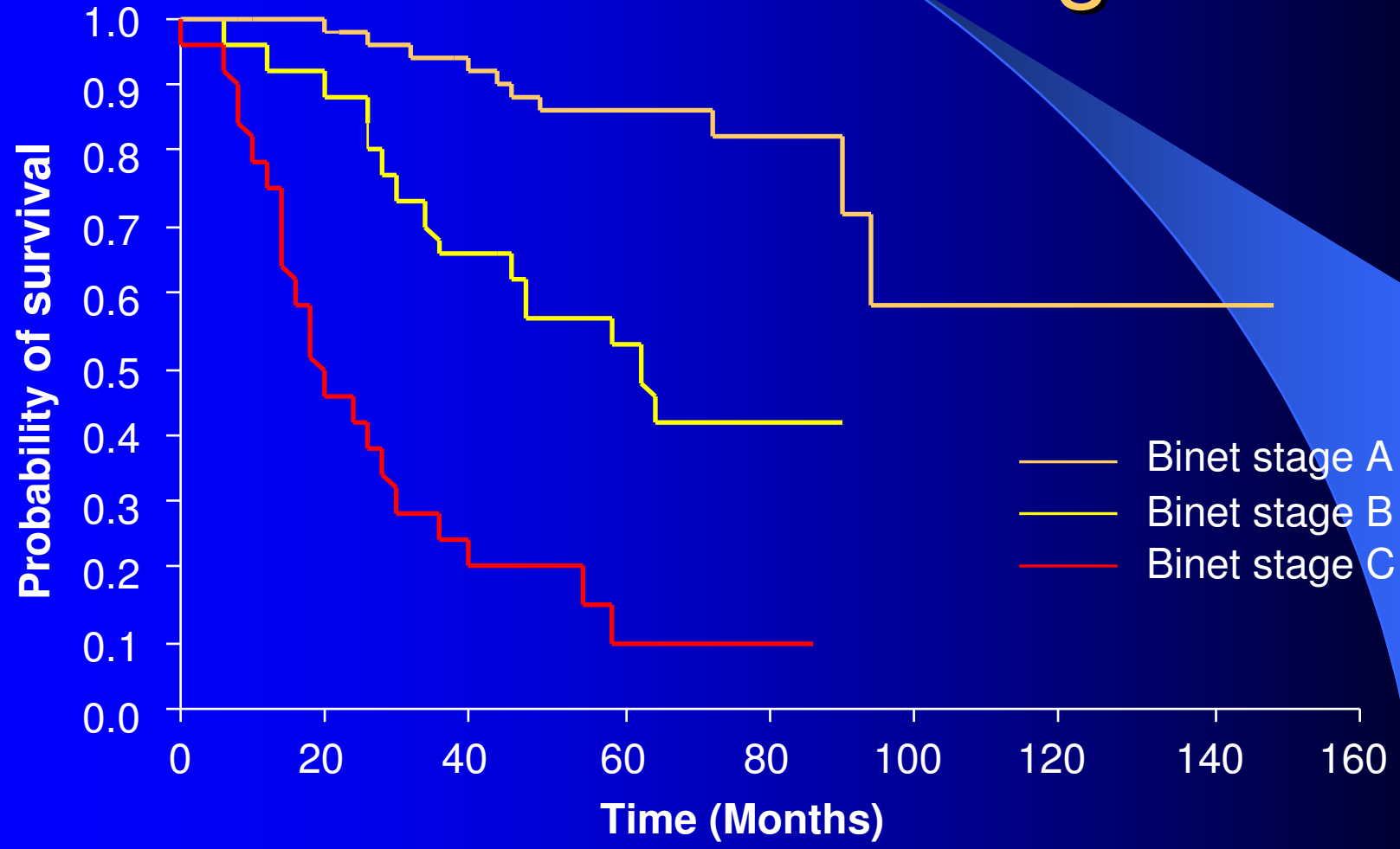
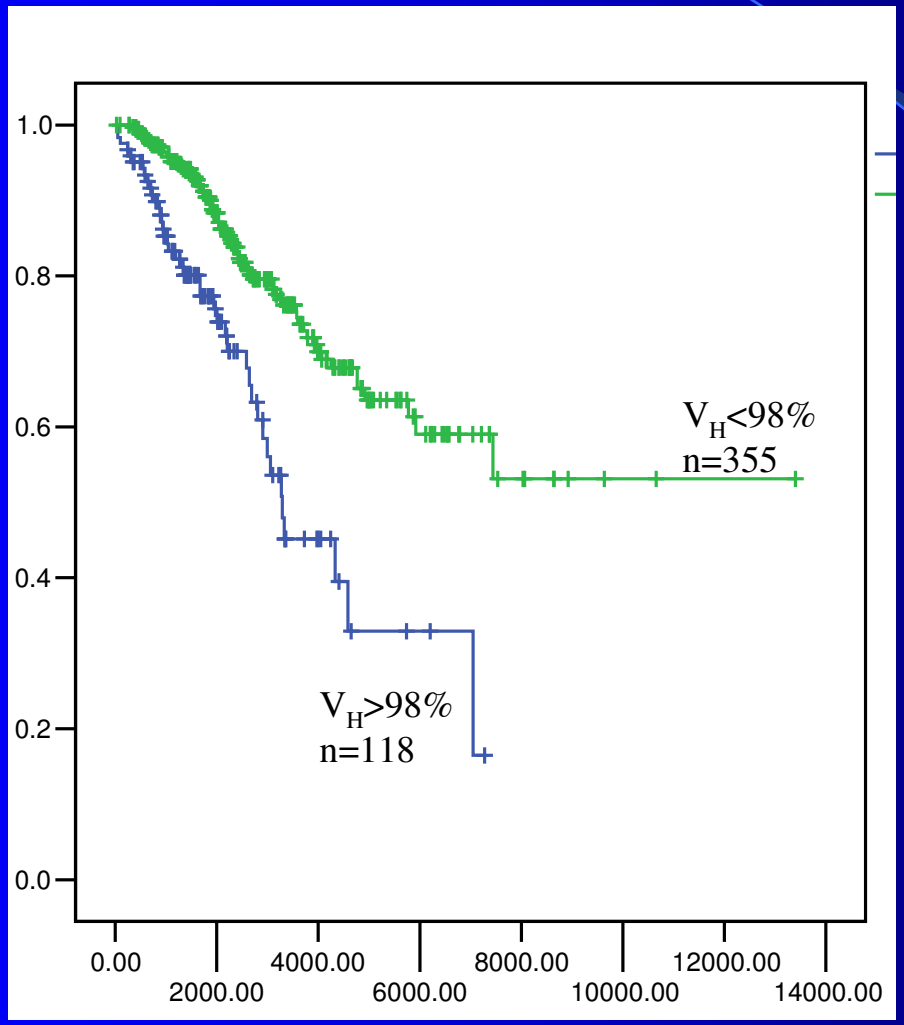
Role of antigen in the perpetuation of CLL

Dr David Allsup

Overall Survival According to Binet Clinical Stage



OS by V_H in 473 CLL cases.



p<0.0000

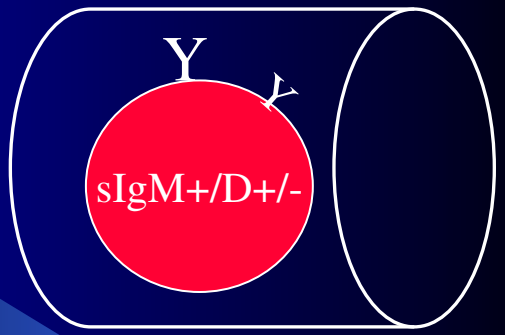
Data derived from Birmingham, Cardiff, Hull and Liverpool Haematology Depts

Role of antigen in CLL

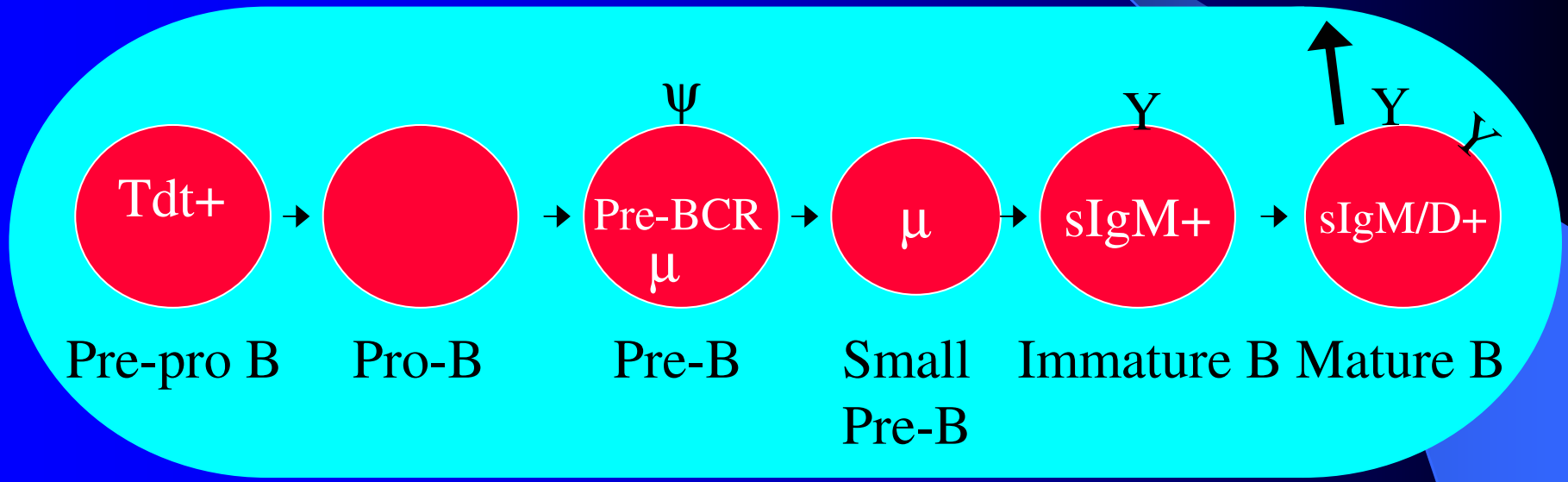
- CLL cells have memory cell immunophenotype.
- Micro array data shows similarity to memory cells.
- Gene expression profiling reveals up regulation of genes involved in B-cell receptor signalling.
- Somatic hypermutation in some cases implies previous antigen encounter.
- Selected V_H gene usage in some.
- Restrictive diversity of BCR, suggesting prior exposure to common antigens.
- CLL is a proliferative disease, not one characterised by a failure of apoptosis.
- CLL thought to be antigen driven.

