B cell development and tolerance

Micro 204

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2018

5 Themes in B cell development

Theme 1: Checkpoints in B cell development: feedback from Ig gene rearrangements

Theme 2: Bone marrow microenvironment

Theme 3: Lineage commitment: transcription factors

Theme 4: Central and peripheral tolerance of B cells

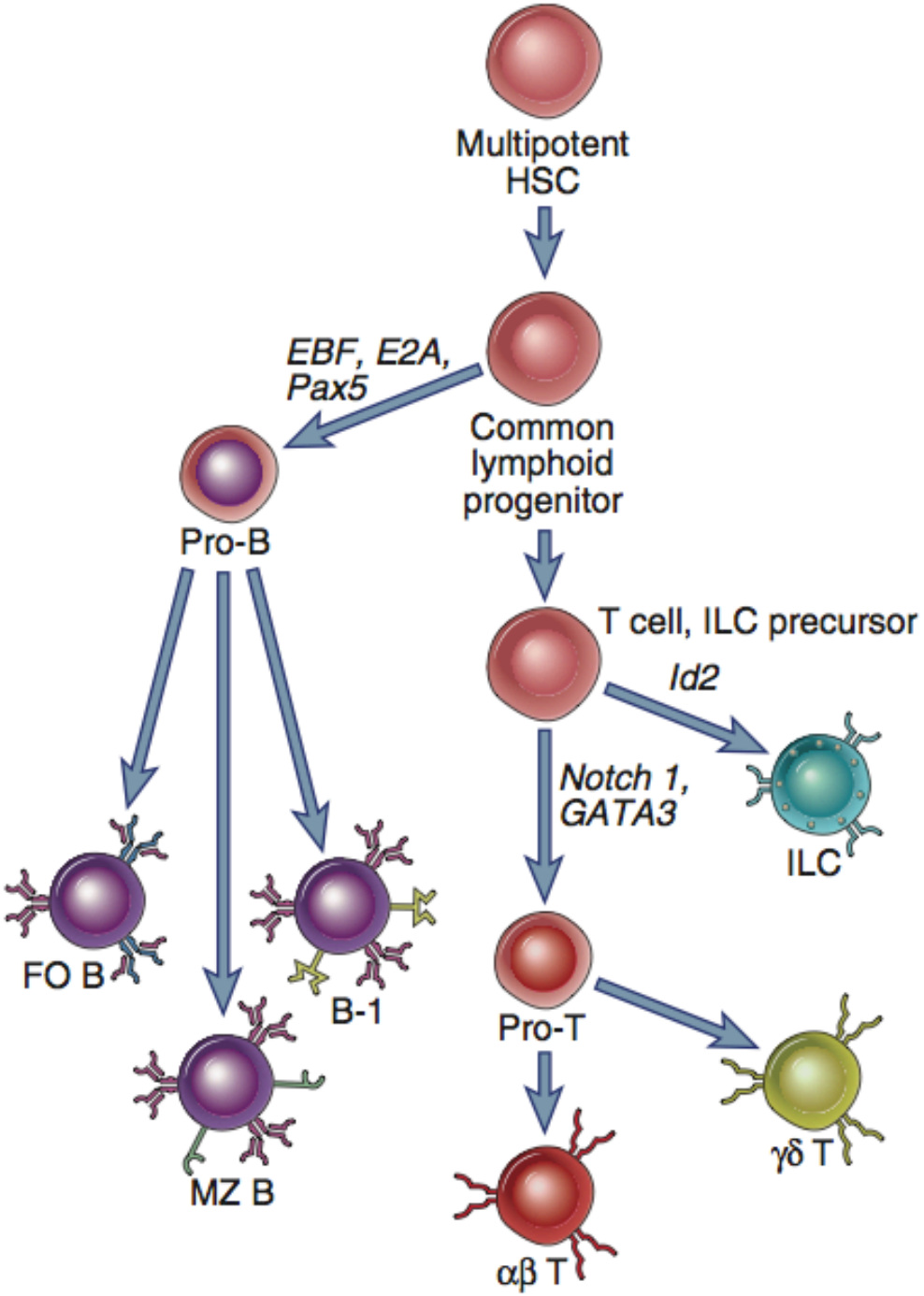
Theme 5: Three different types of mature B cells

