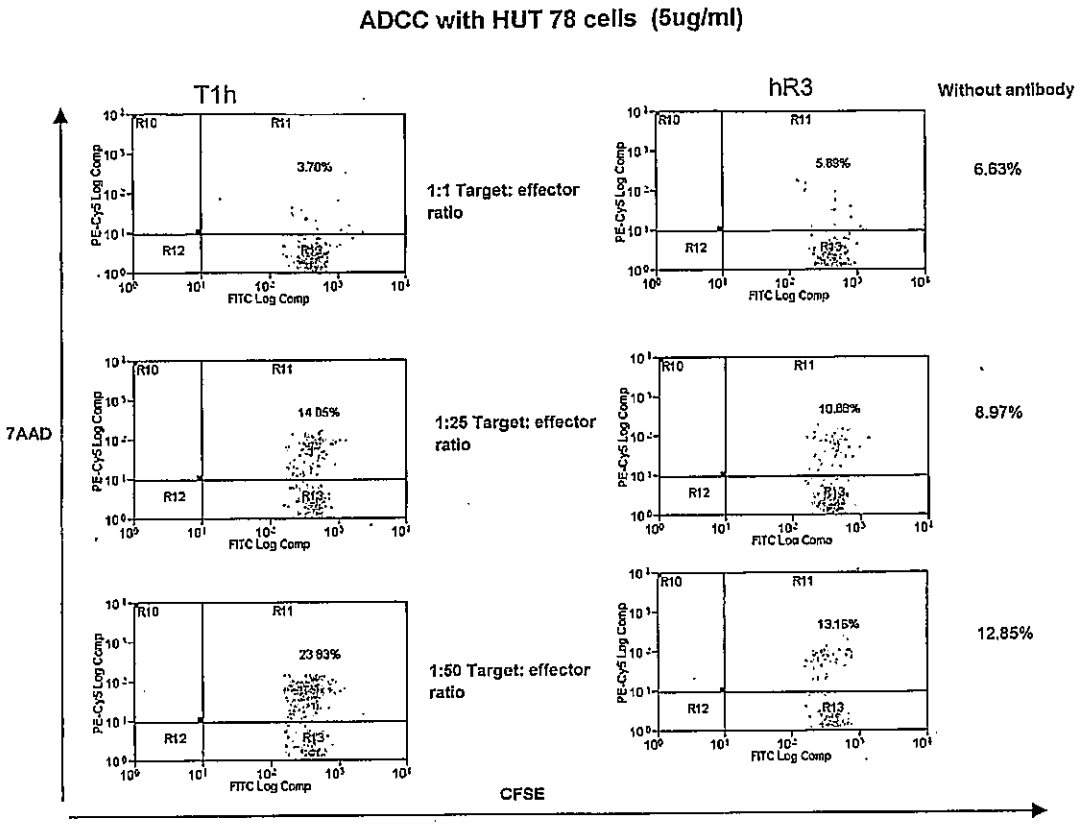

ADCC with HUT 78 cells (5ug/ml)
T1h
hR3
Without antibody
1:1 Target: effector ratio
1:25 Target: effector ratio
1:50 Target: effector ratio
3.70%
5.89%
6.63%
14.05%
10.88%
8.97%
23.93%
13.15%
12.85%
R10
R11
R12
R13
PE-Cy5 Log Comp
FITC Log Comp
7AAD
CFSE

Figure 9

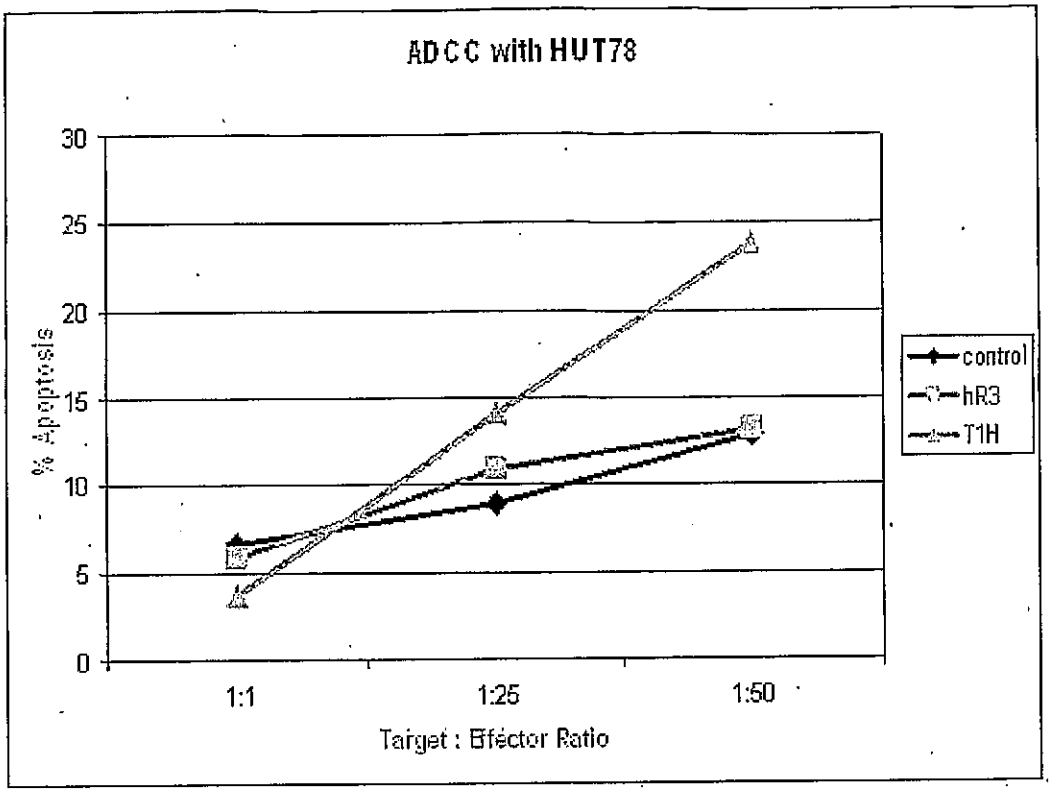
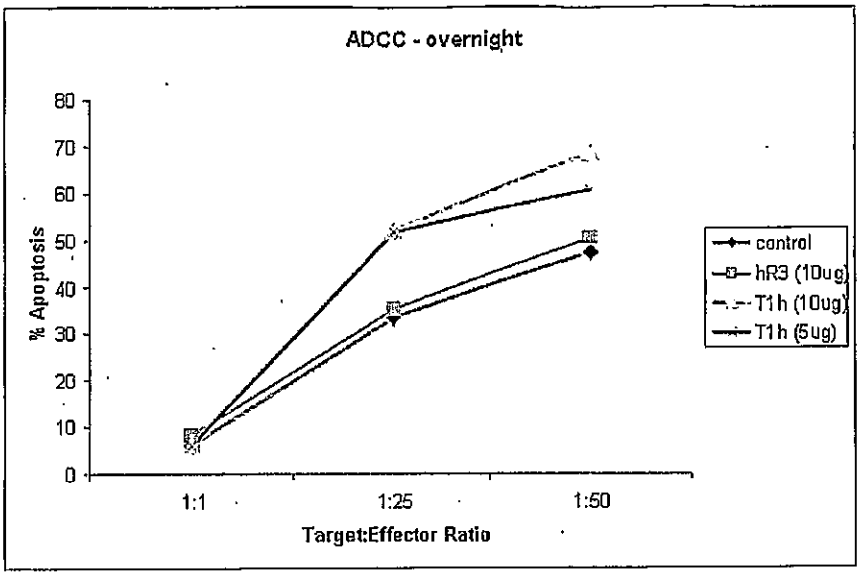

