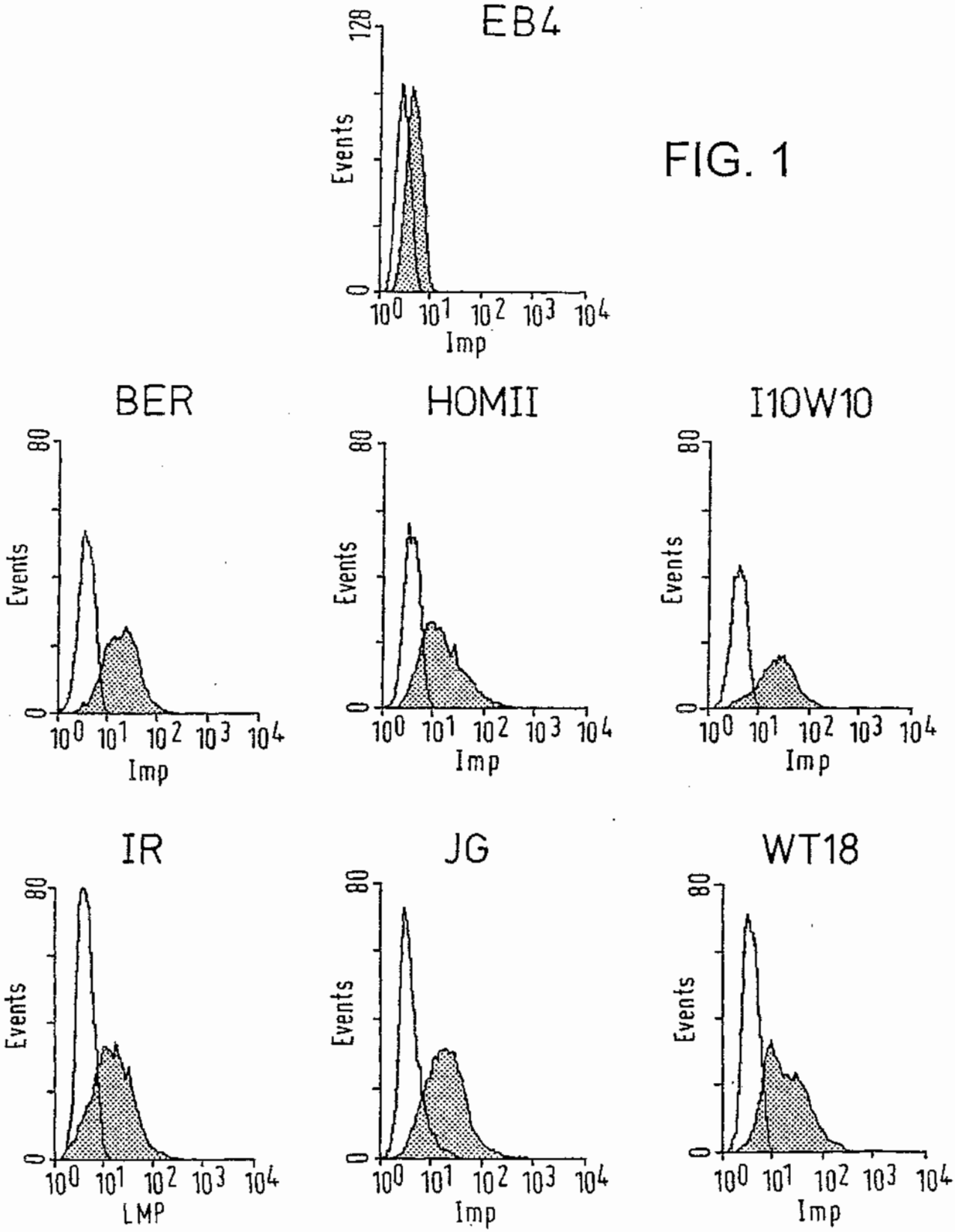


FIG. 1

LMP1 has been labelled on six defferent LCL lines using identical conditions. EB4 was used as a negative control cell line. Using flow cytometry analysis, the aount of LMP1 on these cell lines appears to be similar

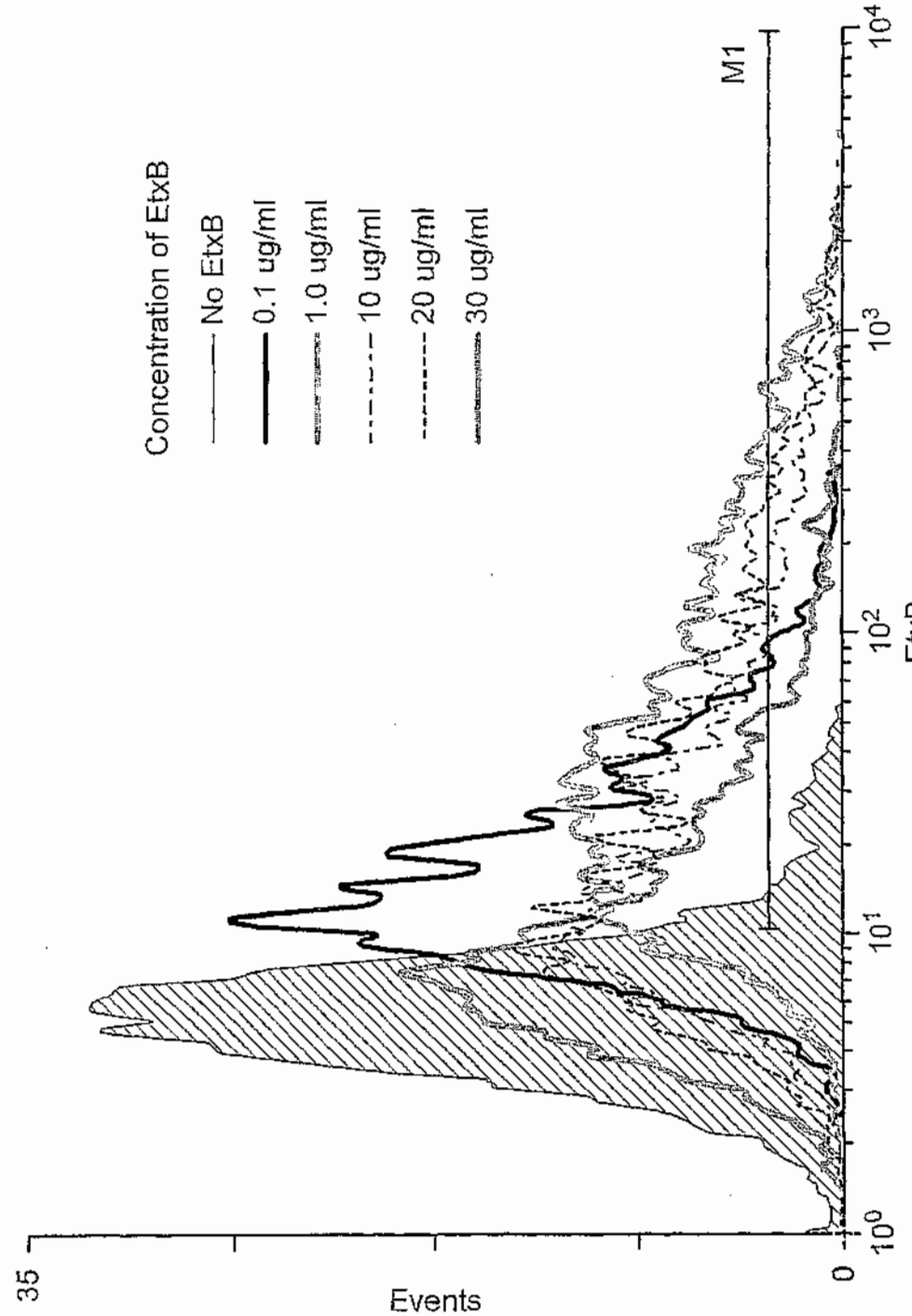
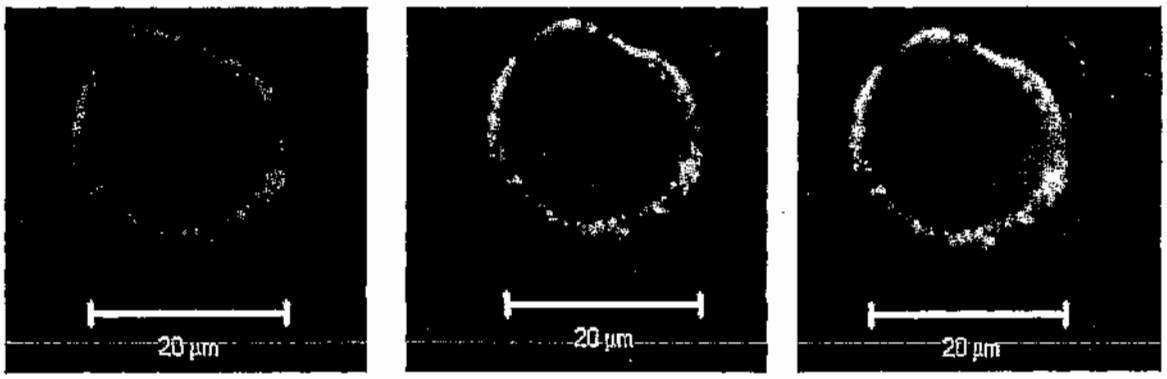