Lymphocyte Development

- Lymphocyte development is designed to generate functional lymphocytes with useful antigen receptors that are not self-reactive
- Much of what happens during lymphocyte development is designed to improve the efficiency of adaptive immunity

B Cell Development: Clinical Relevance

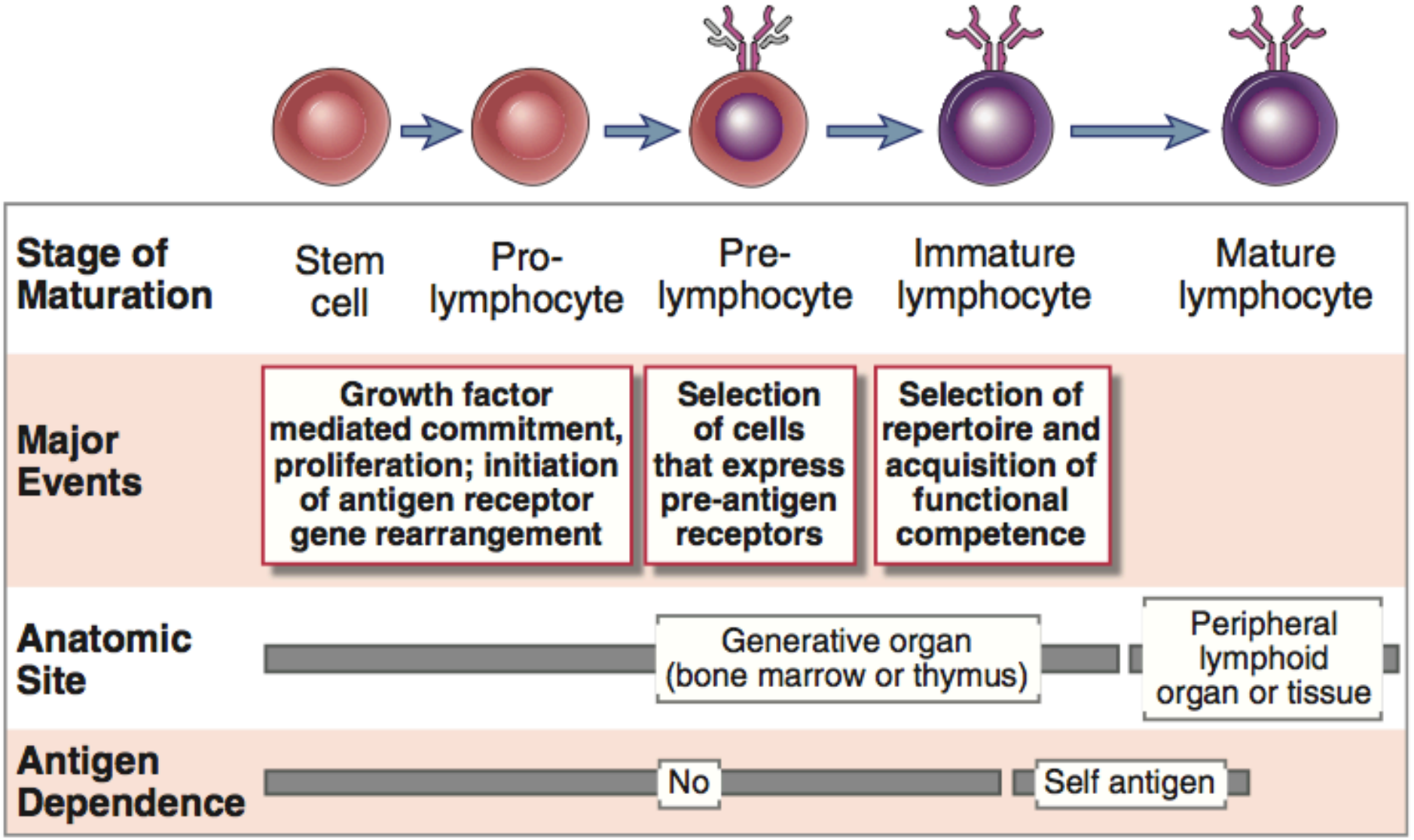
- Immunodeficiencies that affect B cell development
- B cell malignancies (pre-B ALL, etc.)
- Defects in B cell tolerance may underlie some autoimmune diseases
- B cell development is an especially well understood example of mammalian cell development