ADCC with HUT78
30
25
20
15
10
5
0
% Apoptosis
1:1
1:25
1:50
Target : Effector Ratio
control
hR3
T1H

Figure 10



% Apoptosis as compared to hR3

	T1h (5ug)	T1h (10ug)
1:1	-2.03	-1.7
1:25	16.43	16.57
1:50	10.57	18.37

Figure 11

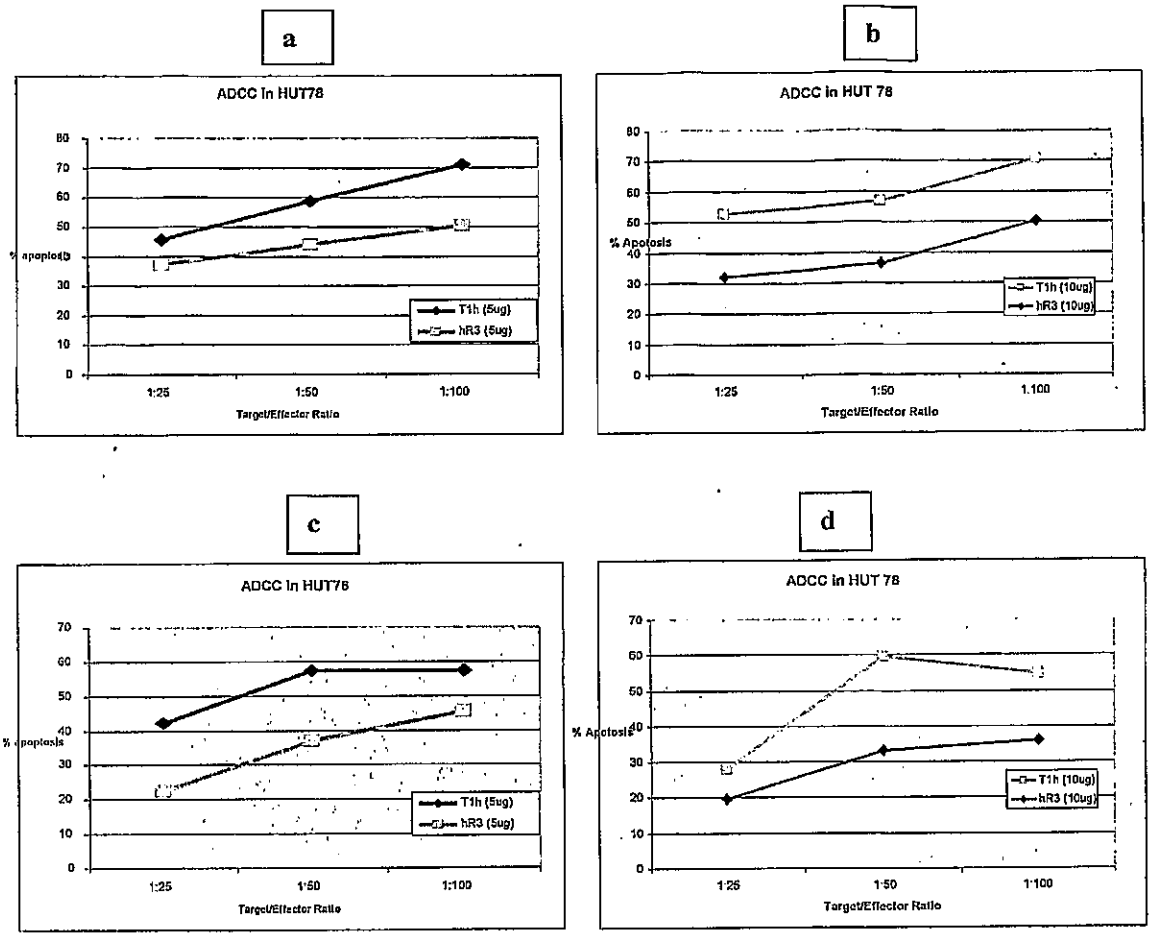

a
ADCC In HUT78
% apoptosis
Target/Effector Ratio
T1h (5ug)
hR3 (5ug)
b
ADCC in HUT 78
% Apotosis
Target/Effector Ratio
T1h (10ug)
hR3 (10ug)
c
ADCC In HUT78
% apoptosis
Target/Effector Ratio
T1h (5ug)
hR3 (5ug)
d
ADCC in HUT 78
% Apotosis
Target/Effector Ratio
T1h (10ug)
hR3 (10ug)

Figure 12

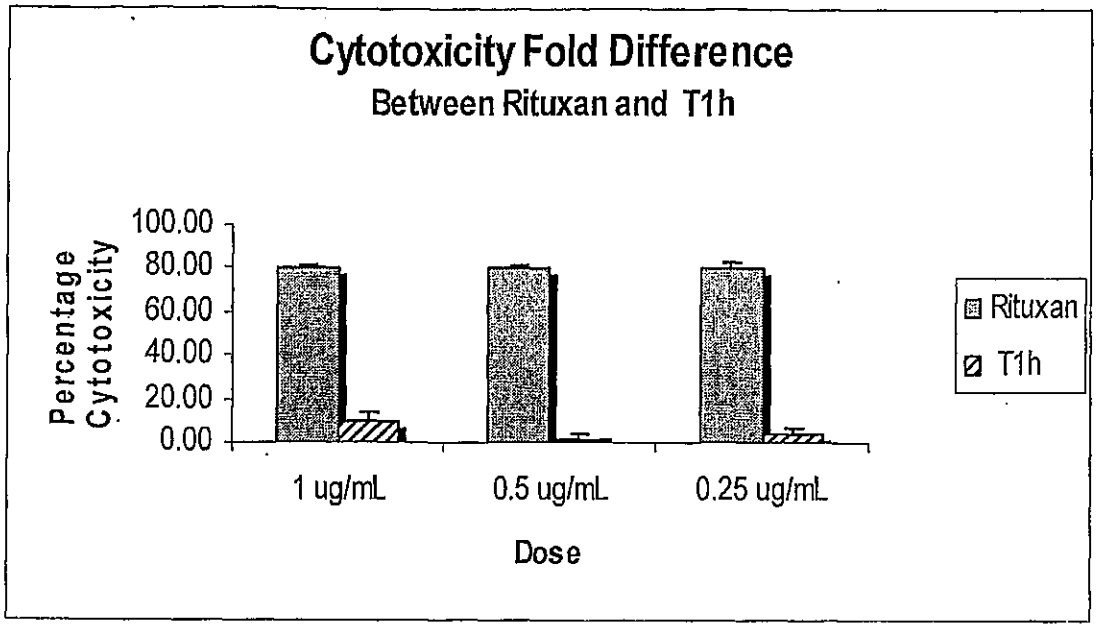

Cytotoxicity Fold Difference
Between Rituxan and T1h
Percentage Cytotoxicity
100.00
80.00
60.00
40.00
20.00
0.00
1 ug/mL
0.5 ug/mL
0.25 ug/mL
Dose
Rituxan
T1h