Variable role of antigen in normal B-lymphocyte development and responses

B-lymphopoiesis is not subject to positive regulation by antigen



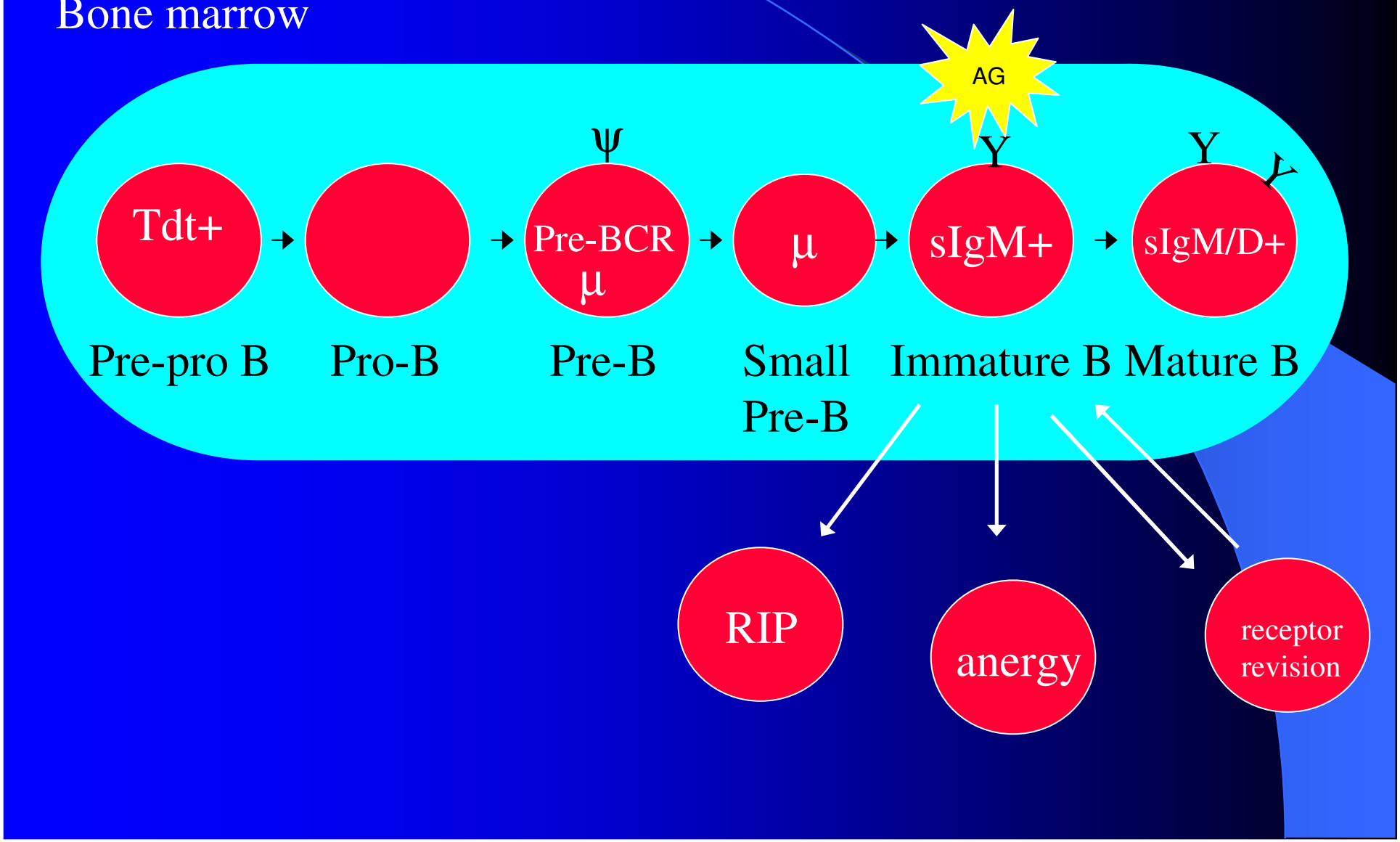
Bone marrow



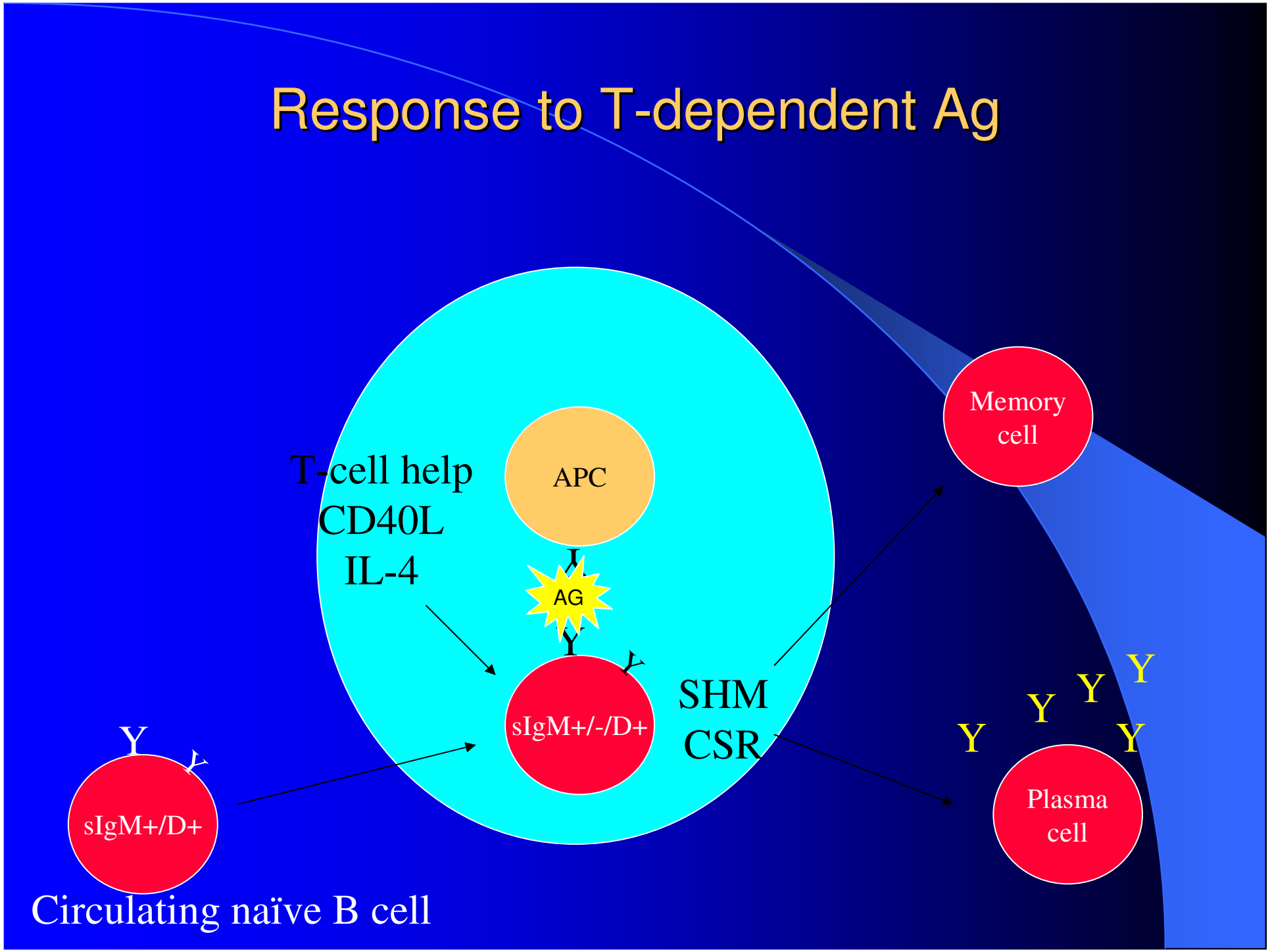
D_H to J_H

V_H to D_HJ_H

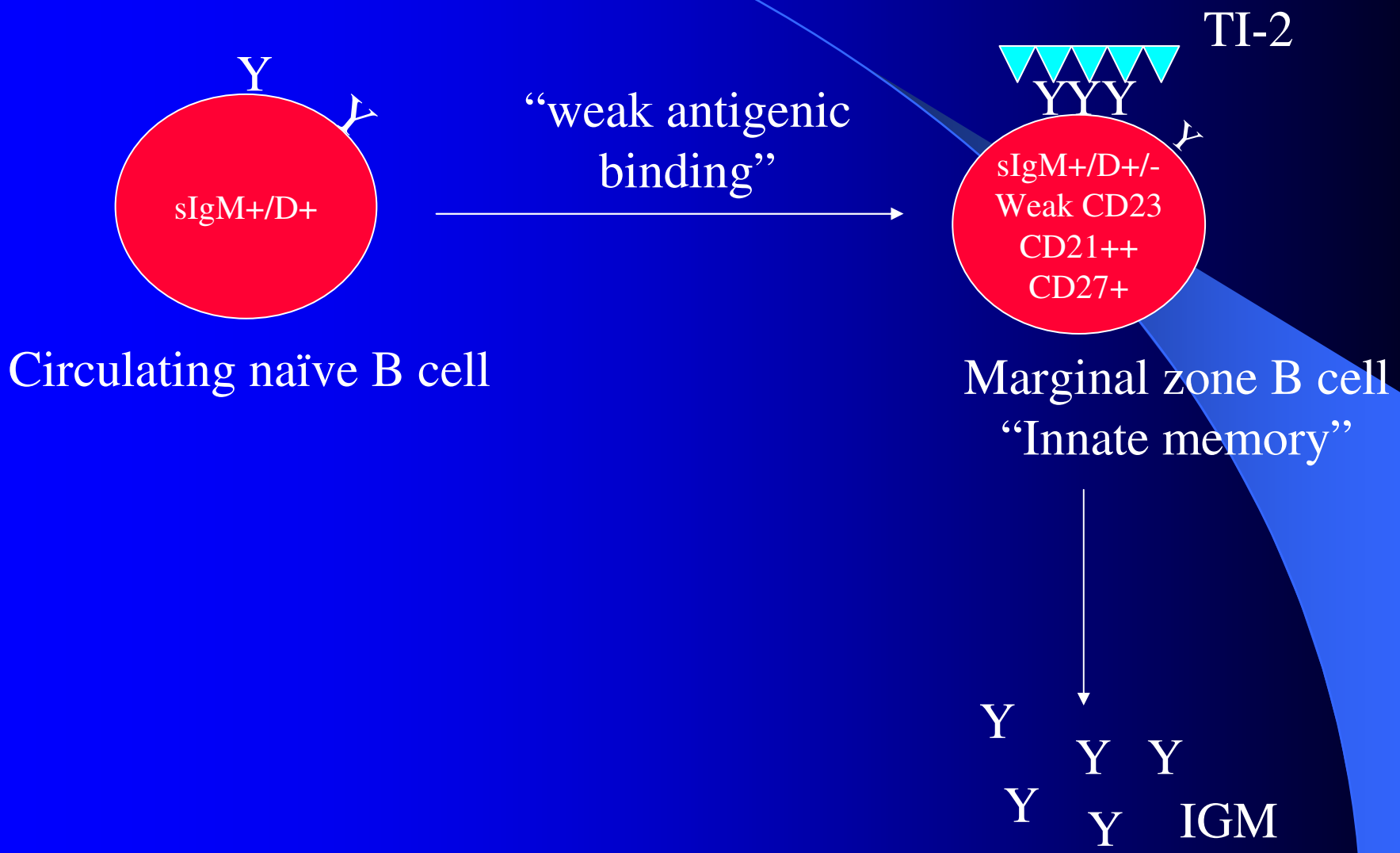
Role of antigen in negative regulation of B-lymphopoiesis