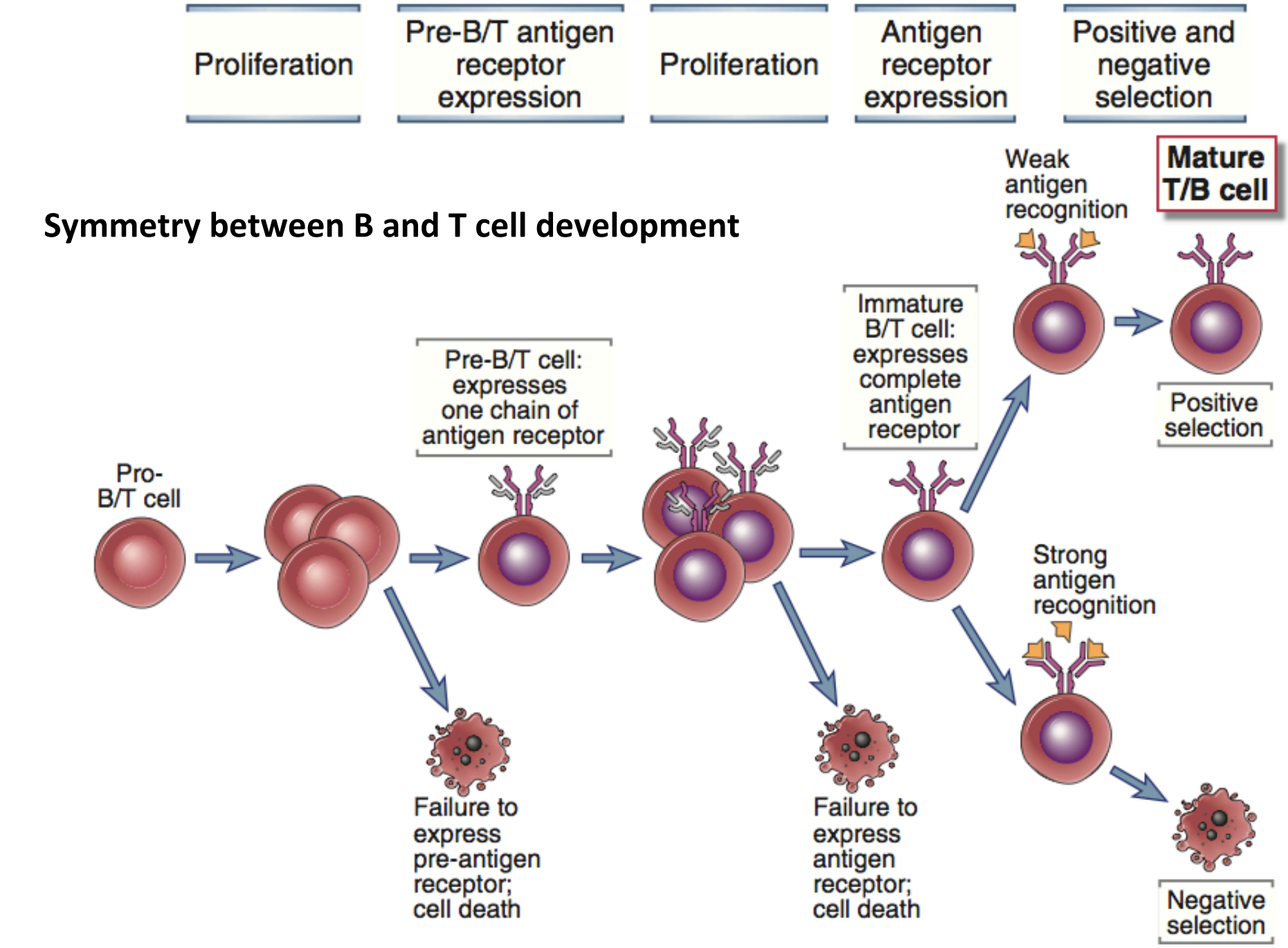
Lymphopoiesis

- Before birth, B lymphocytes develop from committed precursors in the fetal liver, and after birth, B cells are generated in the bone marrow.