FIG. 2

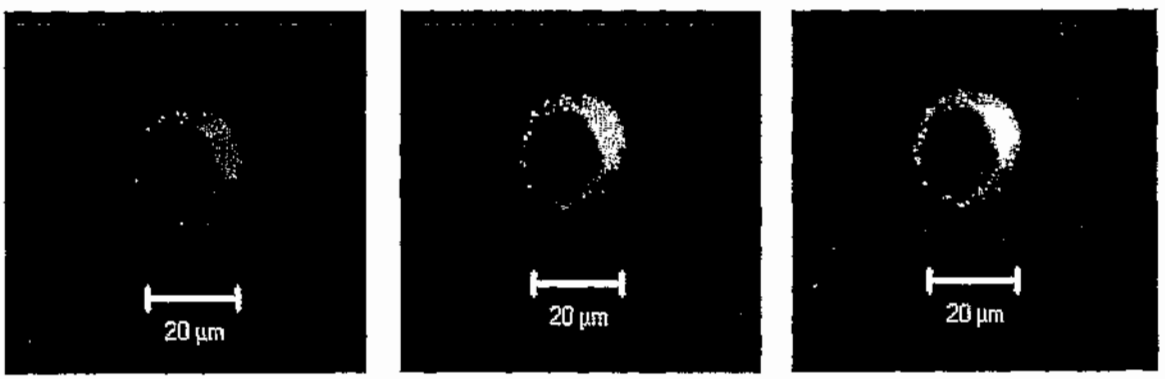
Binding of EtxB to LCL at varying concentrations. The region M1 represents the population of cells that are positively labelled for EtxB. The geometric means of each sub population is shown in table 1



EtxB LMP1 Overlay

FIG. 3

LCL treated with EtxB at 4°C showing binding of EtxB (red) onto the plasma membrane. LMP detected on the same cell was represented in green. Colocalisation of ExtB and LMP1 (seen in yellow), was clearly seen in the overlay.



EtxB LMP1 Overlay

FIG. 4

LCL treated with EtxB at 37°C showing capping of EtxB to one pole of the cell, resulting in a similar redistribution of LMP1. Both ExtB and LMP1 were not clearly seen within the cell. Again, colocalisation of ExtB and LMP1 was evident.

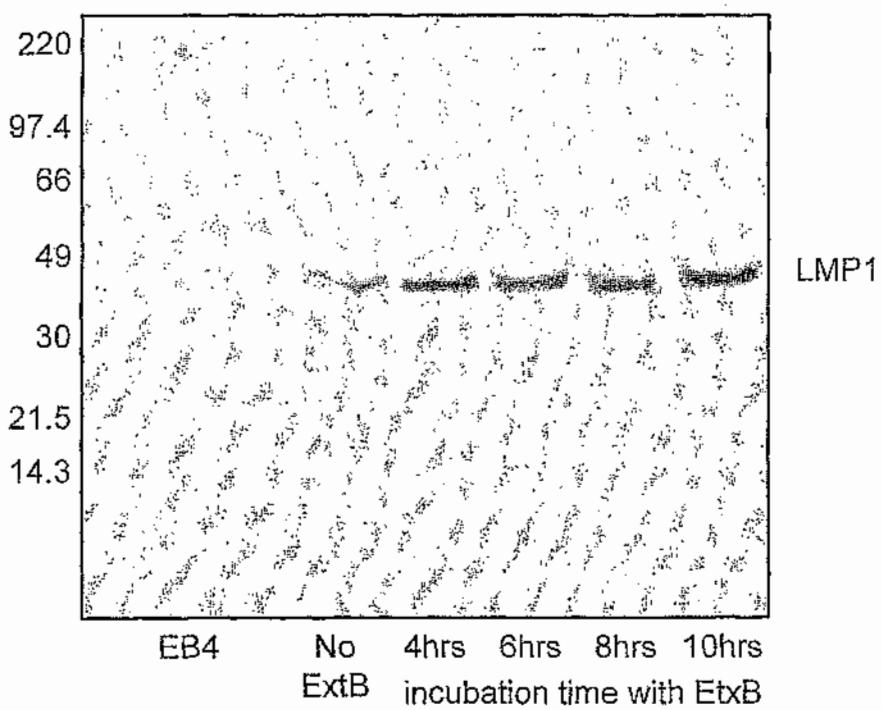


FIG. 5

Detection of LMP1 in LCLs following ExtB treatment using a minigel. The amount of intact LMP1 remains unchanged even after treating the cells with EtxB for up to 10 hours. Degradation fragments of LMP1 was also not seen.

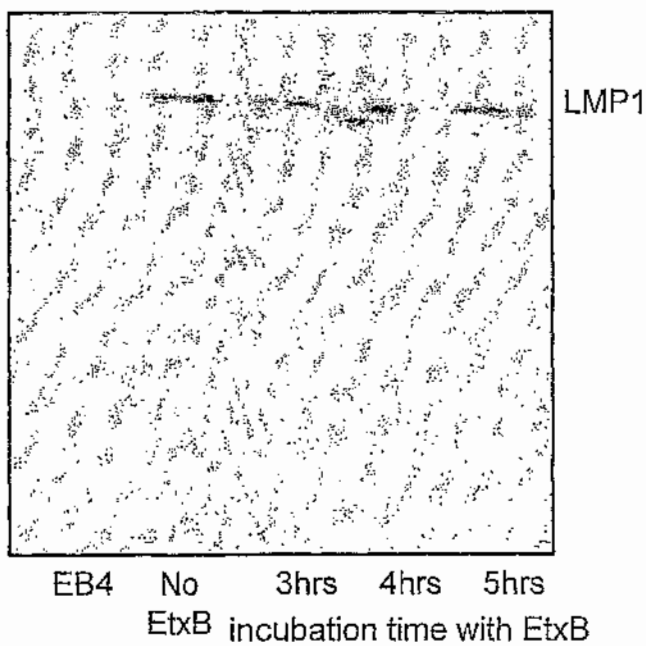


FIG. 6

Detection of LMP1 by Western blot following standard SDS-PAGE gel electrophoresis. Results were similar to that obtained using a minigel. No decrease in full-length LMP1 or any LMP1 fragments was seen.