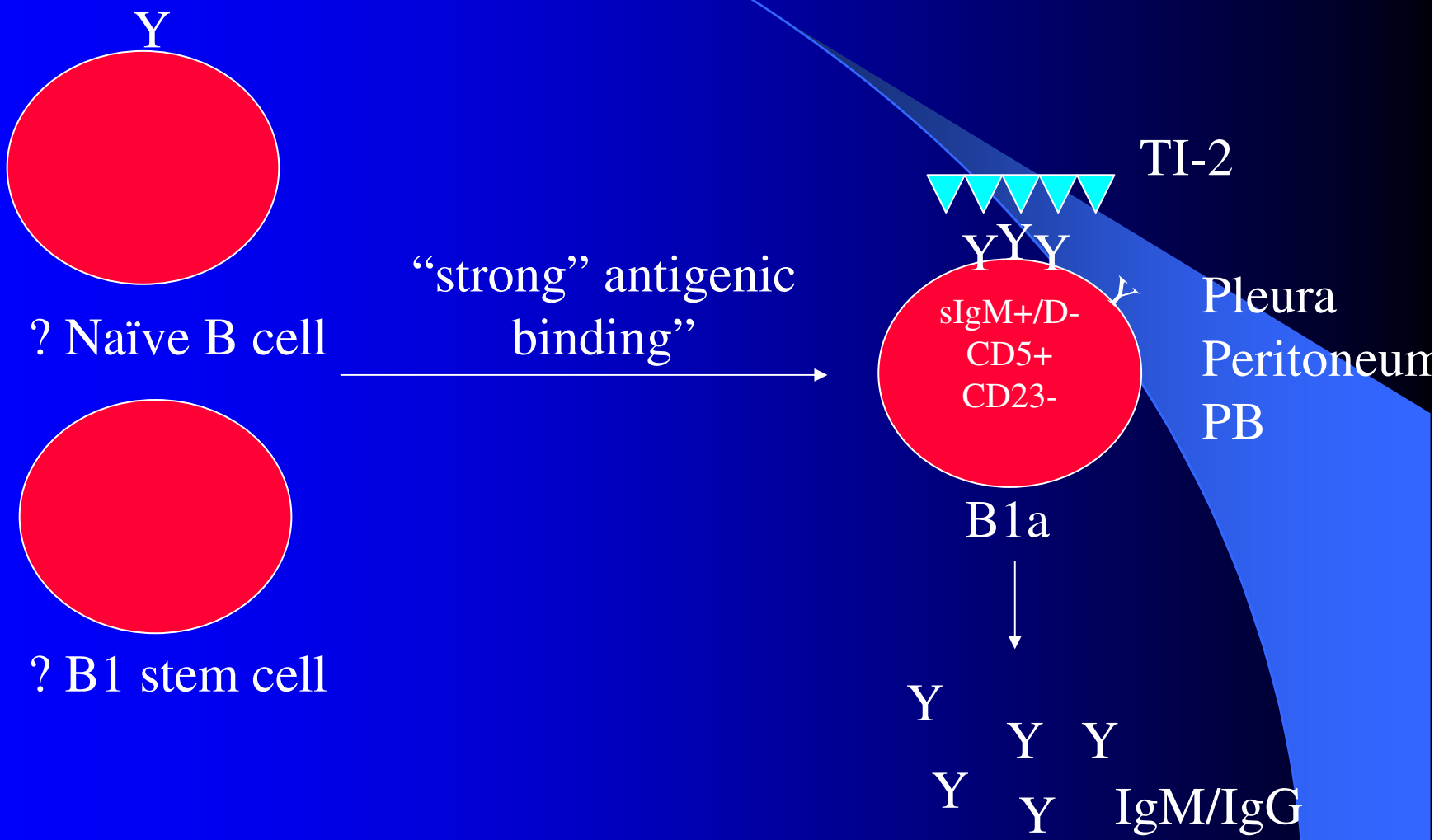
Response to T-dependent Ag



MZ B-cells response to T-independent Ag



B1 cells response to T-independent Ag



B-cell receptor signalling

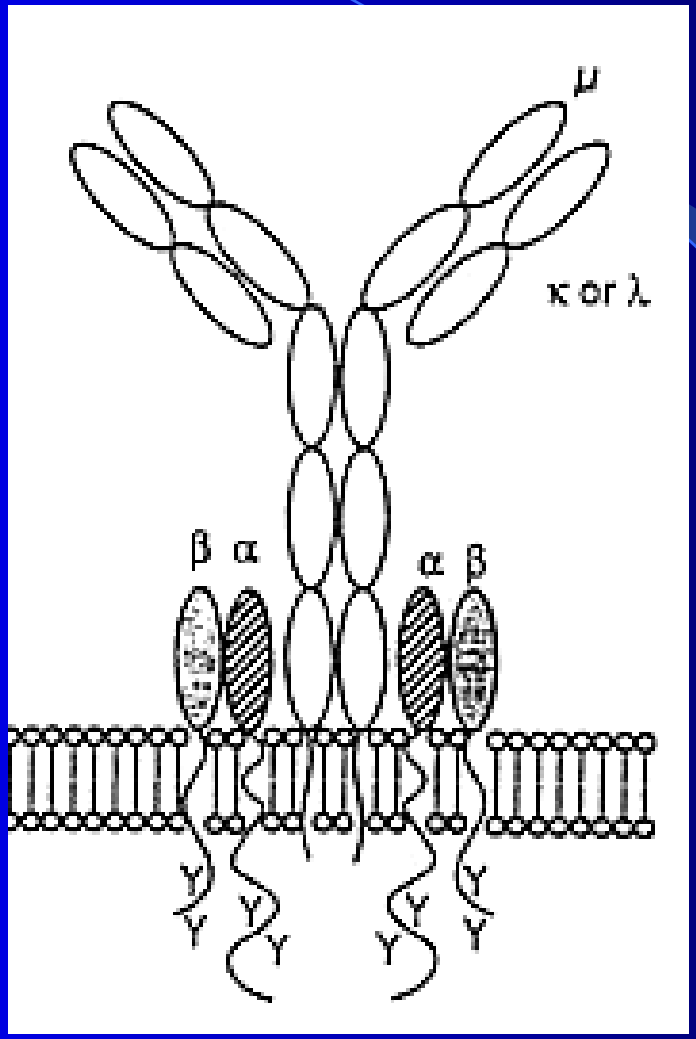
- Antigen binding to BCR can have multiple different outcomes.
- Dependent on:-

  Nature of antigen.

  Developmental stage of B-cell.

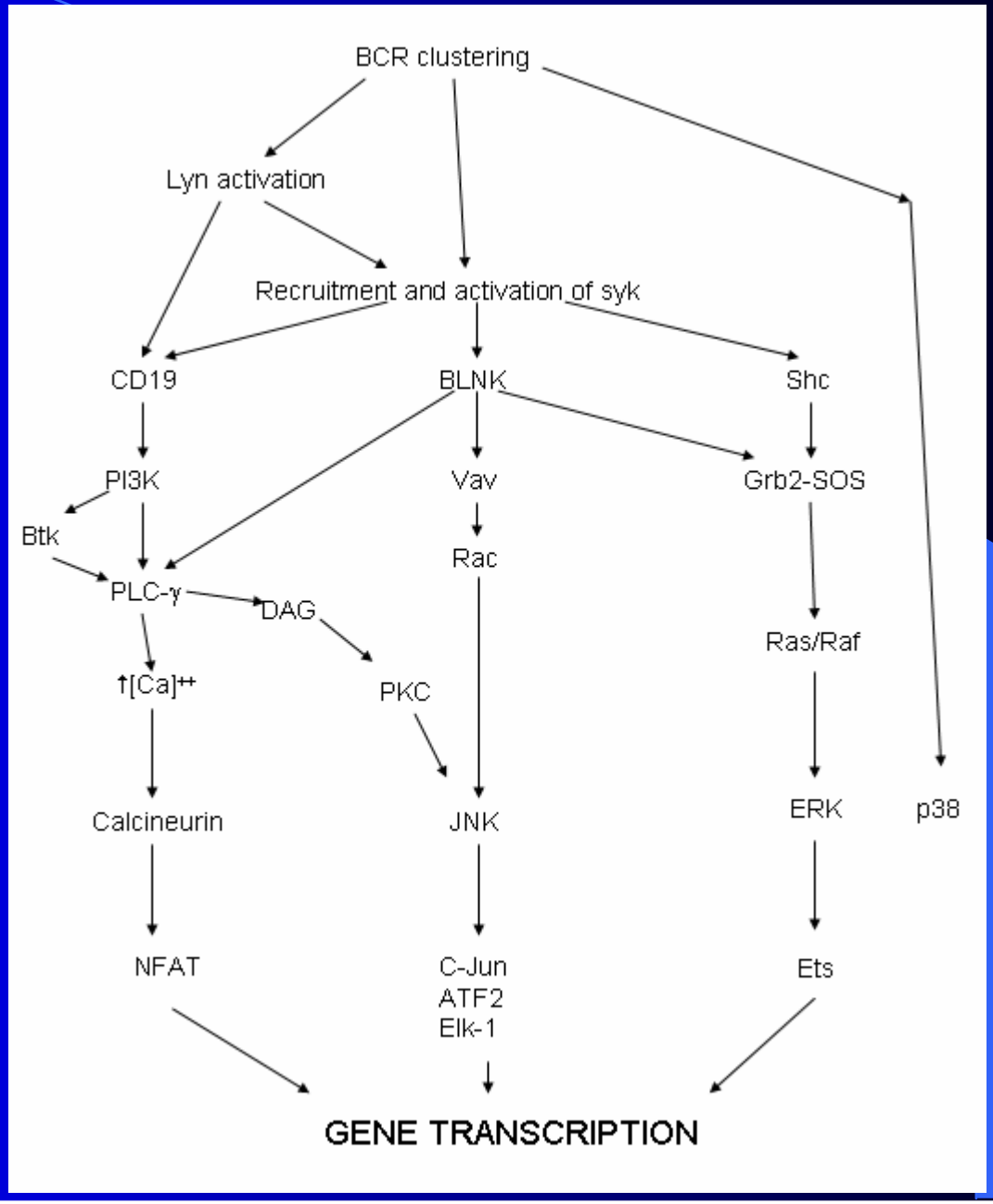
  Presence of co-stimuli.