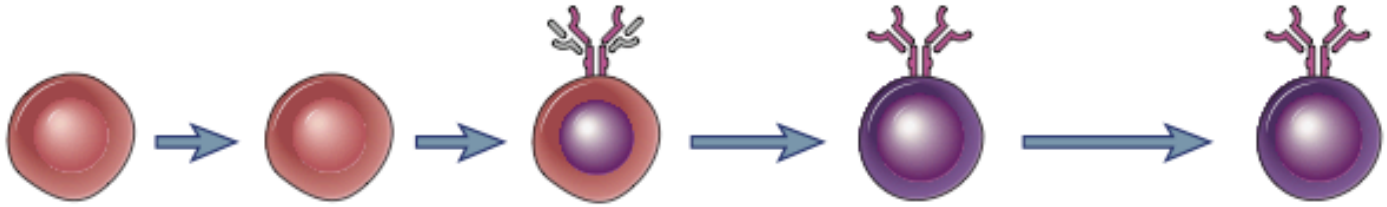
HSC, hematopoietic stem cell
CLP, common lymphoid progenitor
ILC, innate lymphoid cell



- The greatest proliferative expansion of B lymphocyte precursors occurs after successful rearrangement of the genes encoding one of the two chains of the BCR, producing a pre-BCR

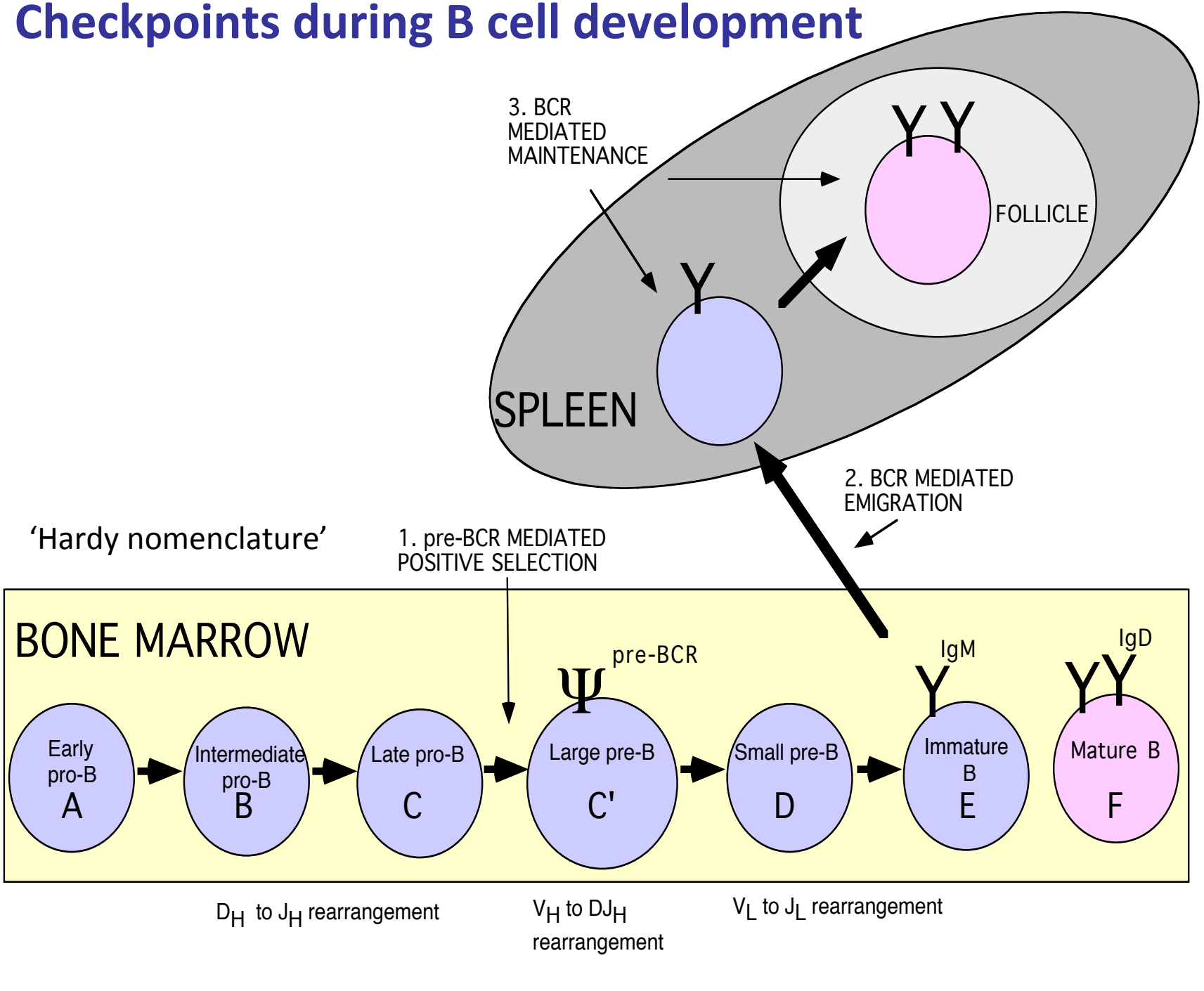

Proliferation
Pre-B/T antigen receptor expression
Proliferation
Antigen receptor expression
Positive and negative selection
Symmetry between B and T cell development
Pro-B/T cell
Pre-B/T cell: expresses one chain of antigen receptor
Immature B/T cell: expresses complete antigen receptor
Weak antigen recognition
Mature T/B cell
Positive selection
Strong antigen recognition
Failure to express pre-antigen receptor; cell death
Failure to express antigen receptor; cell death
Negative selection