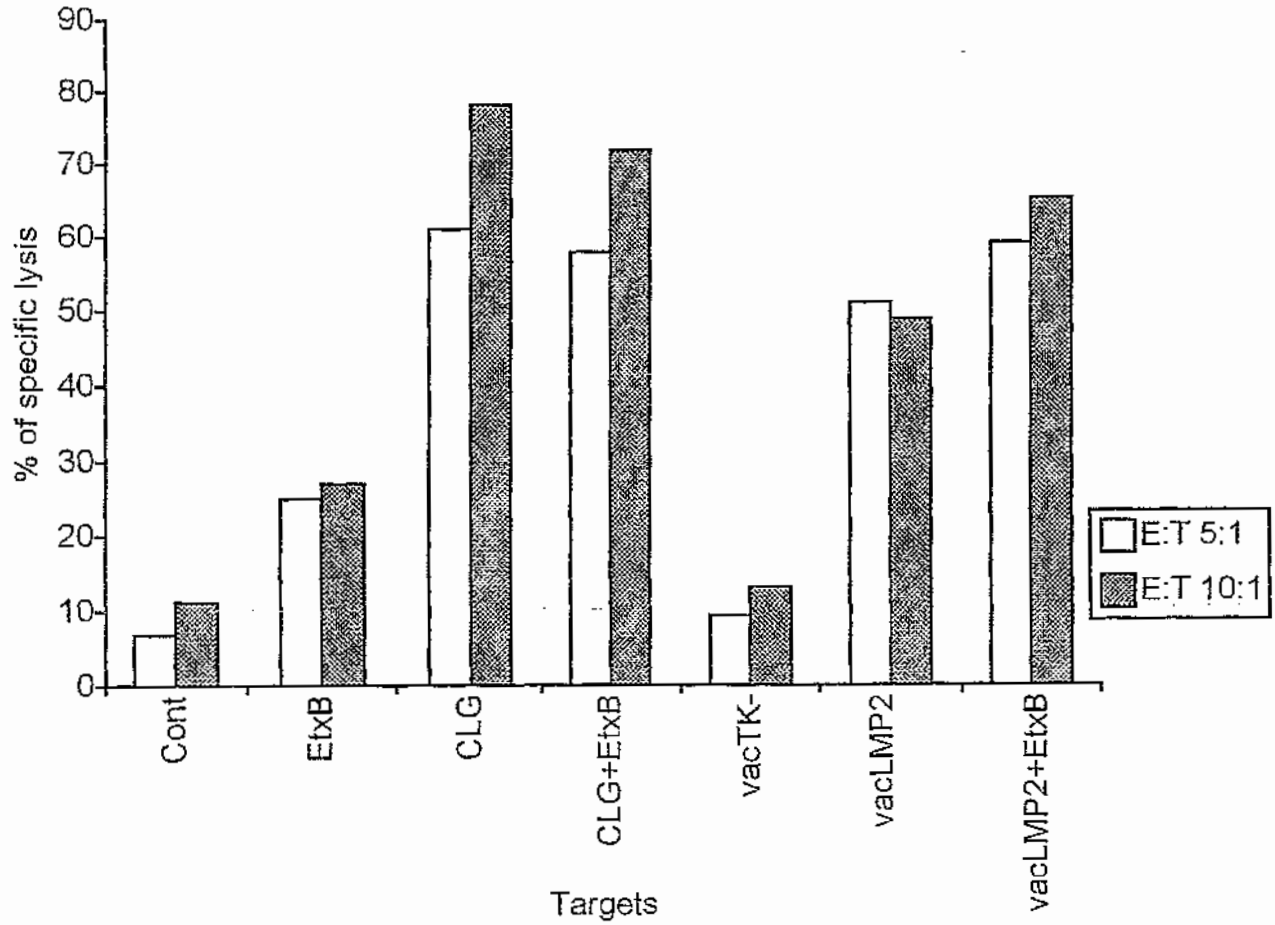

90
80
70
60
50
40
30
20
10
0
% of specific lysis
Cont
EtxB
CLG
CLG+EtxB
vacTK-
vacLMP2
vacLMP2+EtxB
Targets
E:T 5:1
E:T 10:1

FIG. 7

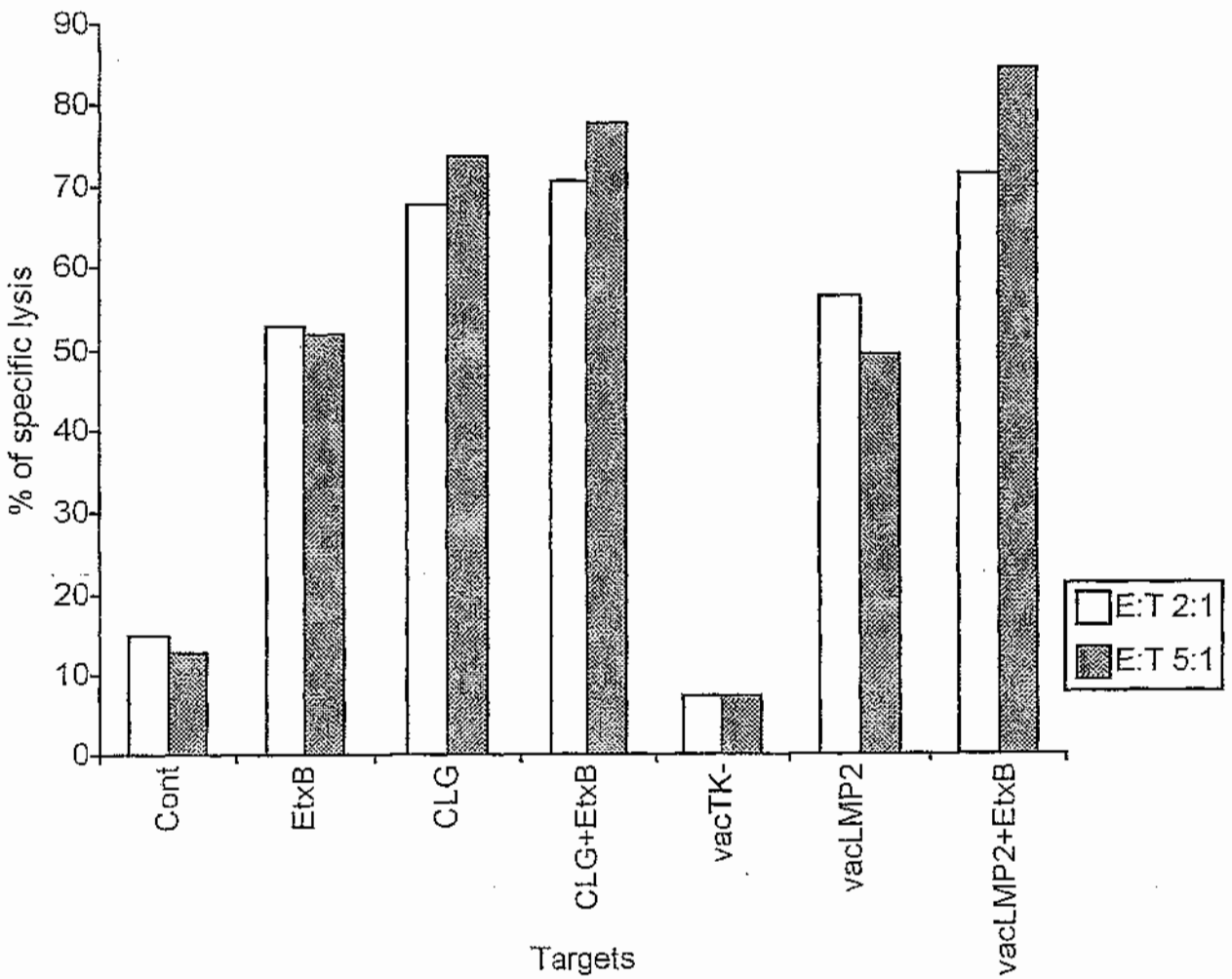

90
80
70
60
50
40
30
20
10
0
% of specific lysis
Cont
EtxB
CLG
CLG+EtxB
vacTK-
vacLMP2
vacLMP2+EtxB
Targets
E:T 2:1
E:T 5:1