Figure 13

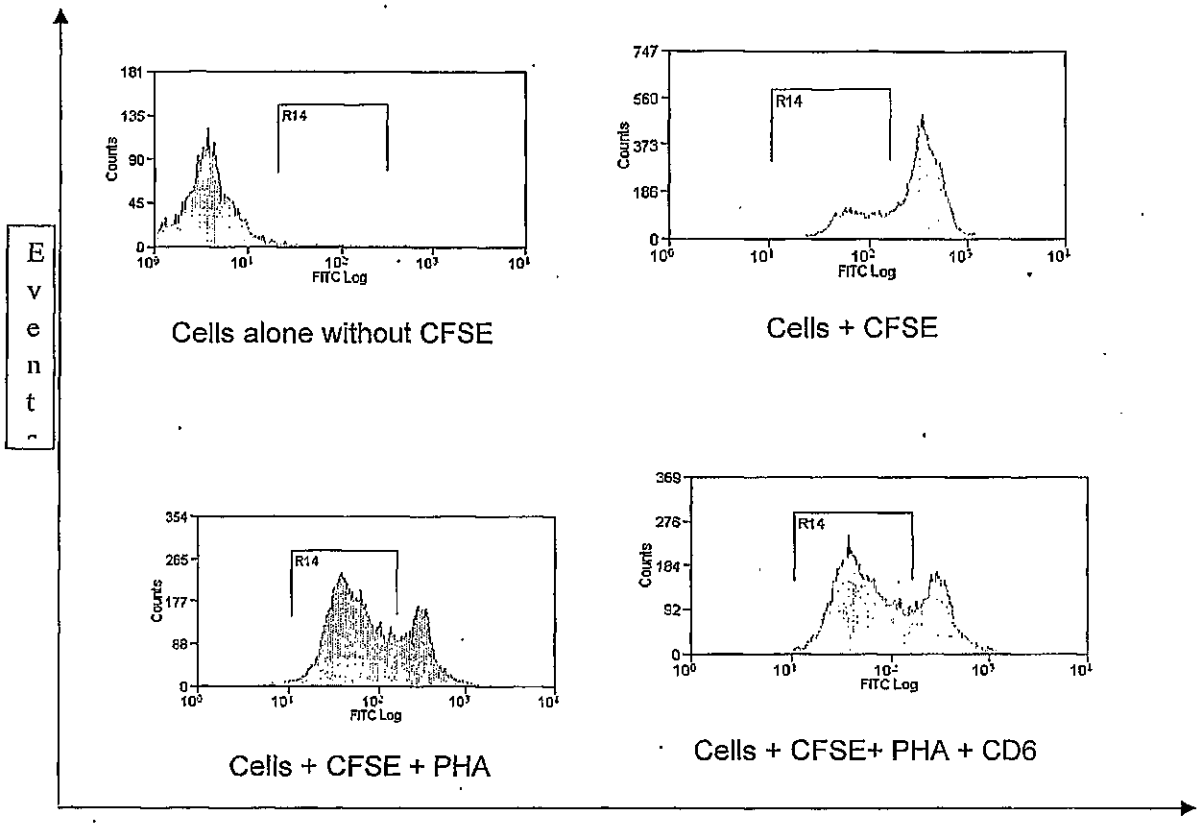

Event
Counts
FITC Log
R14
Cells alone without CFSE
Cells + CFSE
Cells + CFSE + PHA
Cells + CFSE+ PHA + CD6

Figure 14

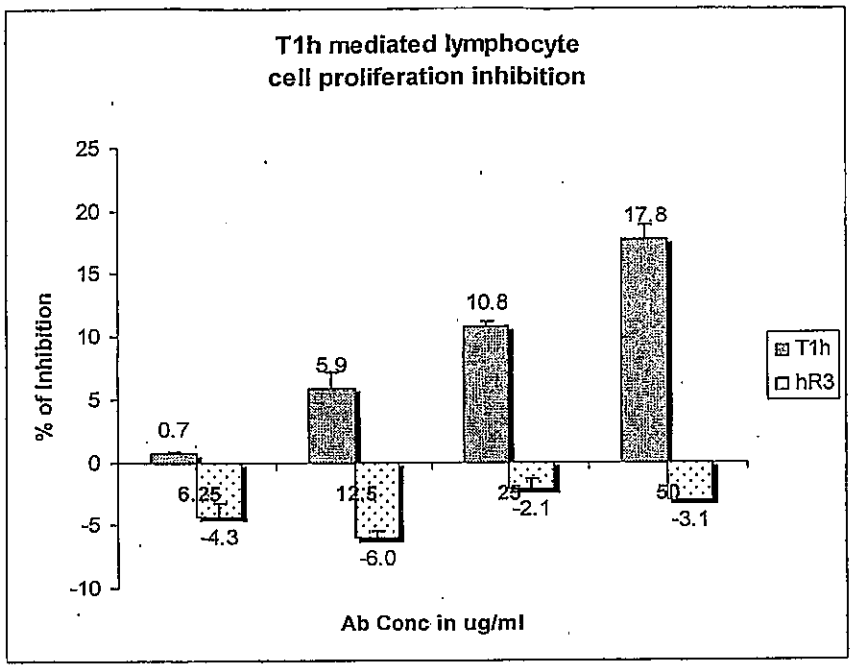

T1h mediated lymphocyte
cell proliferation inhibition
% of Inhibition
25
20
15
10
5
0
-5
-10
0.7
-4.3
5.9
-6.0
10.8
-2.1
17.8
-3.1
6.25
12.5
25
50
T1h
hR3
Ab Conc in ug/ml