B cell receptor structure

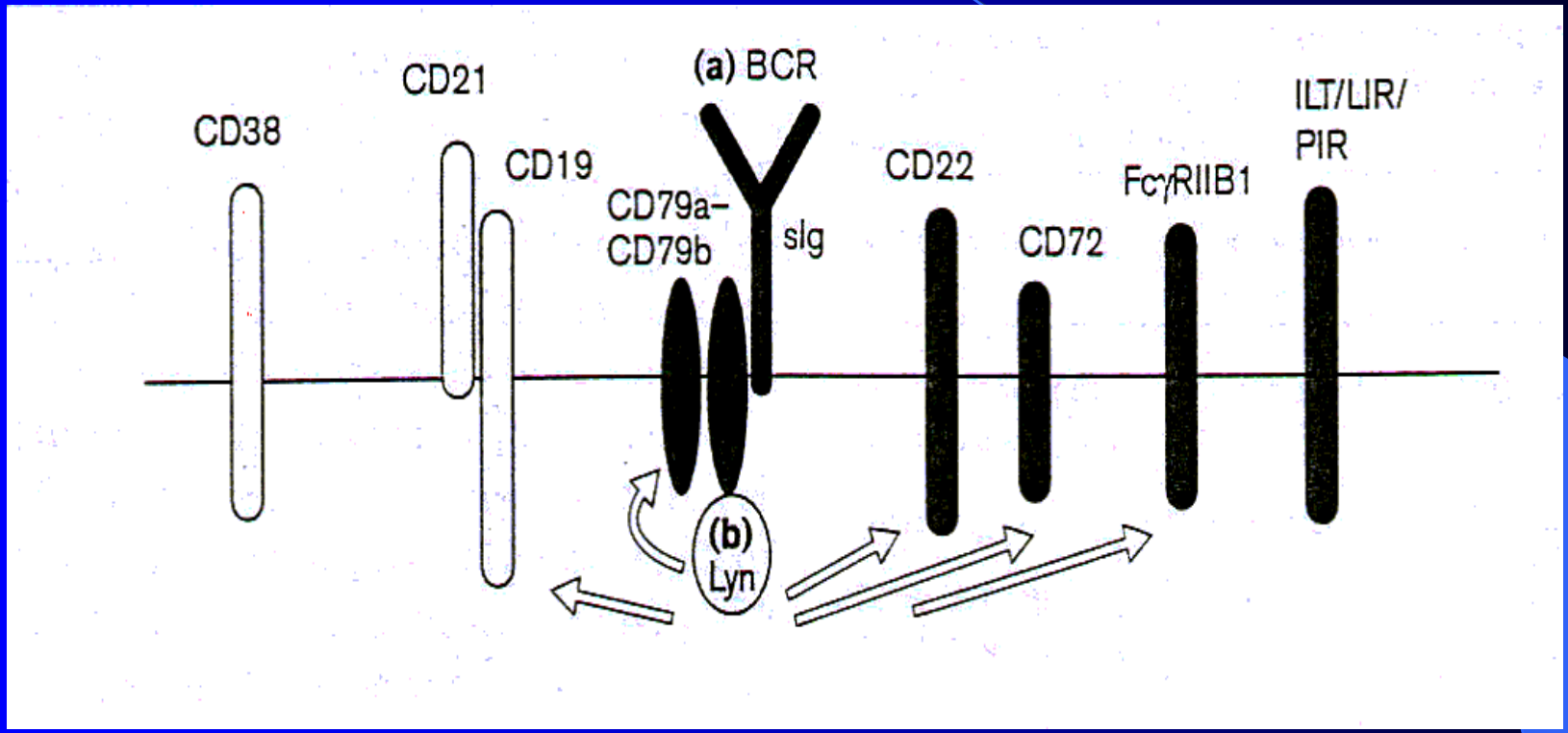


Membrane/proximal signals

Nuclear signals



BCR signals can be modulated

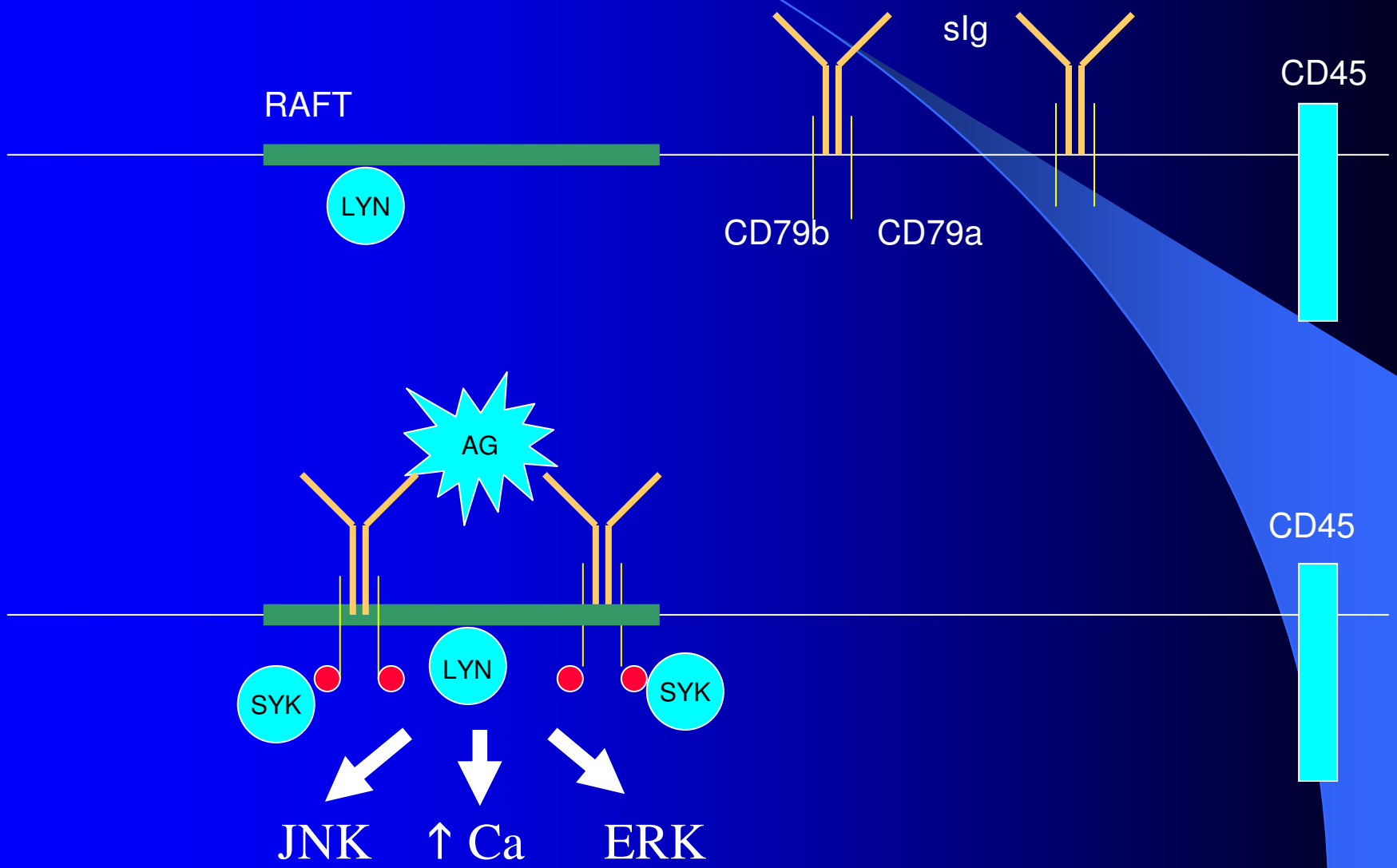


POSITIVE REGULATORS

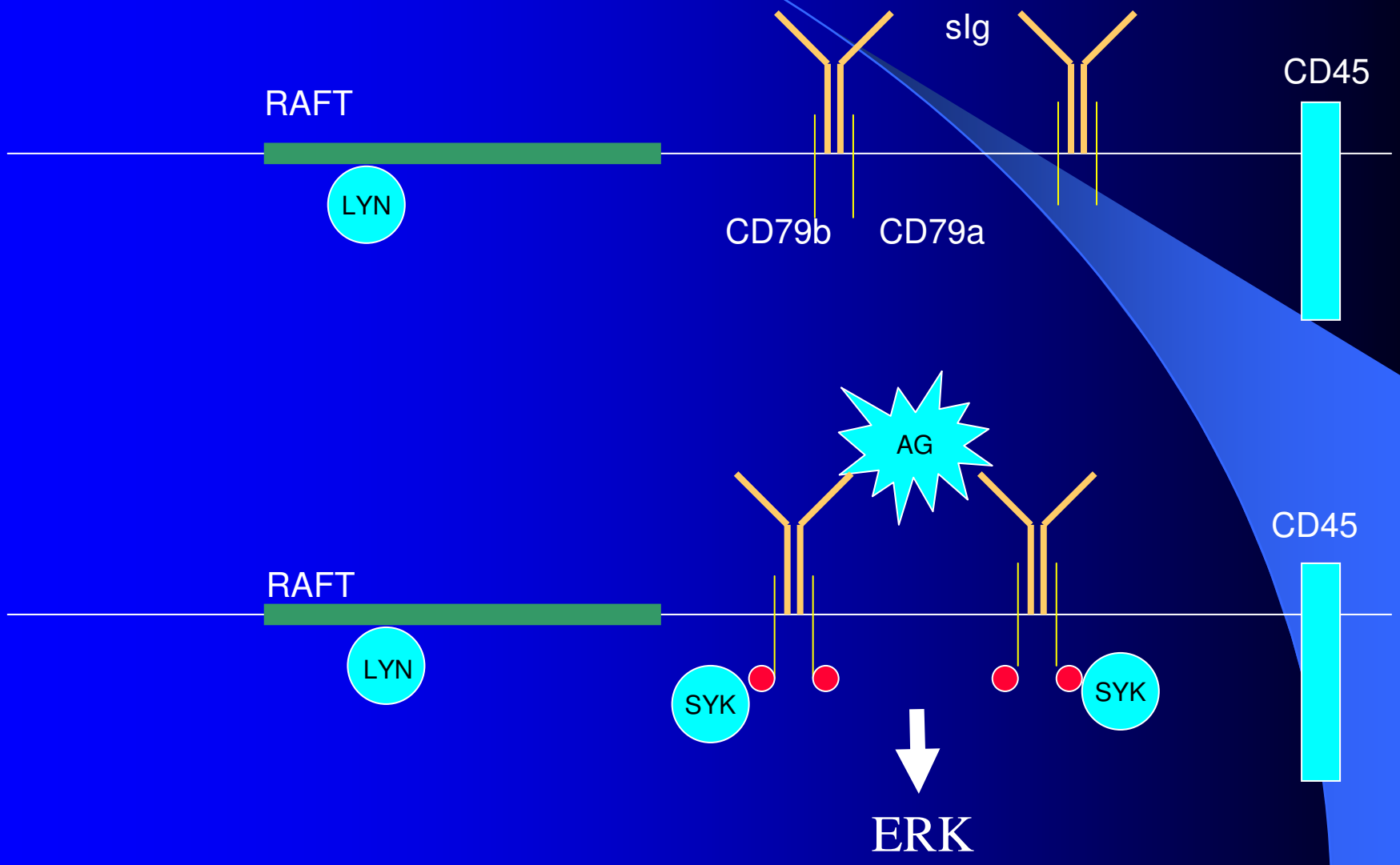
NEGATIVE REGULATORS

From Roitt I, Essential Immunology

BCR lipid raft interactions in mature B-cells



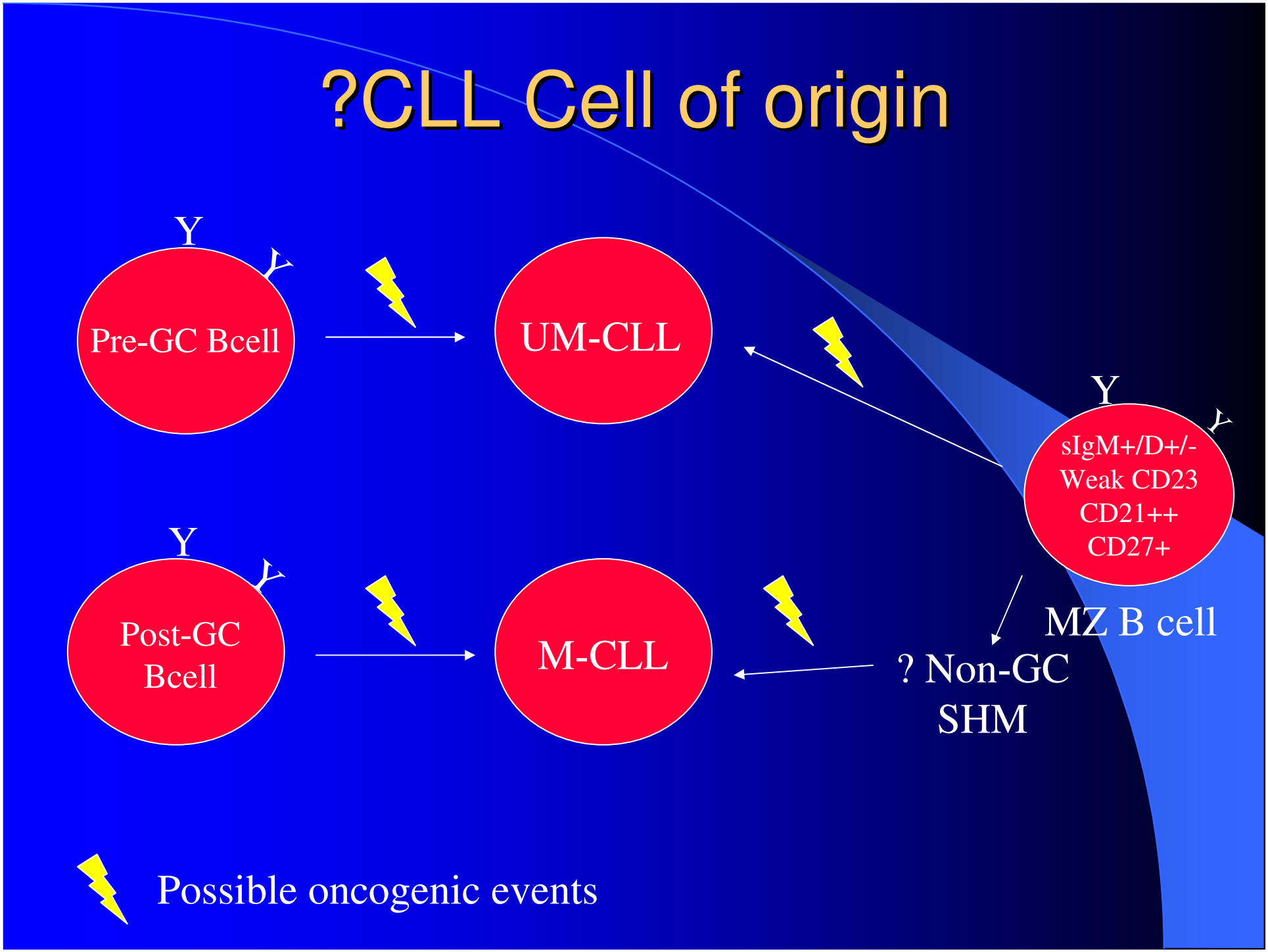
BCR lipid raft interactions in immature and anergic B-cells