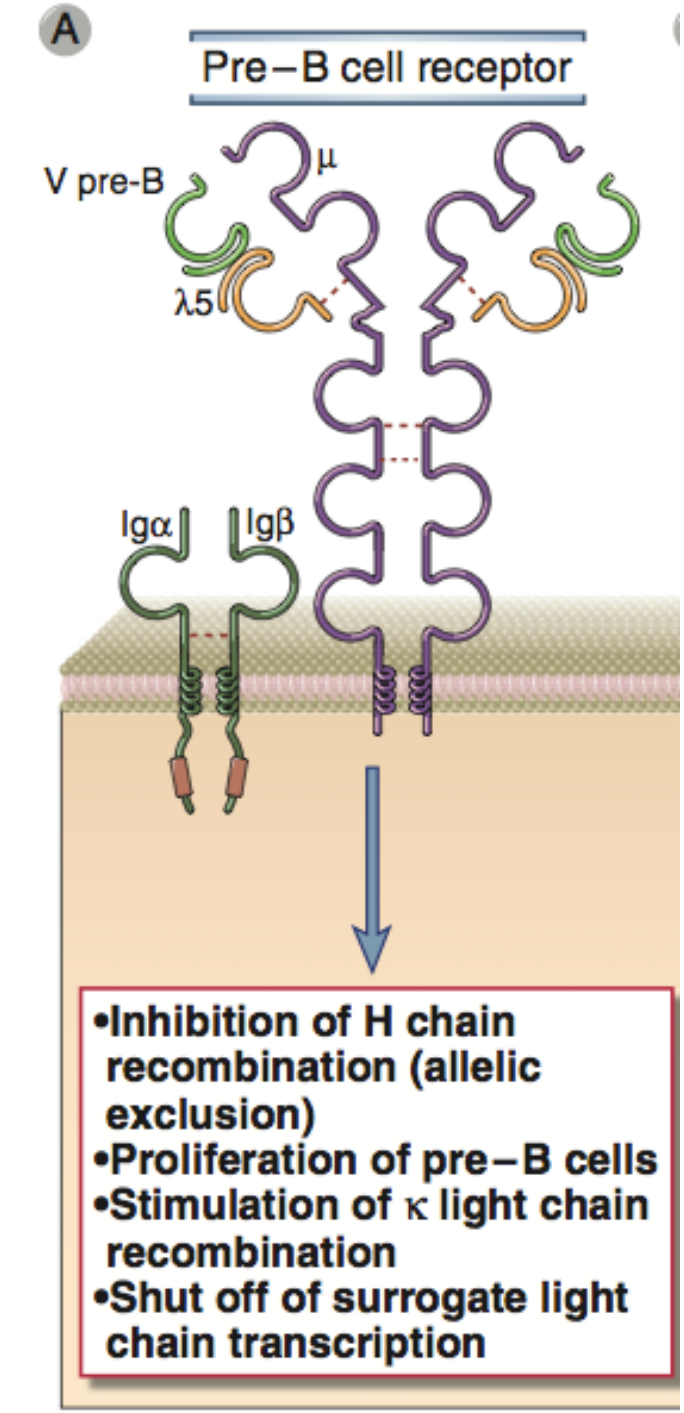
Stages of B cell maturation



Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
RAG expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgM^{hi}
Anatomic site	Bone marrow			Periphery	
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

Checkpoints during B cell development





- The pre–B cell receptor is expressed during the pre–B cell stage of maturation.
- The **pre-BCR** is composed of the μ heavy chain and an invariant **surrogate light chain**.
- The surrogate light chain is composed of the V pre-B protein, which is homologous to a light chain V domain, and a λ5 protein that is attached to the μ heavy chain by a disulfide bond.
- The pre–BCR is associated with the Igα and Igβ signaling molecules that are part of the BCR complex in mature B cells.
- Once formed, the pre-BCR transmits signals that informs the cell the Hc has been successfully rearranged.

B cell deficiency

- Bruton's tyrosine kinase (Btk) is activated downstream of the pre-BCR and is required for delivery of signals that mediate survival, proliferation, and maturation at and beyond the pre-B cell stage