Figure 15

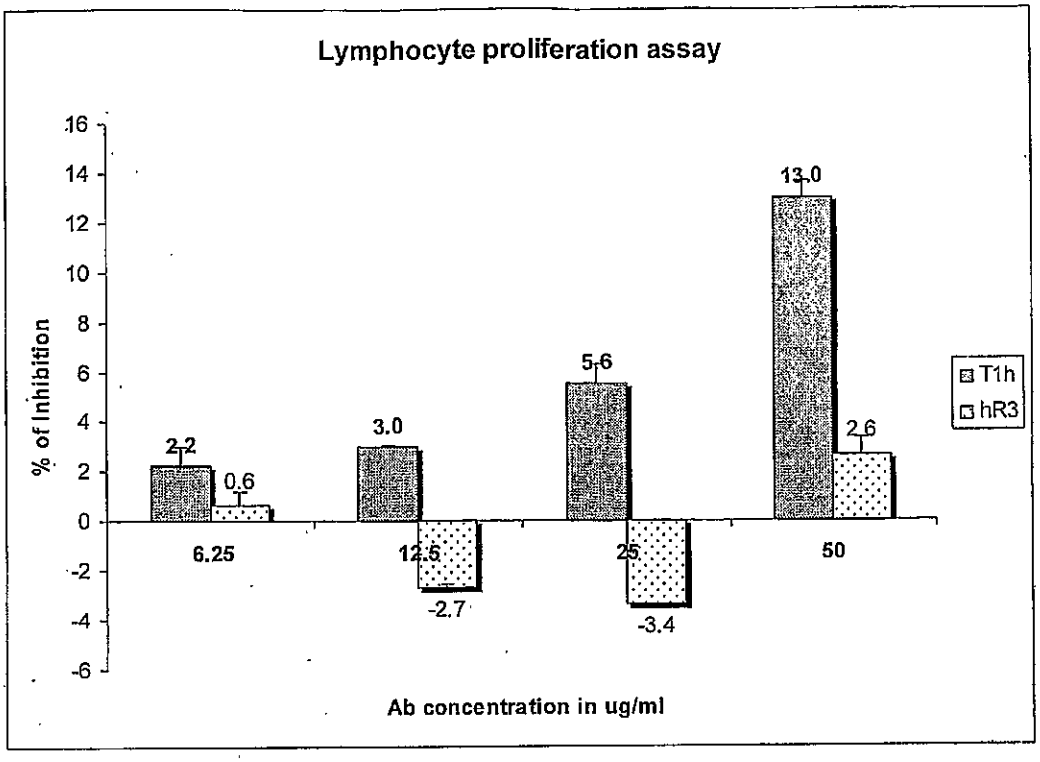
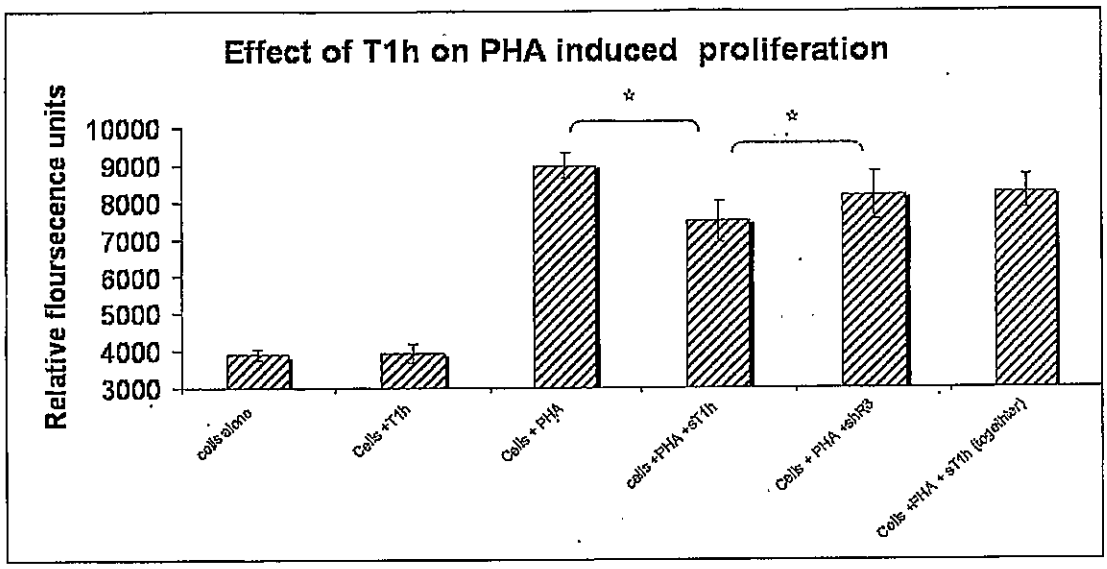

Lymphocyte proliferation assay
% of Inhibition
16
14
12
10
8
6
4
2
0
-2
-4
-6
2.2
0.6
3.0
-2.7
5.6
-3.4
13.0
2.6
6.25
12.5
25
50
Ab concentration in ug/ml
T1h
hR3

Figure 16



	media/sT1h	sT1h / shR3
Ttest	0.0023	0.0225

Figure 17

	1	2	3	4	5	6	7	8	9	10	11	12
A	CD3 1ug/ml	CD3 1ug/ml	CD3 1ug/ml	CD3 1ug/ml	CD3 1ug/ml+ T1h	CD3 1ug/ml + T1h	CD3 1ug/ml + T1h	CD3 1ug/ml + T1h	CD3 1ug/ml + Alcam	CD3 1ug/ml + Alcam	CD3 1ug/ml + Alcam	CD3 1ug/ml + Alcam
B	CD3 0.5ug/ml	CD3 0.5ug/ml	CD3 0.5ug/ml	CD3 0.5ug/ml	CD3 0.5ug/ml + T1h	CD3 0.5ug/ml + T1h	CD3 0.5ug/ml + T1h	CD3 0.5ug/ml + T1h	CD3 0.5ug/ml + alcam	CD3 0.5ug/ml + alcam	CD3 0.5ug/ml + alcam	CD3 0.5ug/ml + alcam
C	CD3 0.25ug/ml	CD3 0.25ug/ml	CD3 0.25ug/ml	CD3 0.25ug/ml	CD3 0.25ug/ml + T1h	CD3 0.25ug/ml + T1h	CD3 0.25ug/ml + T1h	CD3 0.25ug/ml + T1h	CD3 0.25ug/ml + Alcam	CD3 0.25ug/ml + Alcam	CD3 0.25ug/ml + Alcam	CD3 0.25ug/ml + Alcam
D	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml	CD3 0.125ug/ml
E	CD3 0.625ug/ml	CD3 0.625ug/ml	CD3 0.625ug/ml	CD3 0.625ug/ml	CD3 0.625ug/ml + T1h	CD3 0.625ug/ml + T1h	CD3 0.625ug/ml + T1h	CD3 0.625ug/ml + T1h	CD3 0.625ug/ml Alcam	CD3 0.625ug/ml Alcam	CD3 0.625ug/ml Alcam	CD3 0.625ug/ml Alcam
F	No Anitbody	No Anitbody	No Anitbody	T1h1 ug/ml;	T1h1 ug/ml;	T1h1 ug/ml;	hR31 ug/ml	hR31 ug/ml	hR31 ug/ml	hR31 ug/ml	hR31 ug/ml	hR31 ug/ml
G	No Anitbody	No Anitbody	No Anitbody	T1h1 ug/ml;	T1h1 ug/ml;	T1h1 ug/ml;	hR31 ug/ml	hR31 ug/ml	hR31 ug/ml	hR31 ug/ml	hR31 ug/ml	hR31 ug/ml
H												