FIG. 8

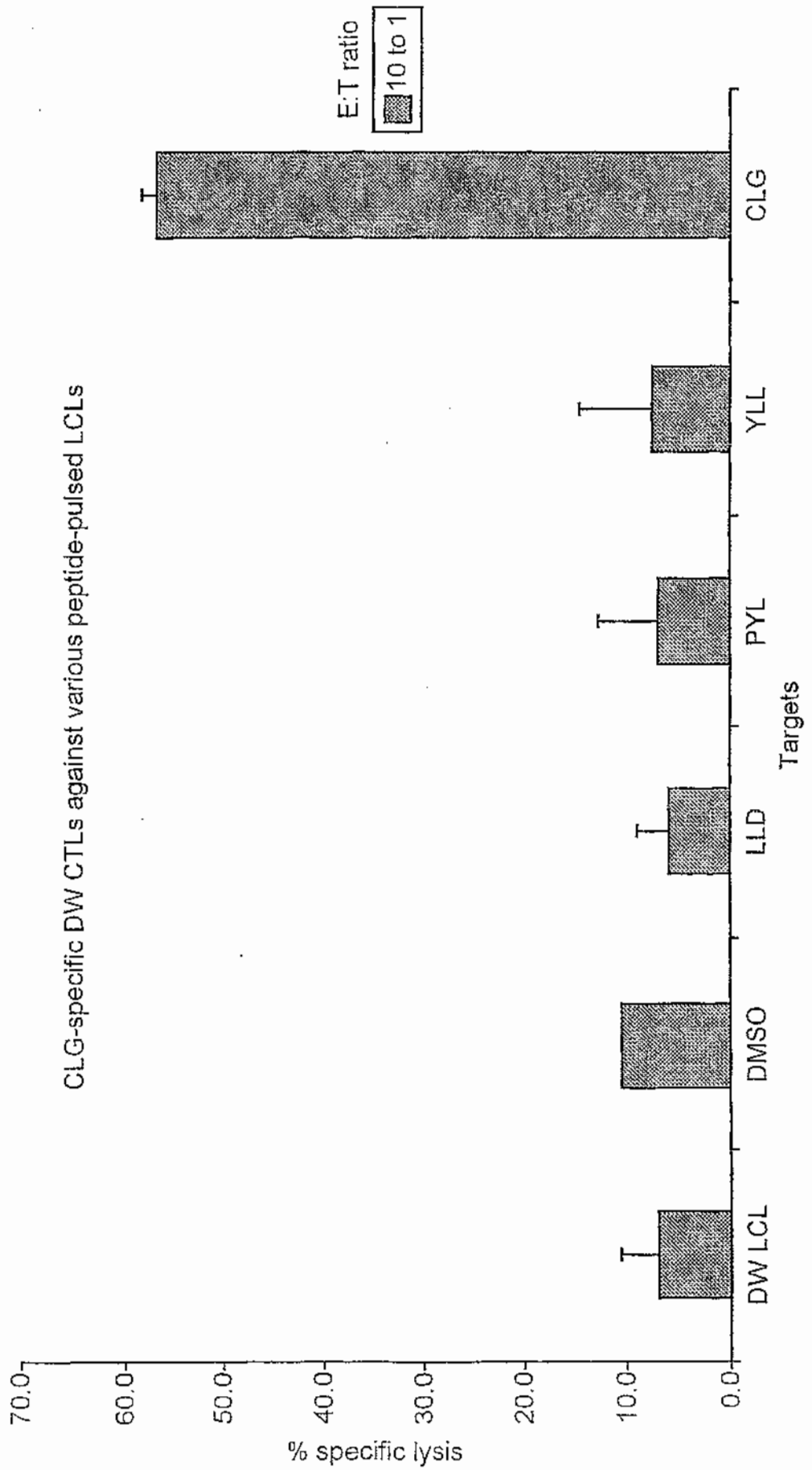

E:T ratio
10 to 1
CLG-specific DW CTLs against various peptide-pulsed LCLs
% specific lysis
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
DW LCL
DMSO
LLD
PYL
YLL
CLG
Targets