Role of antigen in CLL

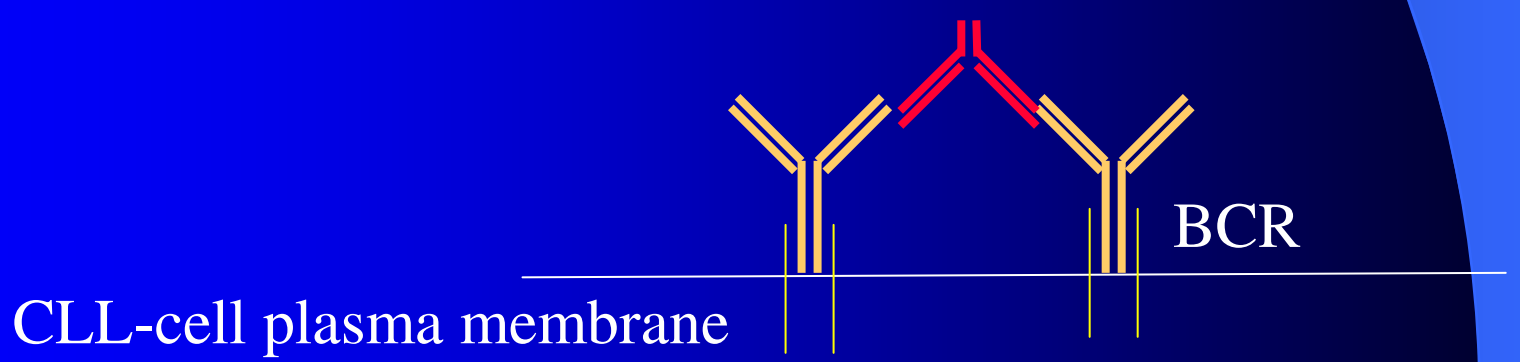
- Cells response to antigen mediated by B-cell receptor (BCR).
- Variable response to BCR ligation in CLL.
- Response to BCR ligation associated with V_H, CD38, progressive disease.
- CLL-cell of origin is probably antigen experienced.
- Are CLL-cells continually responding to antigen *in-vivo*?

?CLL Cell of origin

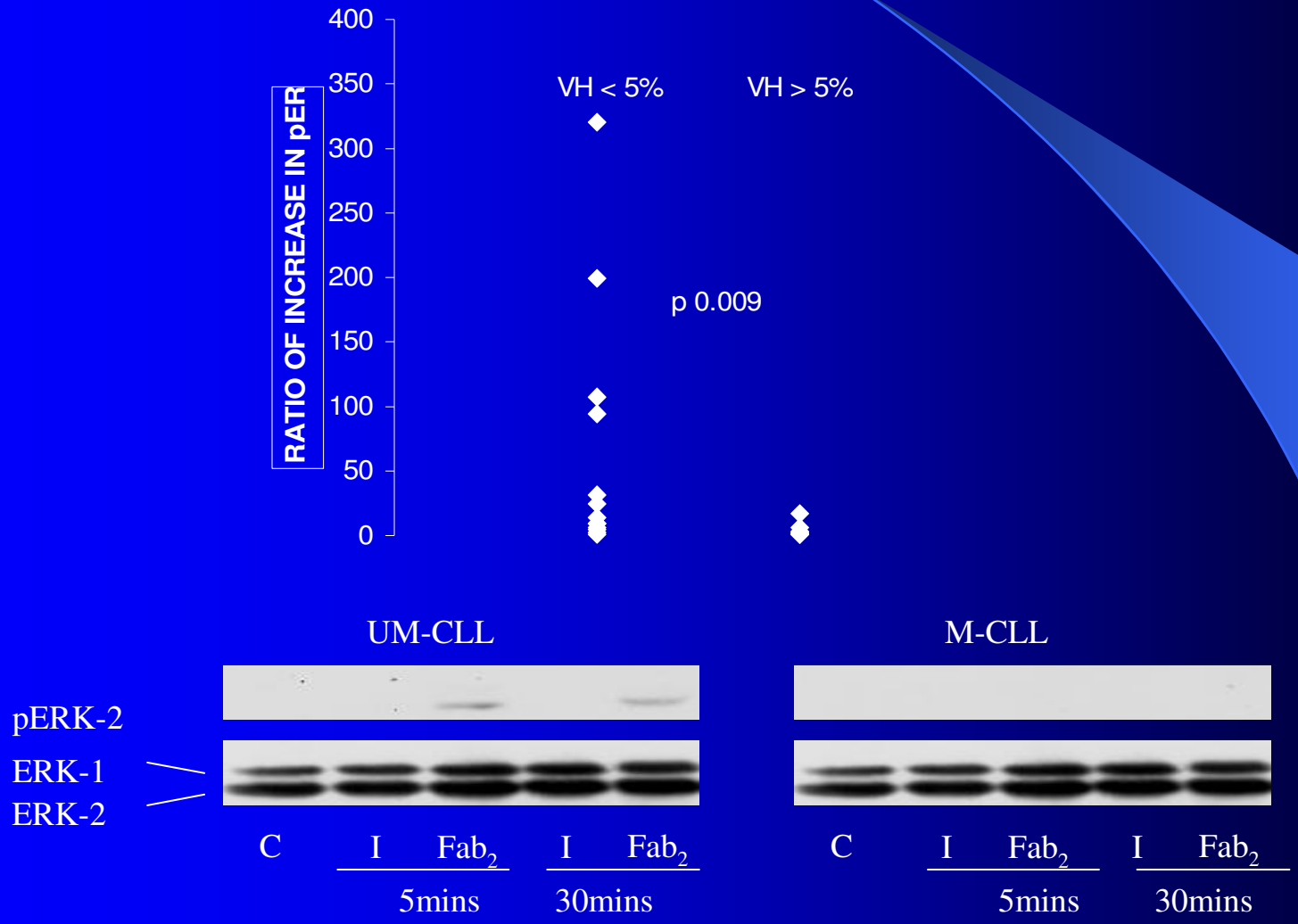


Studies of BCR signalling in CLL

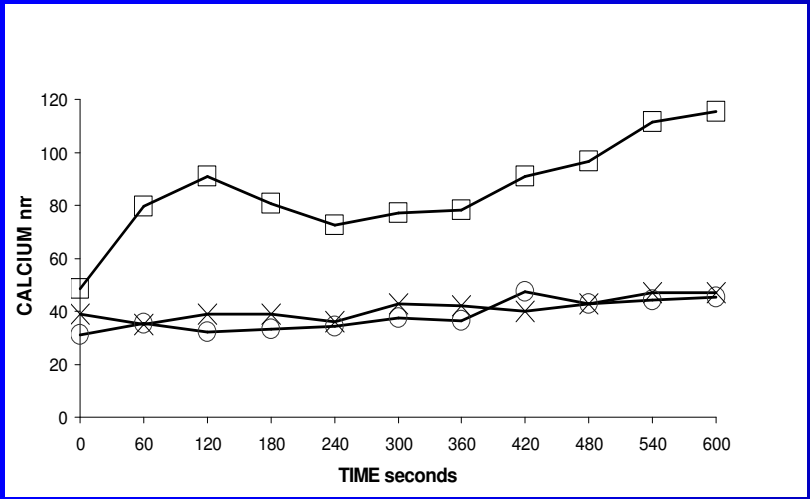
- Aim to understand how CLL-cells respond to Ag.
- In-vitro studies of BCR signalling utilise anti-Ig antibodies to cluster BCR and induce a response.
- Non-physiological stimulus.



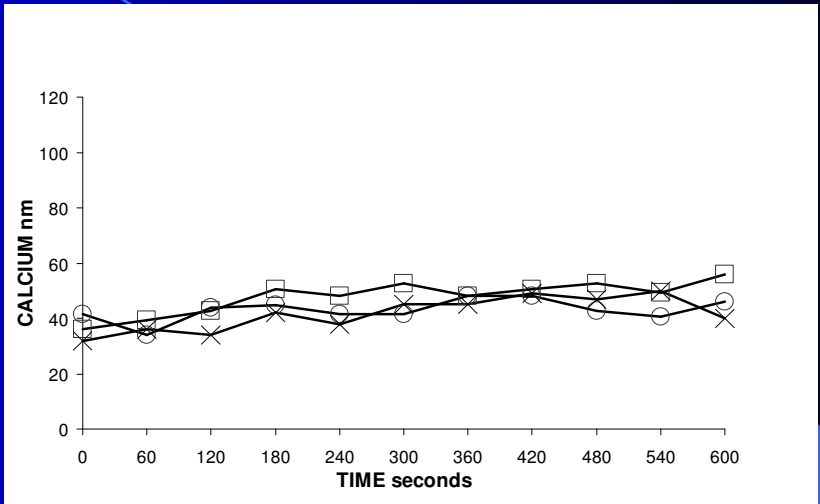
BCR INDUCED ERK PHOSPHORYLATION



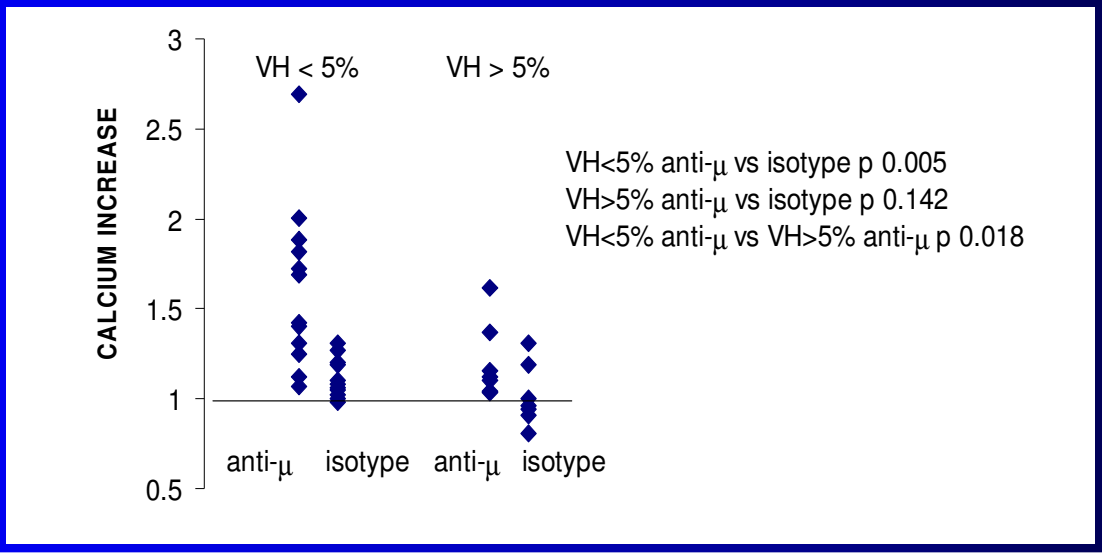
Intracellular calcium rises following BCR crosslinking