Figure 18

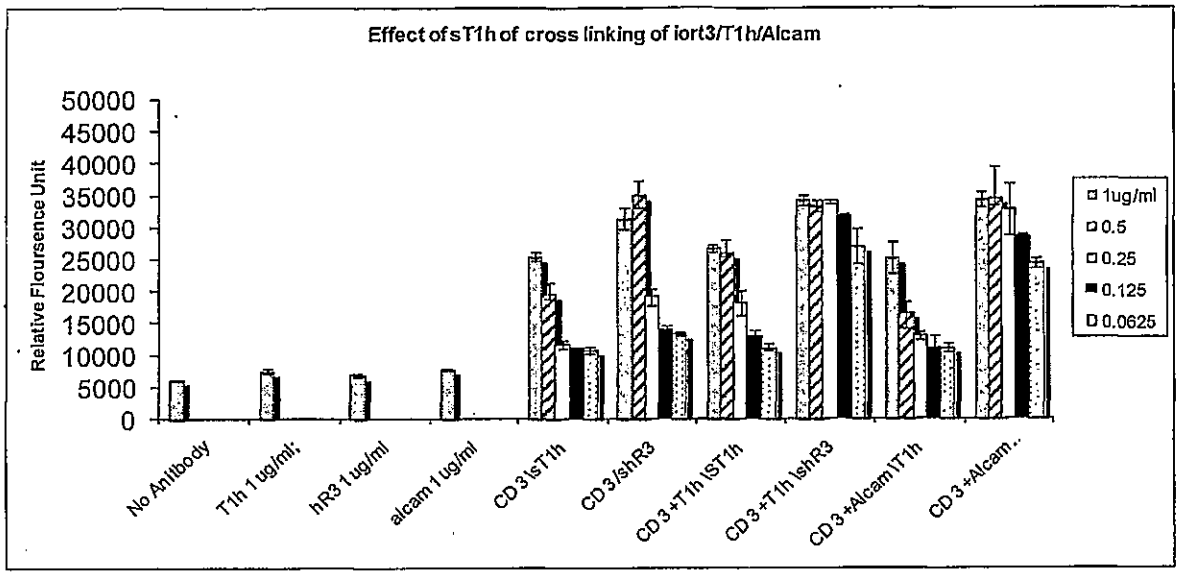
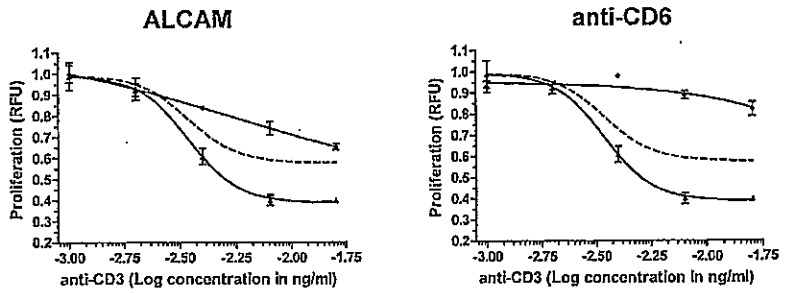

Effect of sT1h of cross linking of iort3/T1h/Alcam
Relative Flourscence Unit
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
No Antibody
T1h 1 ug/ml;
hR3 1 ug/ml
alcam 1 ug/ml
CD3 1sT1h
CD3 /shR3
CD3 +T1h lST1h
CD3 +T1h lshR3
CD3 +AlcamIT1h
CD3 +Alcam..
1ug/ml
0.5
0.25
0.125
0.0625

Figure 19



ALCAM: Four parameters logistic fit

T1h: Sigmoidal dose-response

Control: hR3 (anti-human EGFR mAb)

anti-CD6: T1h (anti-human CD6 mAb)

Concentration: 10µg/mL soluble and 1 µg/mL tethered

RFU: Relative Fluorescence Unit (Alamar Blue)

Incubation: 5 days

Number of cells: 30000 cells/ well

Bliss additivism model

-predicts the combined response *C* for two single compounds with effects *A* and *B* is:

$$C = A + B - A \cdot B$$

where each effect is expressed as fractional inhibition between 0 and 1. These effect-based synergy models make no assumptions about the functional form of the dose-response curves, and do not require dose-response information that lies outside the range sampled by each screening matrix.

Figure 20

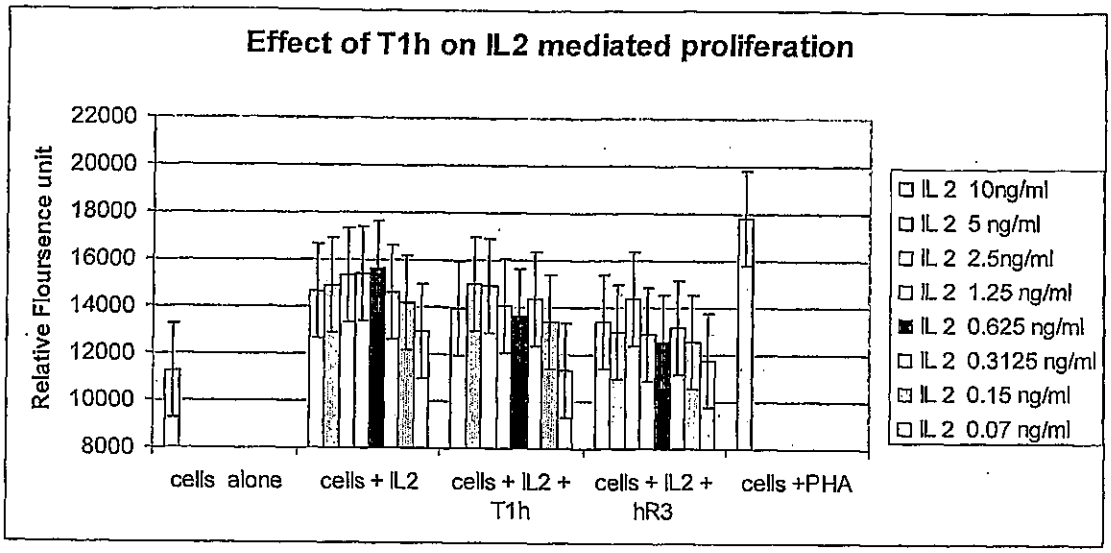

Effect of T1h on IL2 mediated proliferation
Relative Flourscence unit
22000
20000
18000
16000
14000
12000
10000
8000
cells alone
cells + IL2
cells + IL2 + T1h
cells + IL2 + hR3
cells +PHA
IL 2 10ng/ml
IL 2 5 ng/ml
IL 2 2.5ng/ml
IL 2 1.25 ng/ml
IL 2 0.625 ng/ml
IL 2 0.3125 ng/ml
IL 2 0.15 ng/ml
IL 2 0.07 ng/ml