FIG. 9

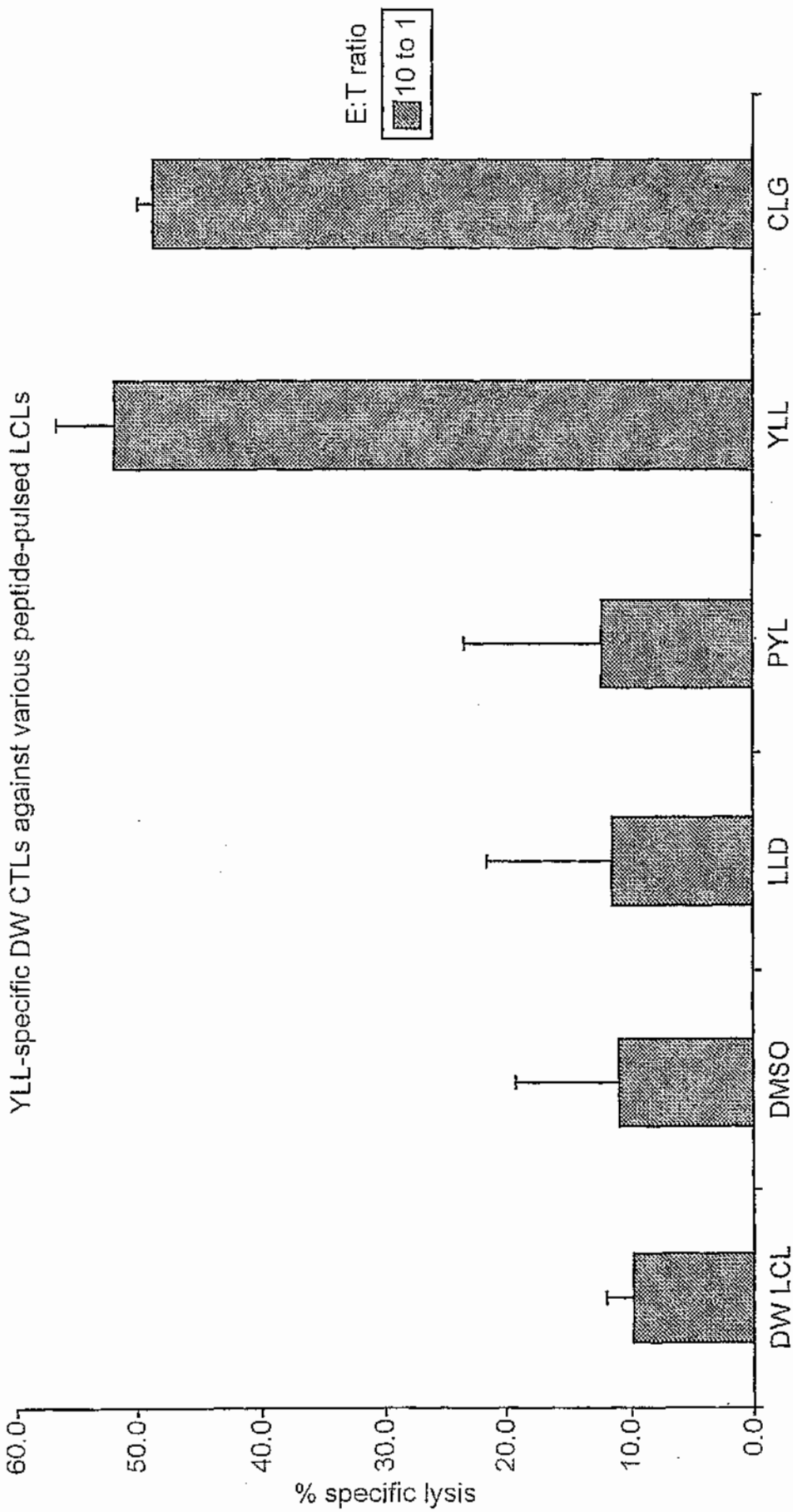

YLL-specific DW CTLs against various peptide-pulsed LCLs
E:T ratio
10 to 1
% specific lysis
60.0
50.0
40.0
30.0
20.0
10.0
0.0
DW LCL
DMSO
LLD
PYL
YLL
CLG
Targets

FIG. 10

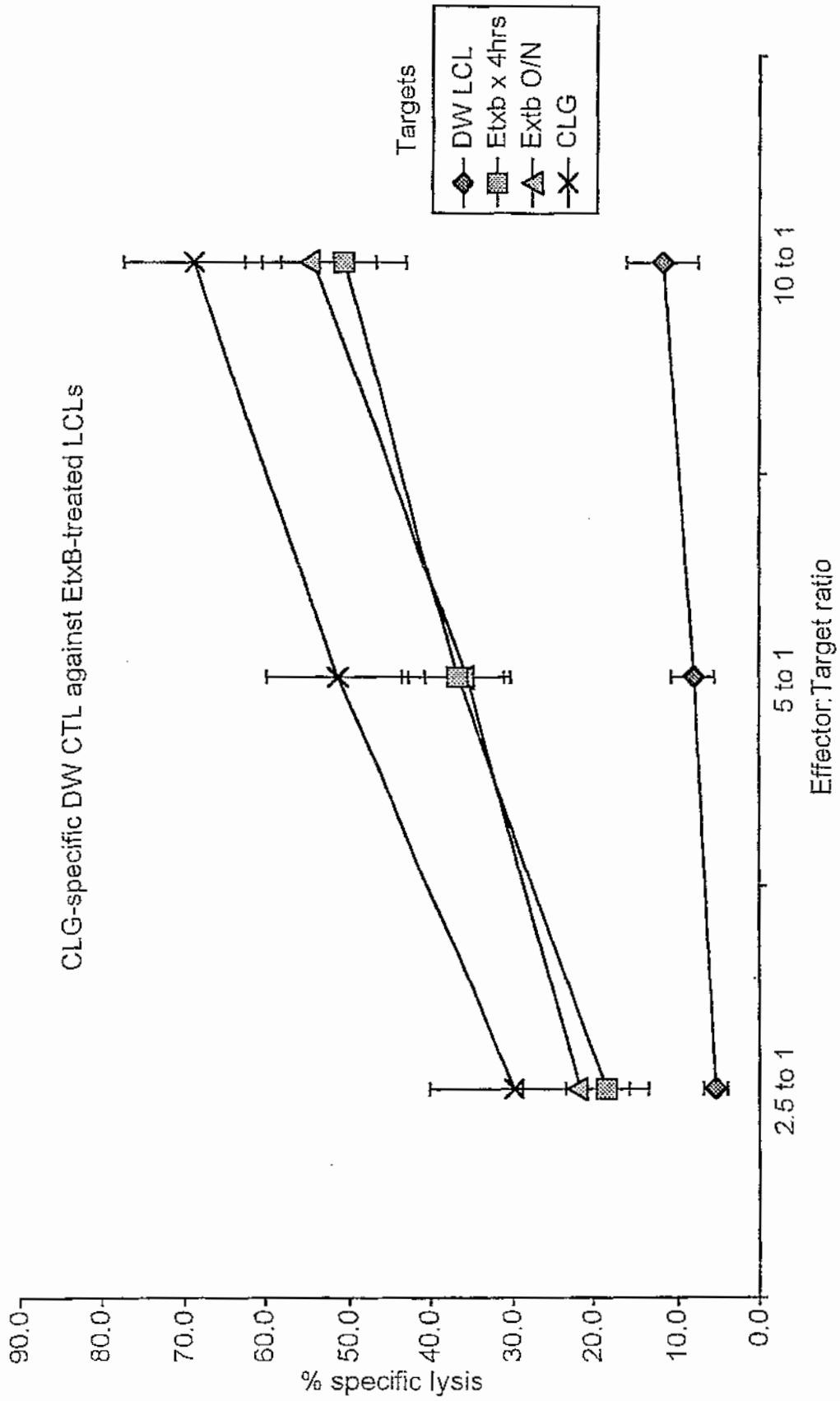

CLG-specific DW CTL against EtxB-treated LCLs
Targets
DW LCL
Etxb x 4hrs
Extb O/N
CLG
% specific lysis
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
2.5 to 1
5 to 1
10 to 1
Effector:Target ratio