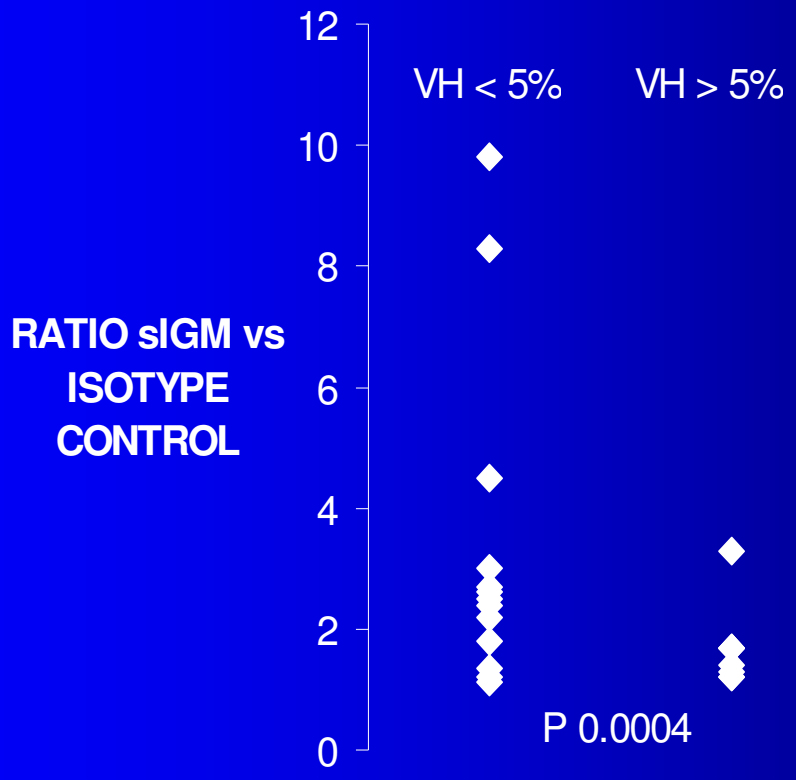
V_H 0.4%, n=13



V_H 11.1%, n=8

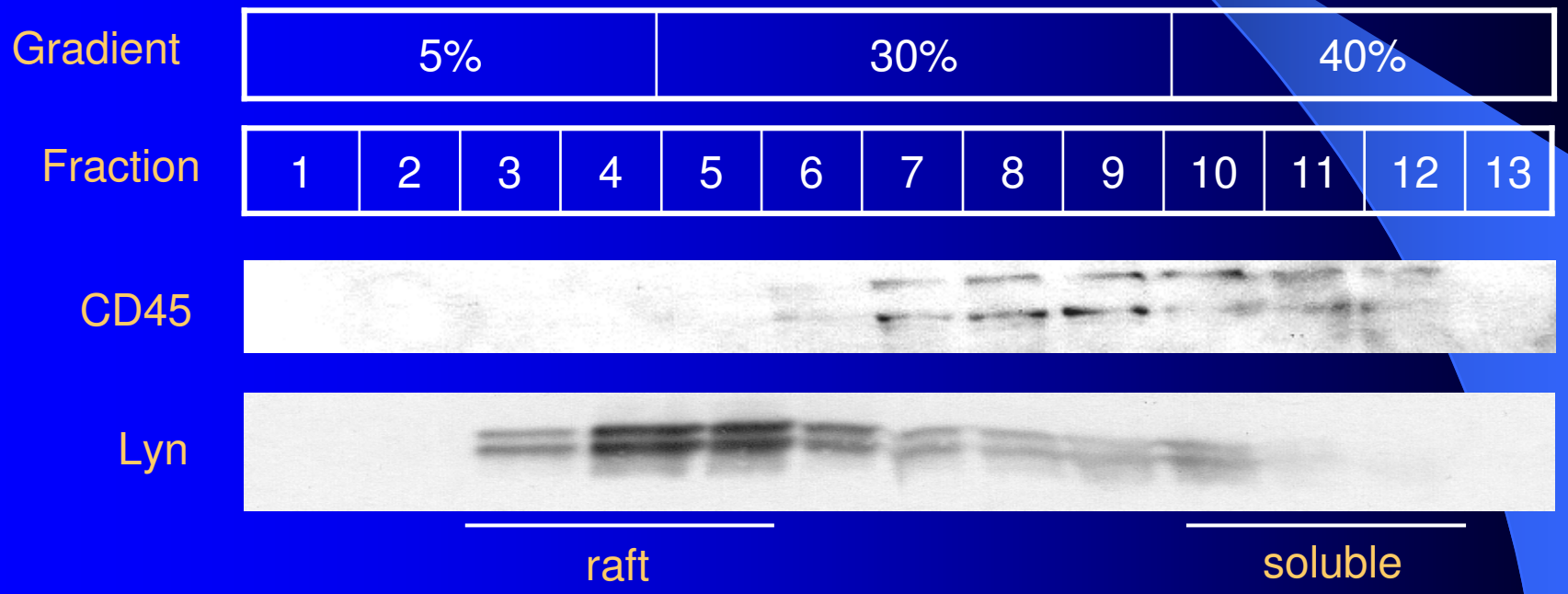


sIgM EXPRESSION VARIES BETWEEN SUBGROUPS

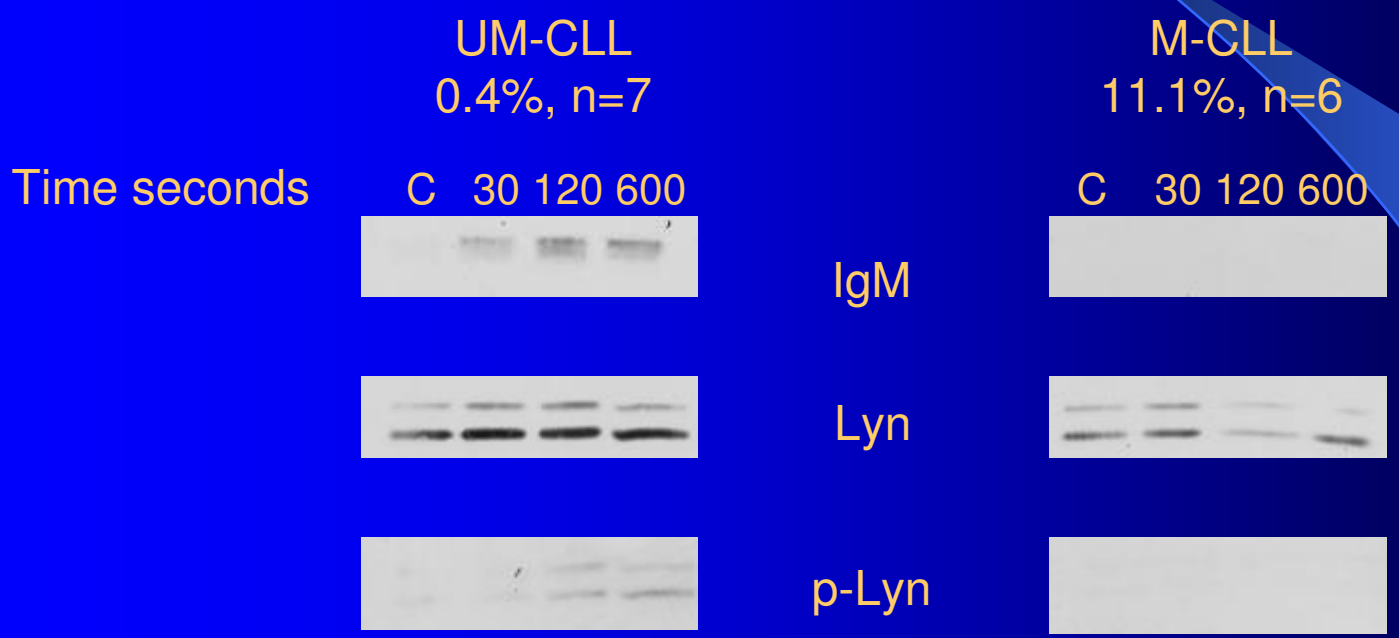


Do BCR lipid raft interactions regulate the response of CLL-cells to BCR crosslinking?

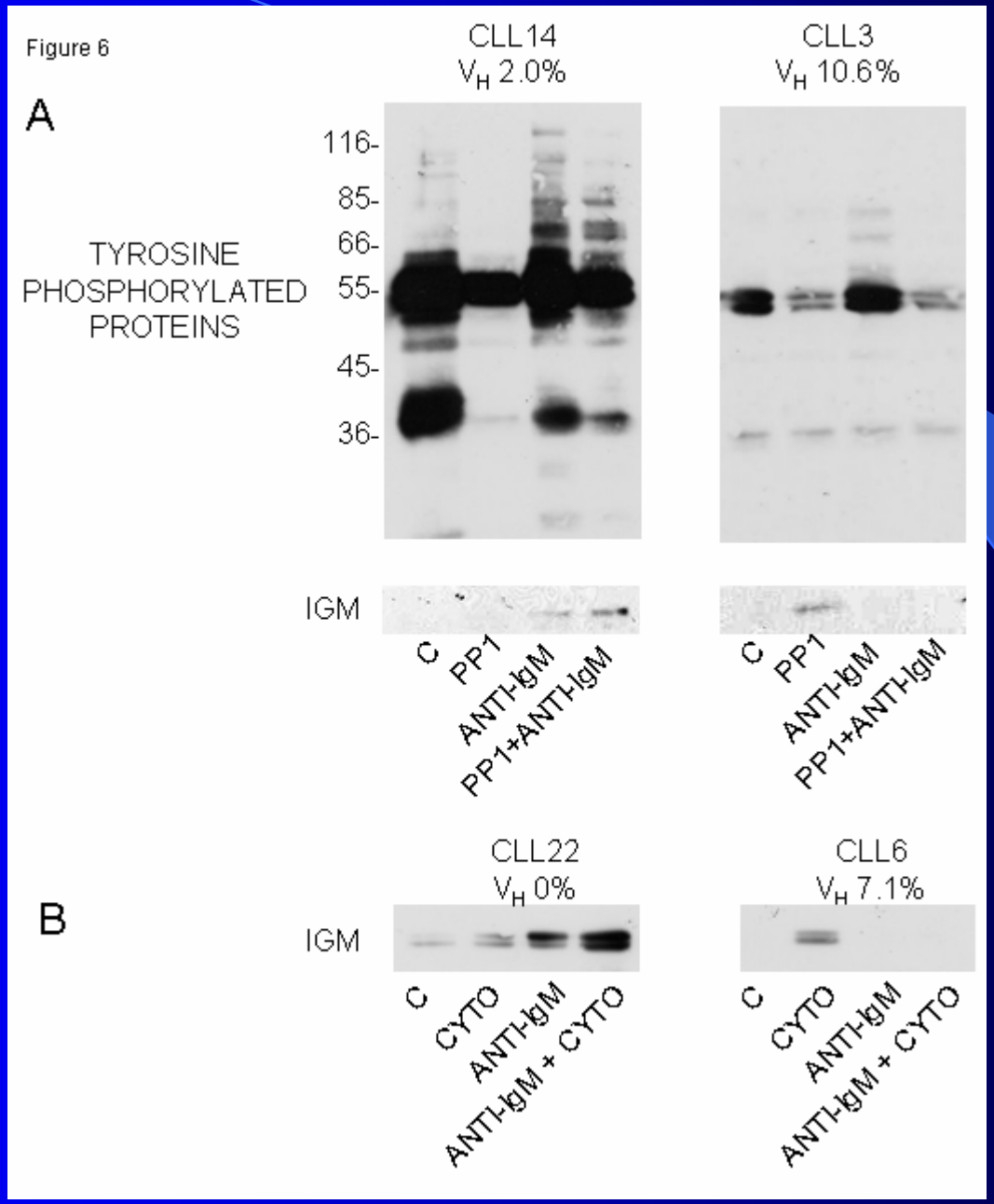
Isolation of lipid rafts by density gradient ultracentrifugation