Figure 21

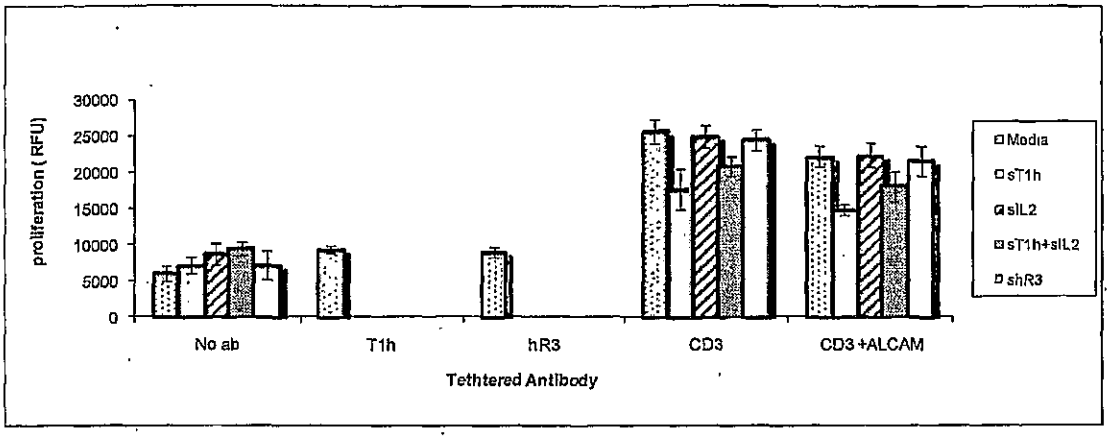

proliferation (RFU)
30000
25000
20000
15000
10000
5000
0
No ab
T1h
hR3
CD3
CD3 +ALCAM
Tethtered Antibody
Media
sT1h
slL2
sT1h+slL2
shR3

Figure 22

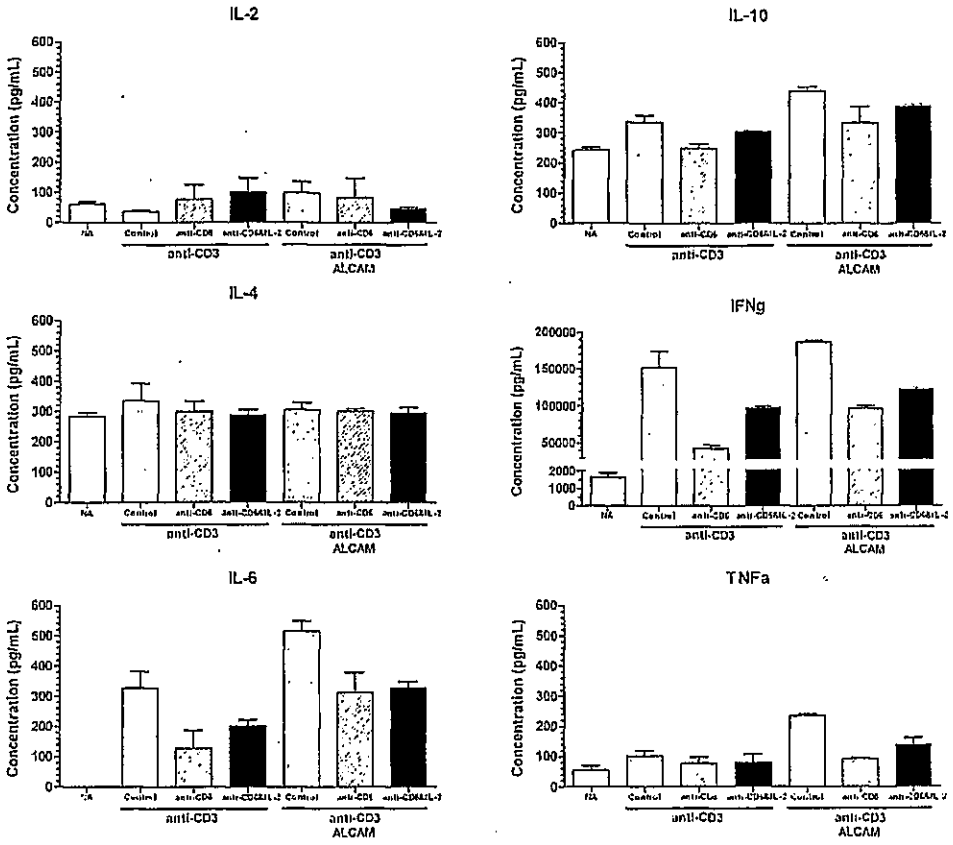

IL-2
IL-10
IL-4
IFNg
IL-6
TNFa
Concentration (pg/mL)
anti-CD3
anti-CD3
ALCAM

Figure 23

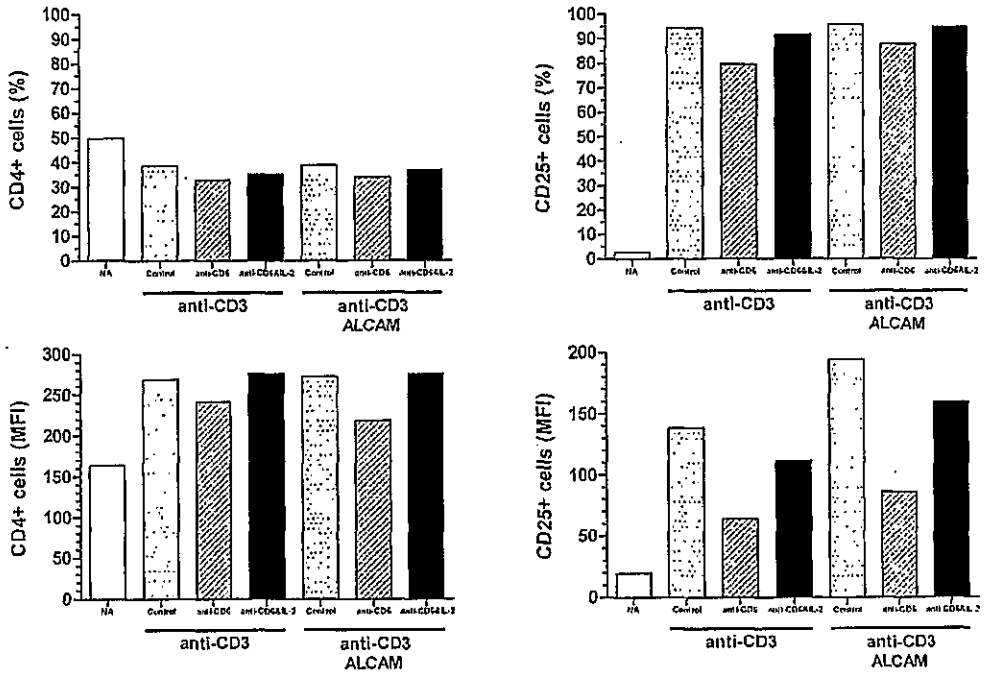

CD4+ cells (%)
CD25+ cells (%)
CD4+ cells (MFI)
CD25+ cells (MFI)
NA
Control
anti-CD3
anti-CD3 ALCAM