FIG. 11

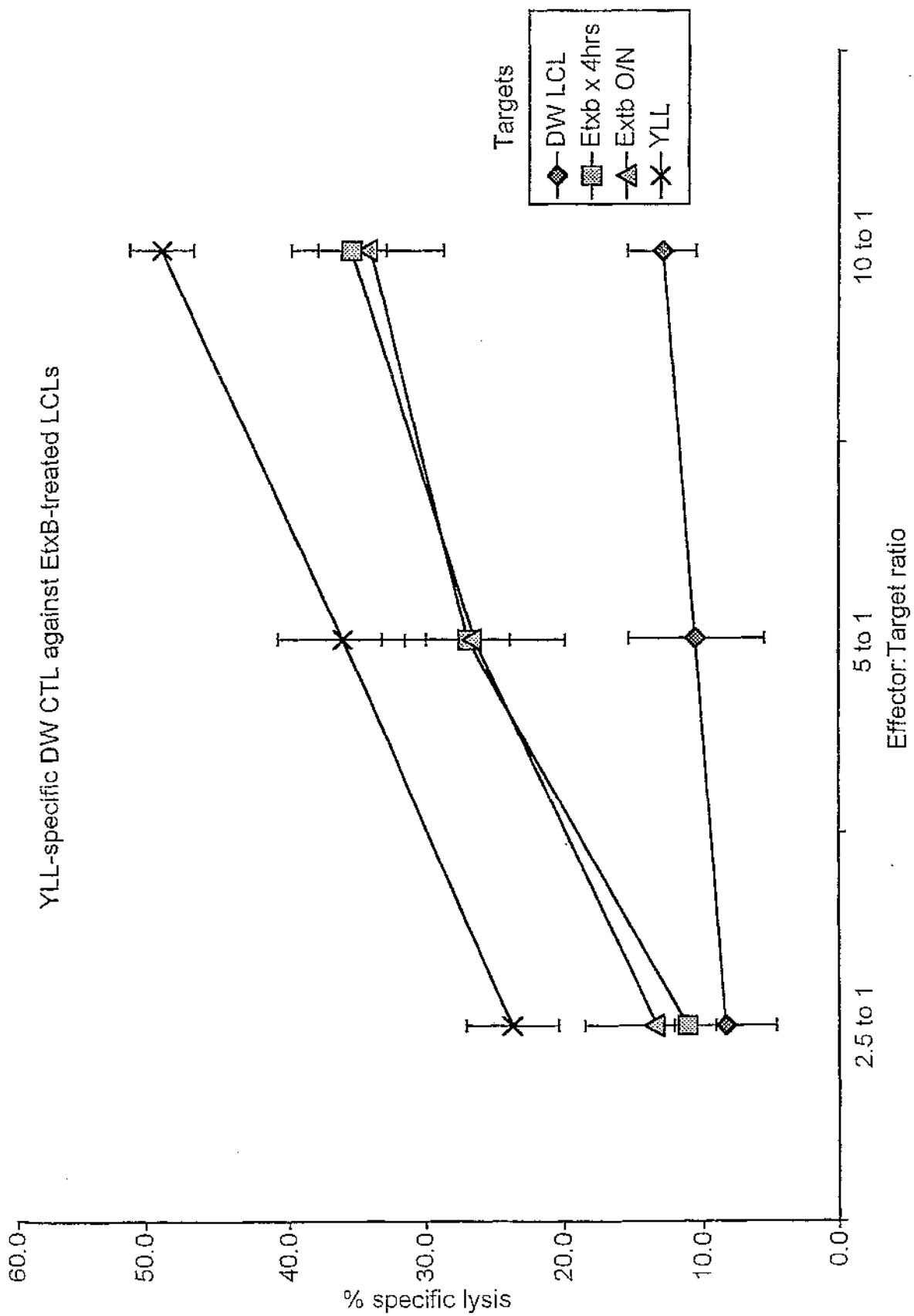

YLL-specific DW CTL against EtxB-treated LCLs
Targets
DW LCL
Etxb x 4hrs
Extb O/N
YLL
% specific lysis
60.0
50.0
40.0
30.0
20.0
10.0
0.0
2.5 to 1
5 to 1
10 to 1
Effector:Target ratio

FIG. 12

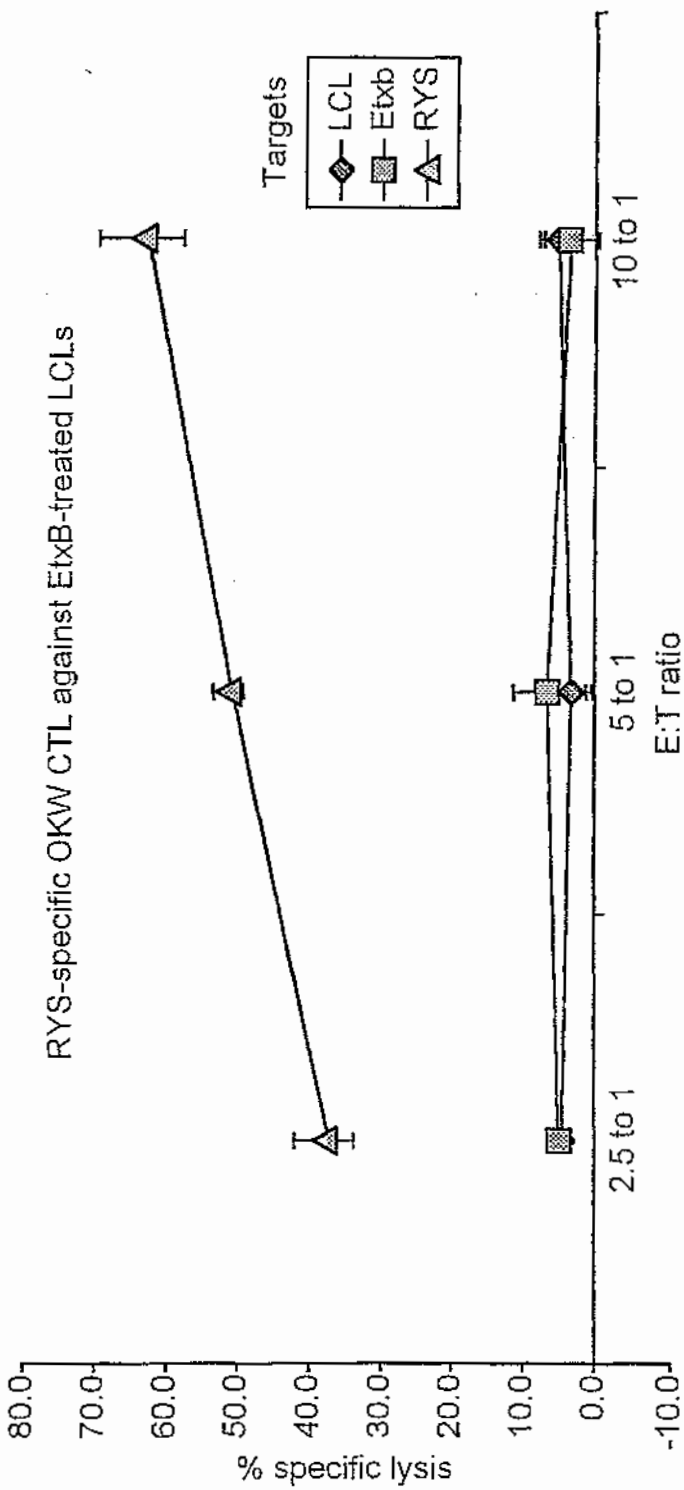

RYS-specific OKW CTL against EtxB-treated LCLs
Targets
LCL
Etxb
RYS
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
% specific lysis
2.5 to 1
5 to 1
10 to 1
E:T ratio