- In humans, mutations in the *BTK* gene result in the disease called **X-linked agammaglobulinemia (XLA)**, which is characterized by a failure of B cell maturation

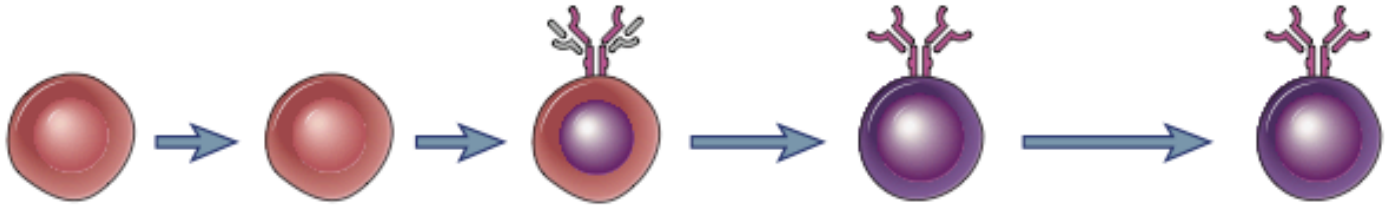
- Loss of Btk or other downstream components of the pre-BCR signalling cascade, including B cell linker protein (BLNK; also known as SLP65) and phospholipase Cγ2 (PLCγ2), block development at the cycling pre-BCR^+ pre-B cell stage

The pre-BCR and allelic exclusion

'One B cell, one receptor'

- If a μ protein is produced from the recombined Ig Hc locus on one chromosome and forms a pre-BCR, this receptor signals to irreversibly inhibit rearrangement of the Hc locus on the other chromosome.
- If the first rearrangement is nonproductive, the Hc allele on the other chromosome can complete VDJ rearrangement.
- So, an individual B cell can express a Hc protein encoded by only one of the two inherited alleles –> '**allelic exclusion**'
- If both Hc alleles undergo nonproductive Hc gene rearrangements, the developing cell cannot generate a pre-BCR–dependent survival signal, and undergoes cell death.
- Ig Hc allelic exclusion involves changes in chromatin structure in the Hc locus that limit accessibility to the V(D)J recombinase.