Figure 24

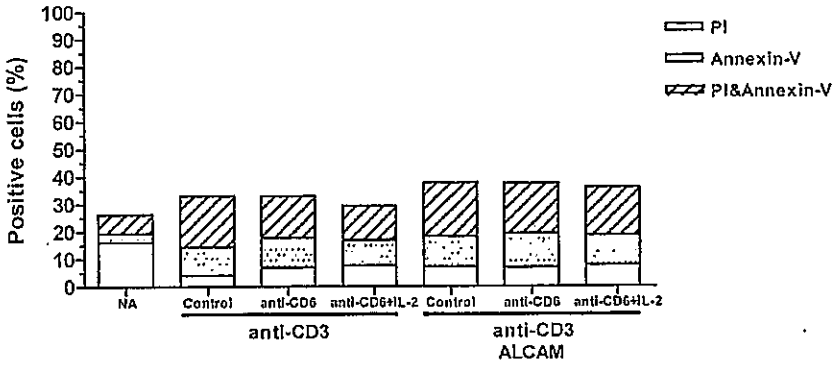

Positive cells (%)
100
90
80
70
60
50
40
30
20
10
0
PI
Annexin-V
PI&Annexin-V
NA
Control
anti-CD6
anti-CD6+IL-2
Control
anti-CD6
anti-CD6+IL-2
anti-CD3
anti-CD3
ALCAM

Figure 25

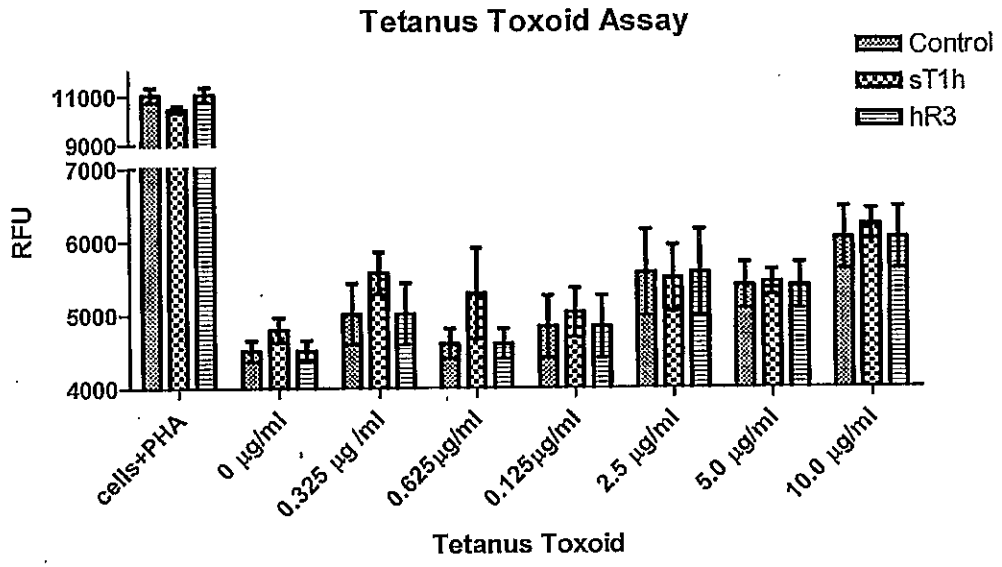

Tetanus Toxoid Assay
Control
sT1h
hR3
RFU
11000
9000
7000
6000
5000
4000
cells+PHA
0 µg/ml
0.325 µg /ml
0.625µg/ml
0.125µg/ml
2.5 µg/ml
5.0 µg/ml
10.0 µg/ml
Tetanus Toxoid

Figure 26

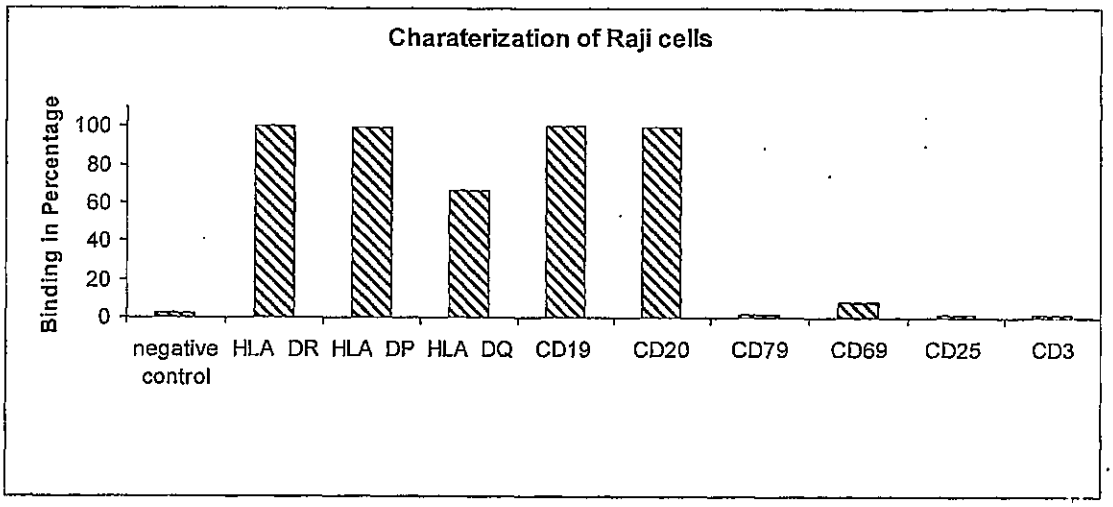

Charaterization of Raji cells
Binding In Percentage
100
80
60
40
20
0
negative control
HLA DR
HLA DP
HLA DQ
CD19
CD20
CD79
CD69
CD25
CD3

Figure 27

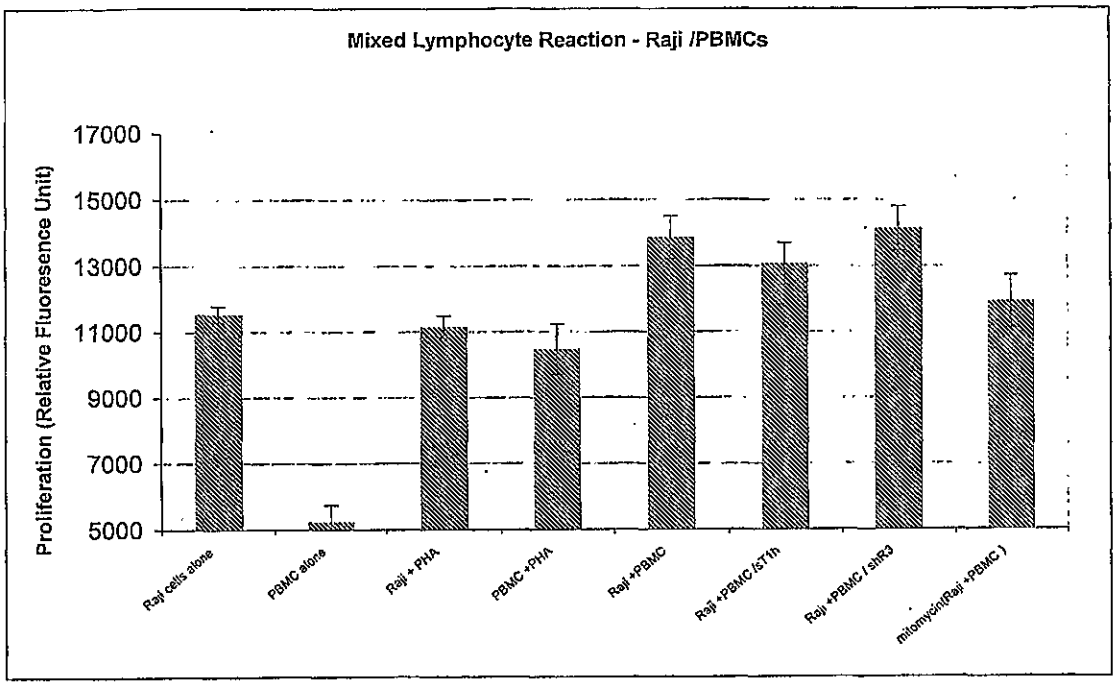

Mixed Lymphocyte Reaction - Raji /PBMCs
Proliferation (Relative Fluoresence Unit)
17000
15000
13000
11000
9000
7000
5000
Raji cells alone
PBMC alone
Raji + PHA
PBMC +PHA
Raji +PBMC
Raji +PBMC /sT1h
Raji +PBMC / shR3
mitomycin(Raji +PBMC)