FIG. 13

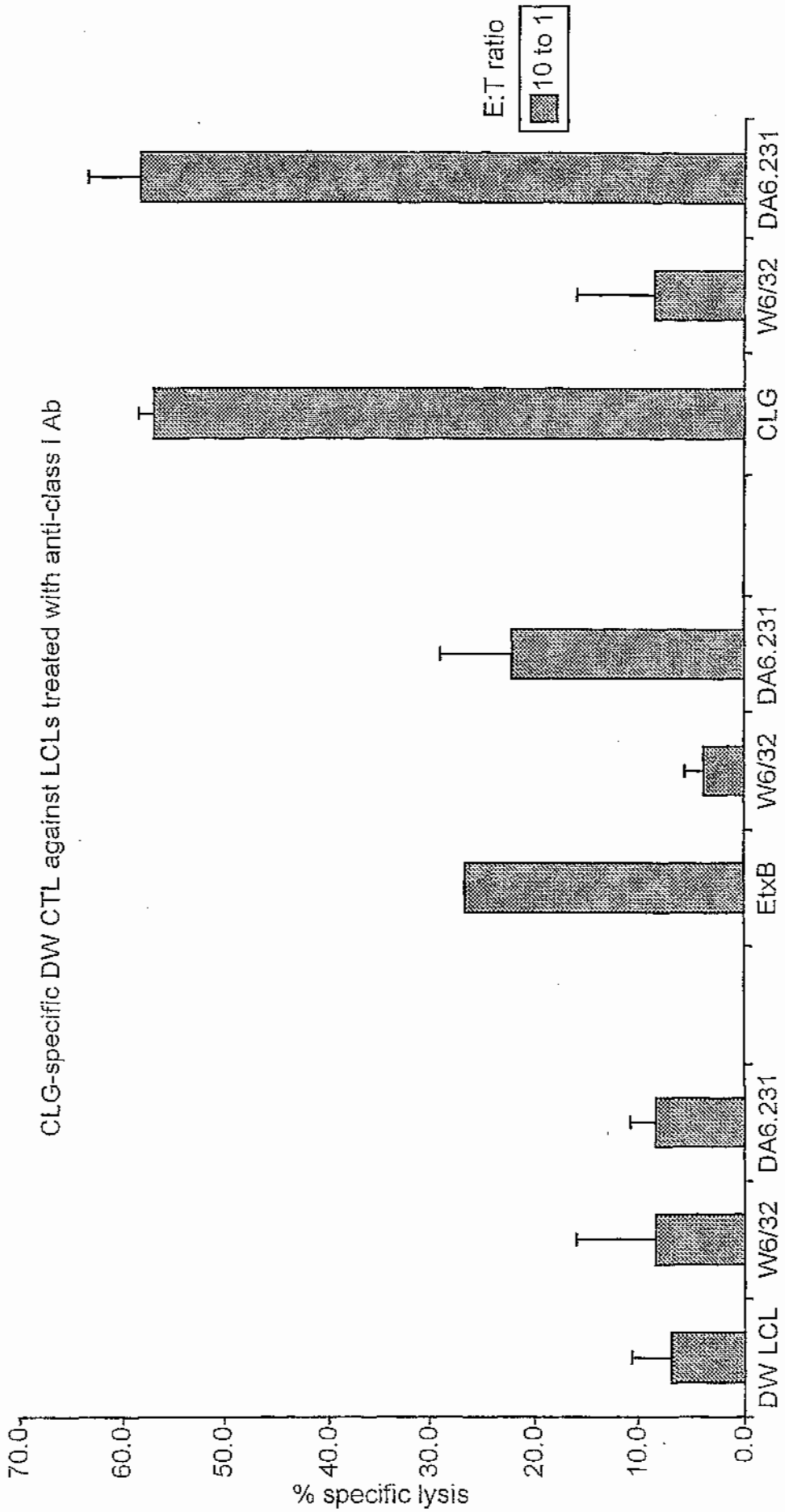

CLG-specific DW CTL against LCLs treated with anti-class I Ab
E:T ratio
10 to 1
% specific lysis
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
DW LCL
W6/32
DA6.231
EtxB
W6/32
DA6.231
CLG
W6/32
DA6.231
Targets

FIG. 14

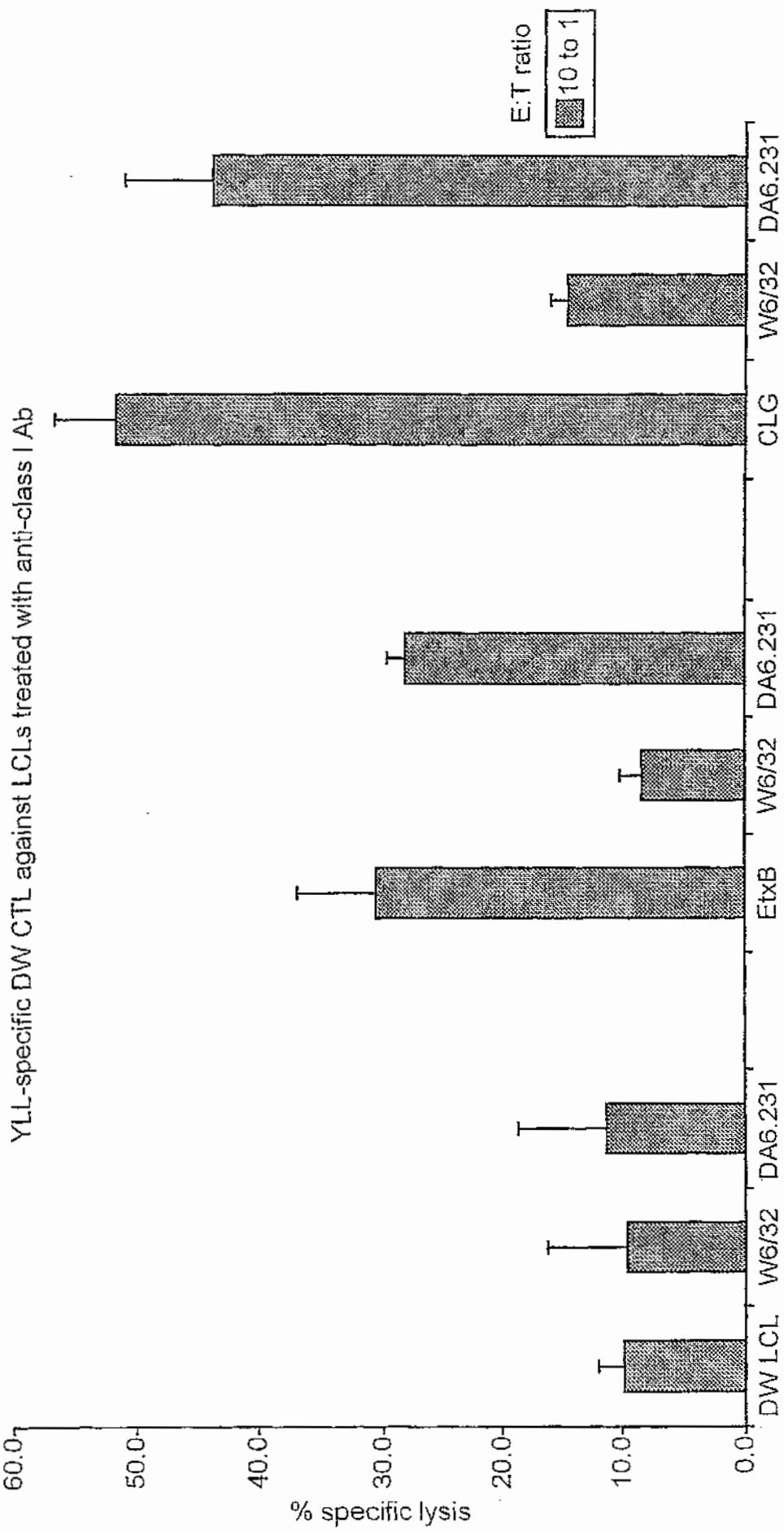

YLL-specific DW CTL against LCLs treated with anti-class I Ab
E:T ratio
10 to 1
% specific lysis
60.0
50.0
40.0
30.0
20.0
10.0
0.0
DW LCL
W6/32
DA6.231
EtxB
W6/32
DA6.231
CLG
W6/32
DA6.231
Targets