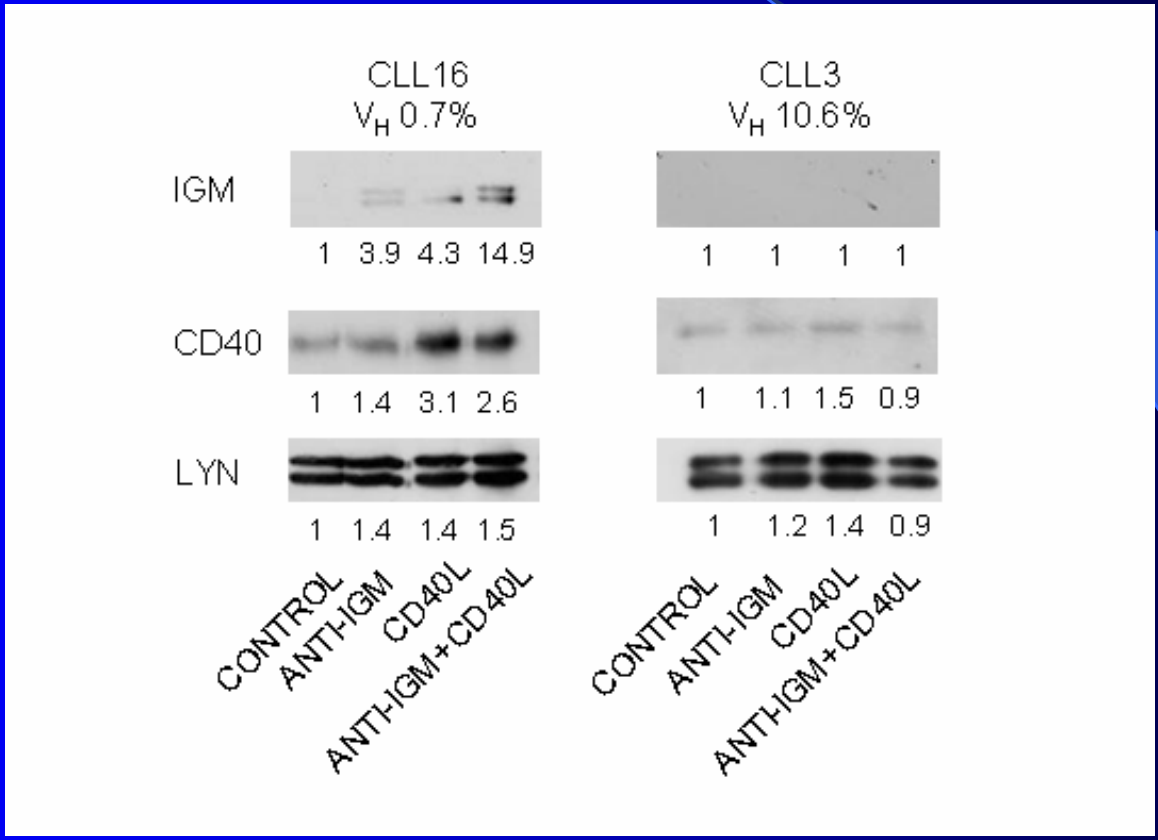
BCR signalling within CLL-cell lipid rafts



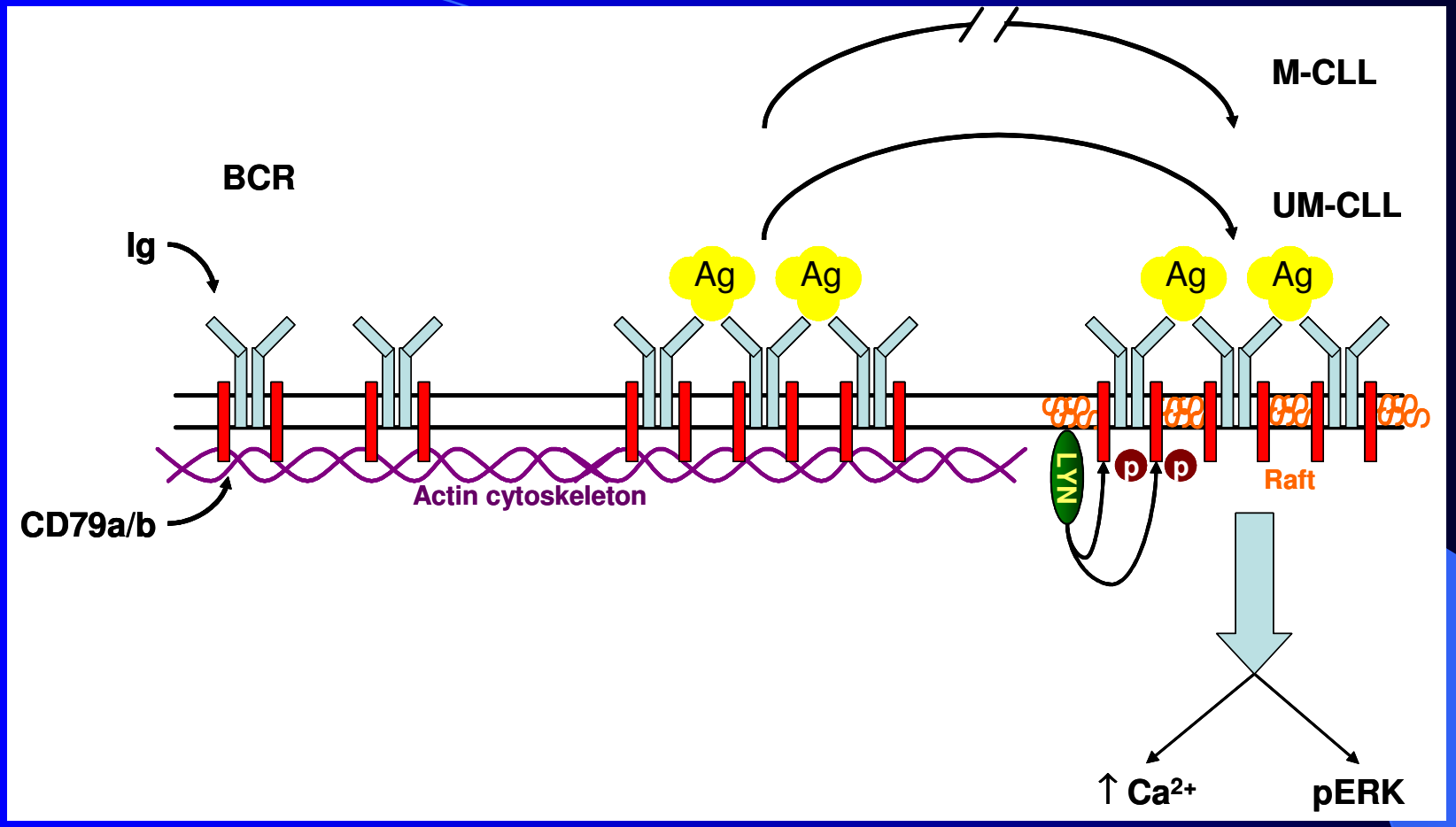
Role of src and cytoskeleton in BCR translocation



CD40L enhances the entry of BCR and CD40 into lipid rafts.

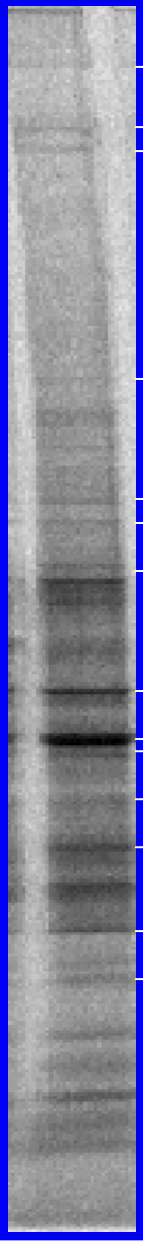


But, CD40L does not further enhance survival in presence of BCR crosslinking.



Translocation to rafts and signalling following BCR crosslinking that take place in UM- but not in M-CLL cells.

Raft-associated proteins in CLL



DNA-dependent protein kinase
α-Spectrin
β-Spectrin

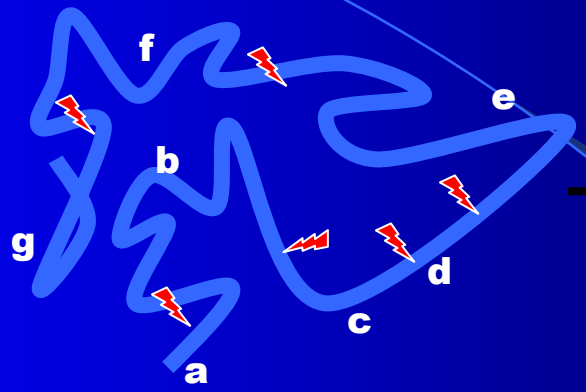
Hypothetical protein

Ezrin
Calnexin
Lamin B1

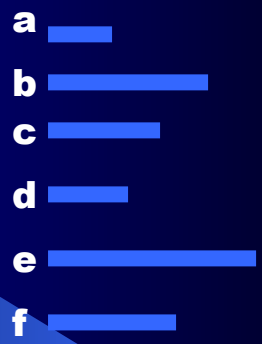
alpha subunit ATP synthase
Actin
Ubiquinol cytochrome C reductase
Prohibitin
Heterogeneous nuclear riboprotein A2/B1
Heterogeneous nuclear riboprotein A2/B1 fragment
Prohibitin fragment

Identification of raft-associated proteins by peptide mass fingerprinting

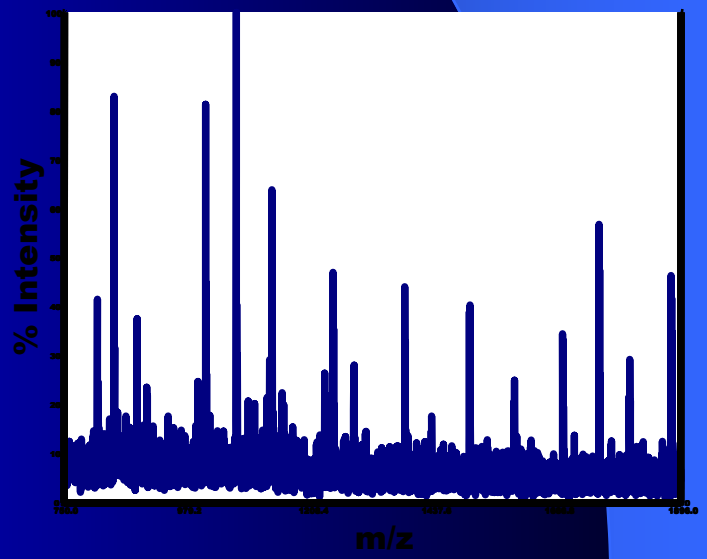
protein separation
1D SDS PAGE



Tryptic Digestion
of band of interest



Tryptic peptides



Measure masses
of tryptic peptides
in MALDI-Tof
Mass Spectrometer

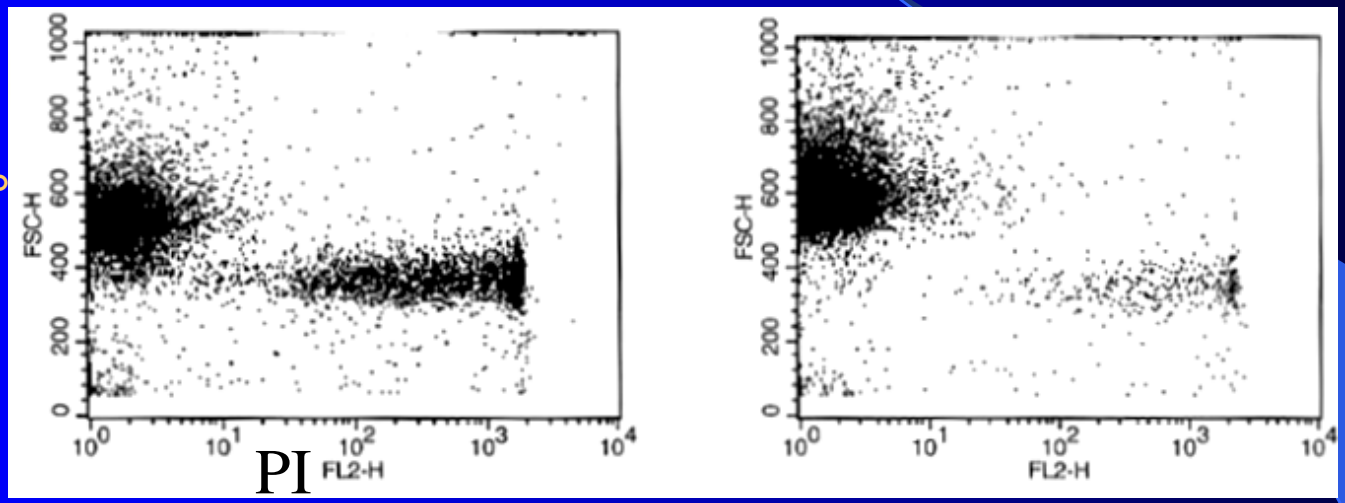
Identification of
protein by
comparison
of peptide masses
with
theoretical digest
masses