Figure 28

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		PBL	PBL	PBL	PBL	PBL	PBL+DC+ T1h40	PBL+DC+ T1h40	PBL+DC+ T1h40	PBL+DC+ T1h40	PBL+DC+ T1h40	
C		DC	DC	DC	DC	DC	PBL+DC+ T1h20	PBL+DC+ T1h20	PBL+DC+ T1h20	PBL+DC+ T1h20	PBL+DC+ T1h20	
D		PBL(M)+ DC	PBL(M)+ DC	PBL(M)+ DC	PBL(M)+ DC	PBL(M)+ DC	PBL+DC+ T1h10	PBL+DC+ T1h10	PBL+DC+ T1h10	PBL+DC+ T1h10	PBL+DC+ T1h10	
E		PBL+DC	PBL+DC	PBL+DC	PBL+DC	PBL+DC	PBL+DC+ Pim100	PBL+DC+ Pim100	PBL+DC+ Pim100	PBL+DC+ Pim100	PBL+DC+ Pim100	
F		PBL+DC+ hR3 40	PBL+DC+ hR3 40	PBL+DC+ hR3 40	PBL+DC+ hR3 40	PBL+DC+ hR3 40	PBL+DC+ Pim50	PBL+DC+ Pim50	PBL+DC+ Pim50	PBL+DC+ Pim50	PBL+DC+ Pim50	
G		PBL+DC+ hR3 20	PBL+DC+ hR3 20	PBL+DC+ hR3 20	PBL+DC+ hR3 20	PBL+DC+ hR3 20	PBL+DC+ Pim25	PBL+DC+ Pim25	PBL+DC+ Pim25	PBL+DC+ Pim25	PBL+DC+ Pim25	
H												

Figure 29

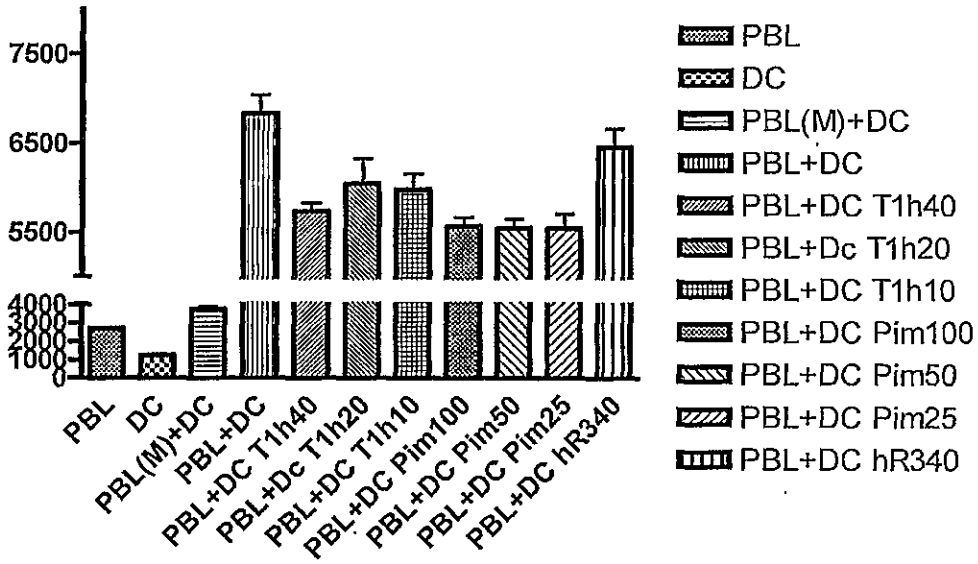

7500
6500
5500
4000
3000
2000
1000
0
PBL
DC
PBL(M)+DC
PBL+DC
PBL+DC T1h40
PBL+Dc T1h20
PBL+DC T1h10
PBL+DC Pim100
PBL+DC Pim50
PBL+DC Pim25
PBL+DC hR340

Figure 30

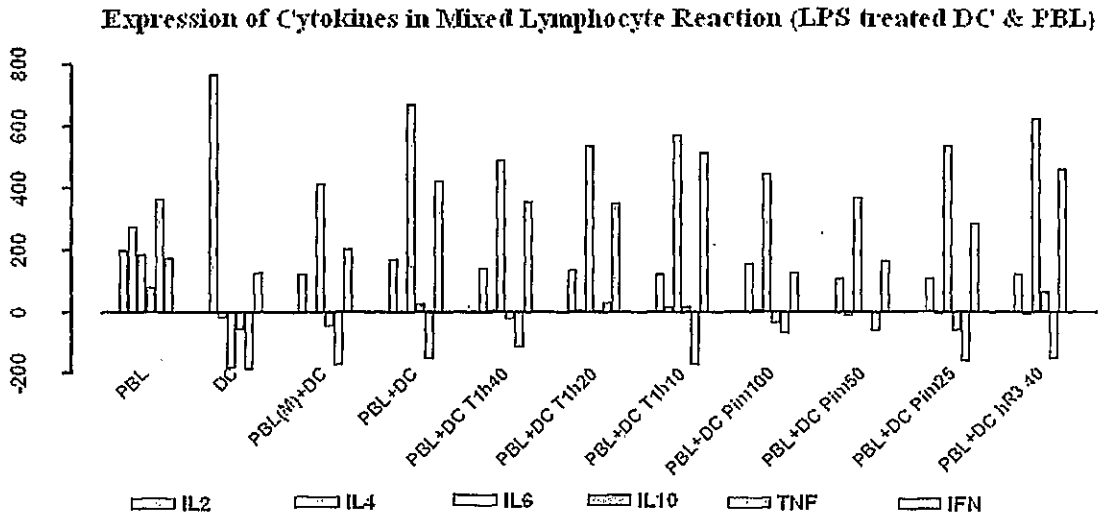

Expression of Cytokines in Mixed Lymphocyte Reaction (LPS treated DC & PBL)
800
600
400
200
0
-200
PBL
DC
PBL(M)+DC
PBL+DC
PBL+DC T1h40
PBL+DC T1h20
PBL+DC T1h10
PBL+DC Pim100
PBL+DC Pim50
PBL+DC Pim25
PBL+DC hR3 40
IL2
IL4
IL6
IL10
TNF
IFN

Figure 31