FIG. 15

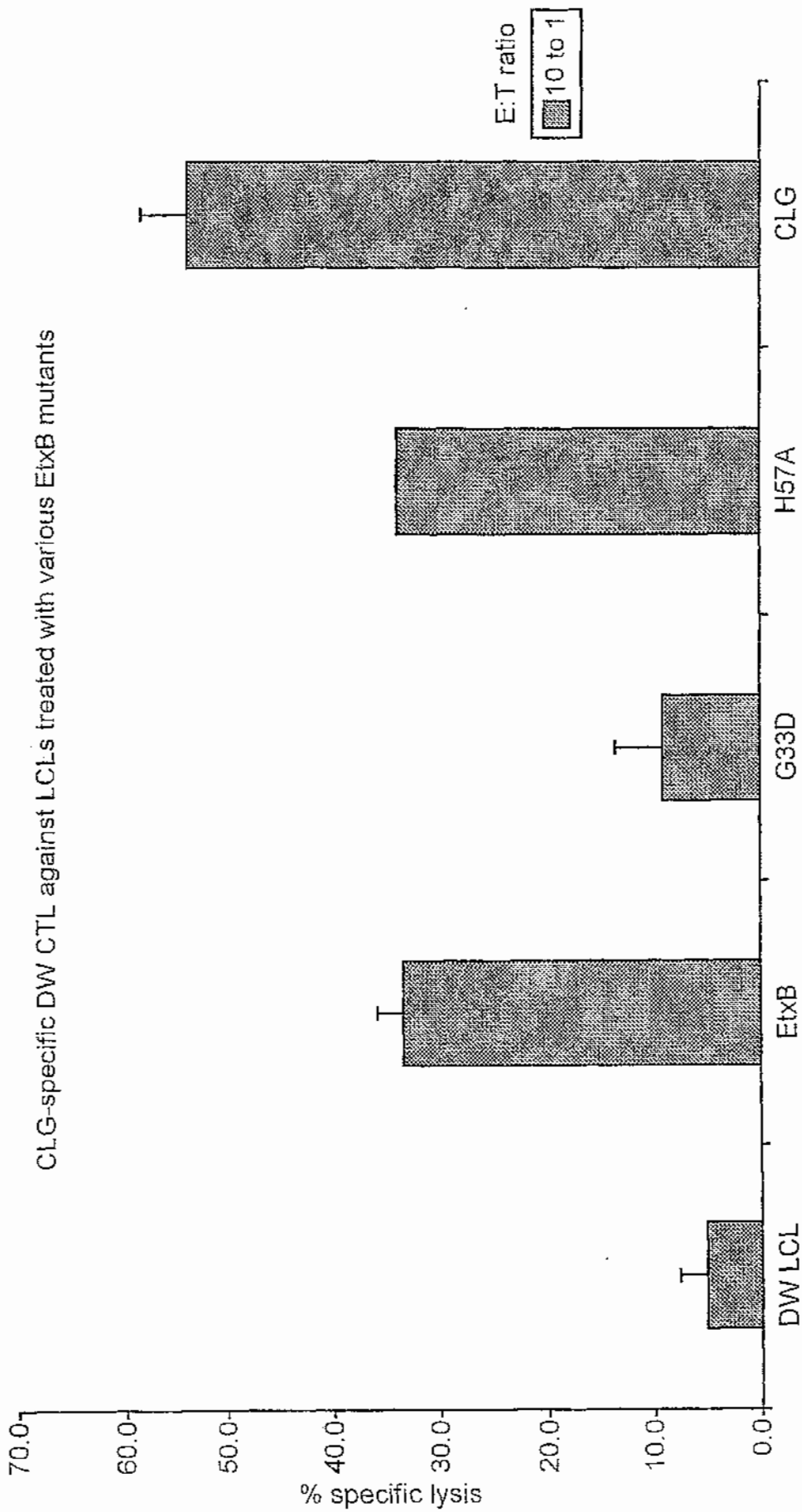

CLG-specific DW CTL against LCLs treated with various EtxB mutants
E:T ratio
10 to 1
% specific lysis
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
DW LCL
EtxB
G33D
H57A
CLG
Targets

FIG. 16

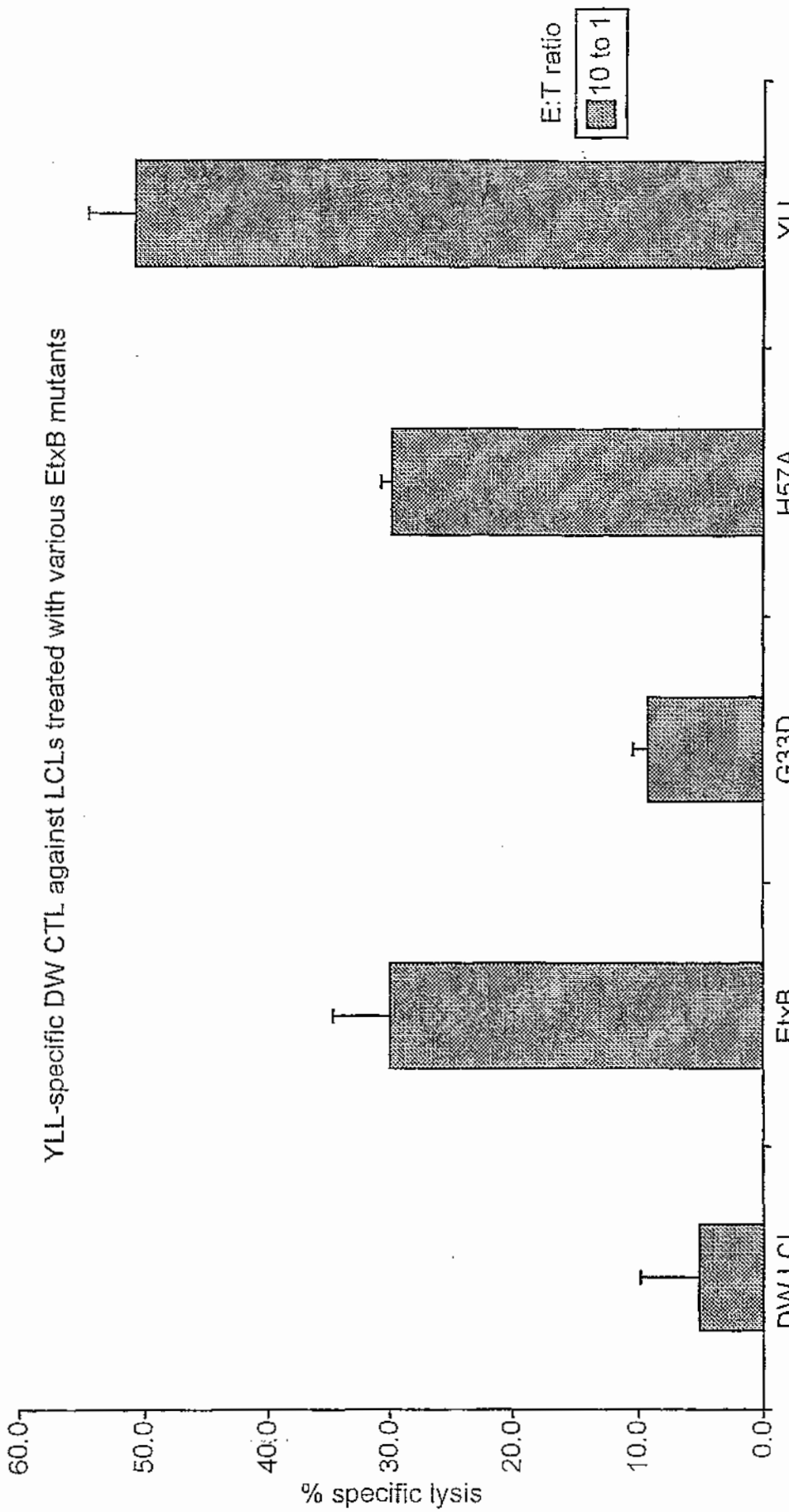

YLL-specific DW CTL against LCLs treated with various EtxB mutants
E:T ratio
10 to 1
% specific lysis
60.0
50.0
40.0
30.0
20.0
10.0
0.0
DW LCL
EtxB
G33D
H57A
YLL
Targets

FIG. 17