BCR crosslinking enhances the survival of CLL cells with relatively unmutated VH genes

MEDIA

F(ab`)$_2$ ANTI-IgM

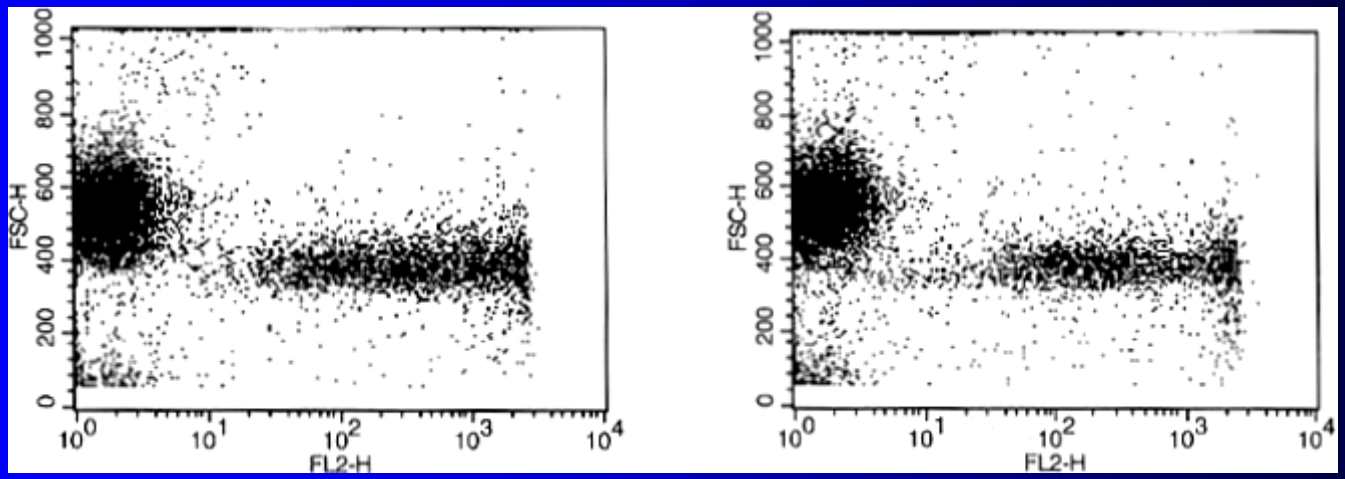
V_H 2.0%



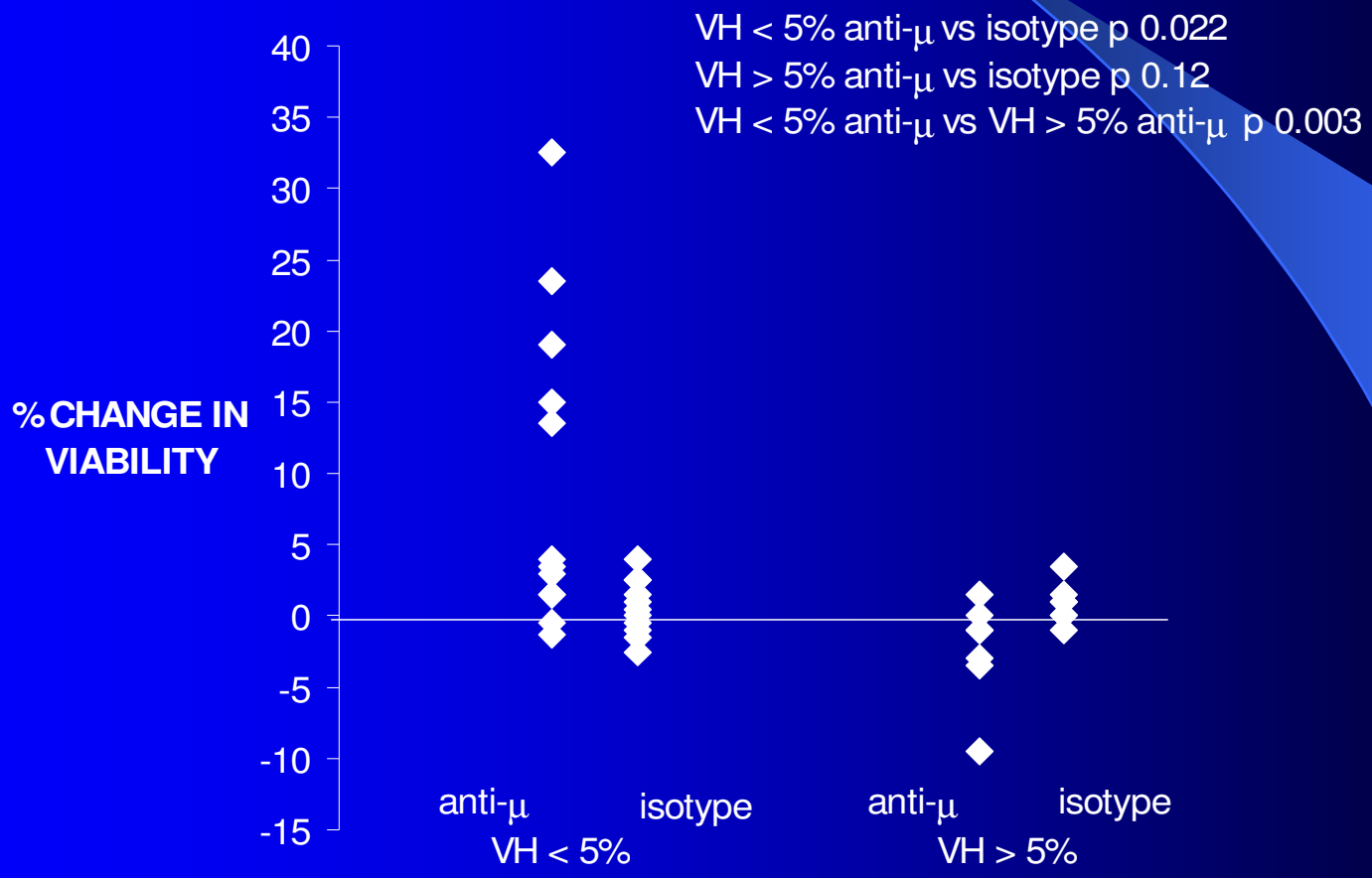
MEDIA

F(ab`)$_2$ ANTI-IgM

V_H 11.1%



BCR CROSSLINKING INDUCES SURVIVAL IN UM-CLL

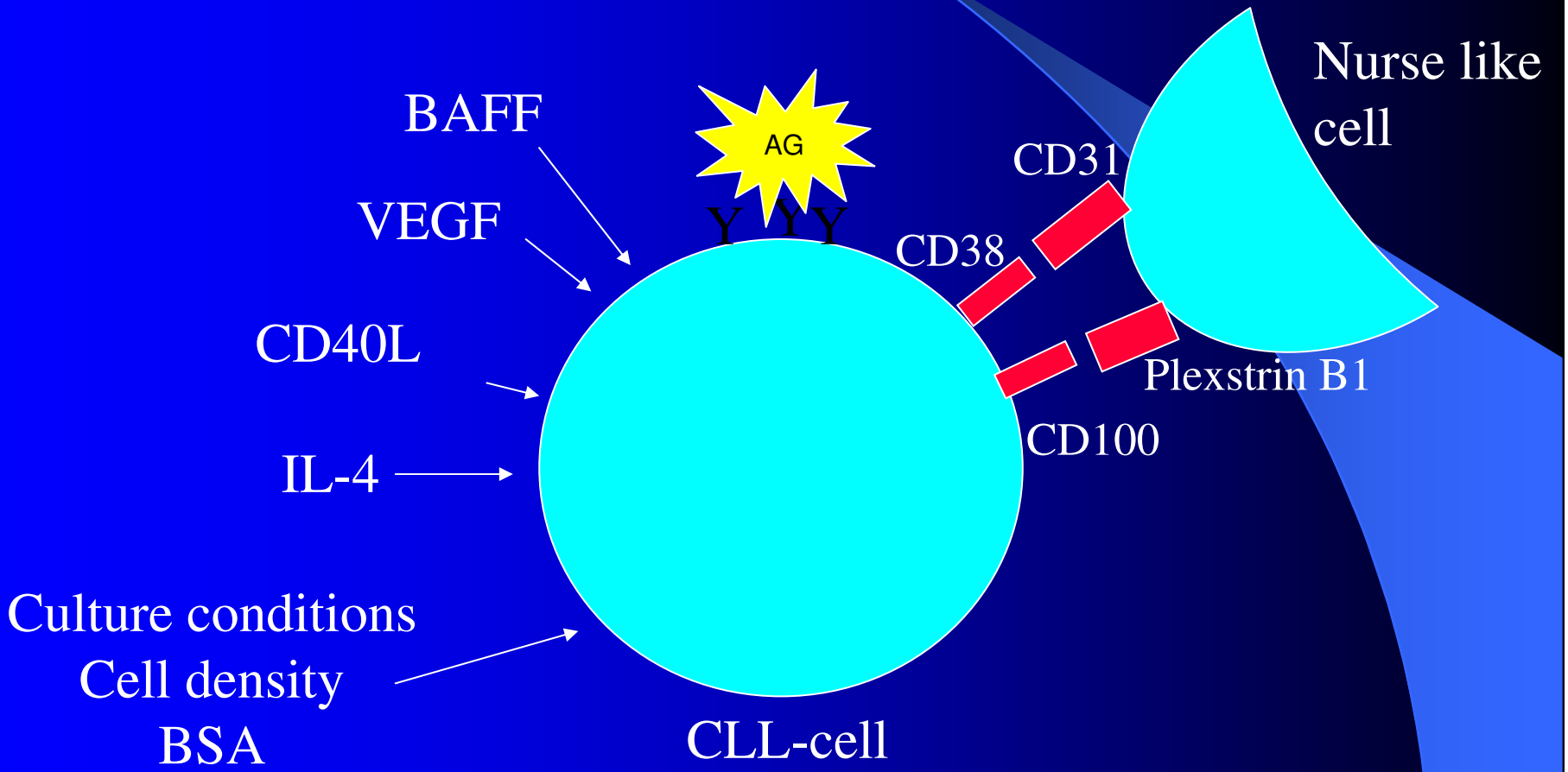


Regulation of CLL-cell viability by BCR crosslinking.

- BCR ligation promotes viability of CLL-cells and inhibits apoptosis (Bernal et al. 2001).
- Extensive, sustained, crosslinking (TI-2 like) promotes CLL-cell viability in all cases (Petlivchovski et al., 2005).

  But
- IgM ligation in CD38+ cases induces apoptosis (Zupo et al. 2000). IgD ligation promotes survival.
- BCR ligation induces expression of pro-apoptotic proteins and apoptosis of cultured cells (Vallat et al., 2007).

Co-stimuli in vitro and in-vivo may modulate the response to BCR ligation



Conclusions (1)

- Ligation of BCR has heterogeneous outcomes in CLL.
- BCR crosslinking prolongs the survival of CLL cells with relatively unmutated VH genes.
- Failure to signal is associated with failure of BCR translocation to rafts on receptor crosslinking.
- In non-responsive cases BCR may be actively excluded from lipid rafts.

Conclusions (2)

- CLL-cells are probably derived from antigen-experienced normal B-cells.
- Continuous antigenic stimulation in-vivo may promote the survival of CLL-cells.
- Nature and location of such antigen is unknown.
- But if a soluble autoantigen is present then the survival of UM-CLL cells may be preferentially promoted.
- If antigen is a multimeric TI-2 antigen then continuous stimulation may promote survival of all CLL-clones.
- Antigenic stimulation may persist due to failure of CLL-cells to differentiate into antibody secreting cells with subsequent failure to clear antigen.

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