Stages of B cell maturation



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Immature B cell

- ***Following the pre-B cell stage, each developing B cell initially rearranges a*** κ ***light chain gene***. If the rearrangement is in-frame, it will produce a κ Lc, which associates with the previously synthesized μ Hc to produce IgM. If the κ locus is not productively rearranged, the cell can rearrange the λ light chain locus.

- The IgM-expressing B cell is called an **immature B cell**.

- In cells that are not strongly self-reactive, the IgM BCR provides ligand-independent tonic signals that keep the B cell alive and also mediate the shutoff of *RAG* gene expression, thus preventing further Ig gene rearrangement.

Questions

- If RAG1 or RAG2 are missing, at what stage would B cell development be blocked?
- If you introduce an Ig Hc transgene (tg) onto a RAG1 KO background, what stage would B cells reach?
- How about if you introduce Ig Hc and Lc tgs?
- Do you expect Ig-tg B cells on a wild-type background to have more than one type of surface Ig?

Theme 2: the bone marrow microenvironment

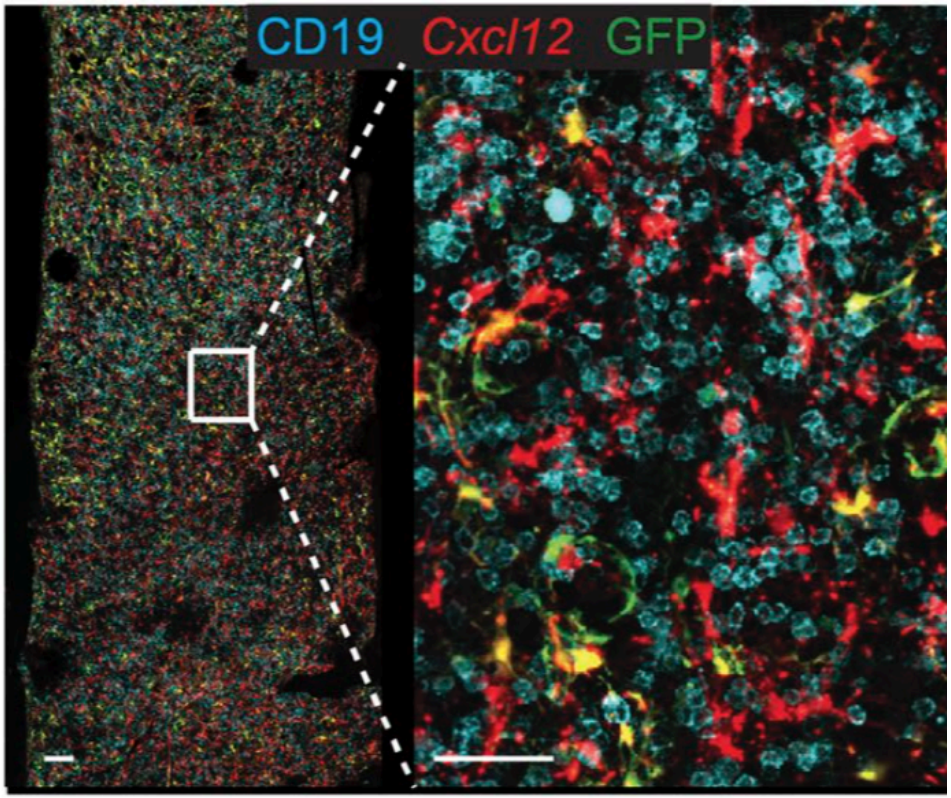
- Role of IL-7
  - In rodents, the cytokine interleukin-7 (IL-7) drives proliferation of both early B and T cell progenitors; in humans, IL-7 is required for the proliferation of T cell progenitors but not of B cell progenitors
    - Mutations in the common γ chain, a protein that is shared by the receptors for several cytokines including IL-2, IL-7, and IL-15, give rise to an immunodeficiency disorder called **X-linked severe combined immunodeficiency disease (X-SCID)**. This disease is characterized by a block in T cell and NK cell development, but normal B cell development.
  - IL-7 is made by a subset of BM stromal cells

- Lack of Notch ligands in the BM favor B lineage development (Notch ligands in thymus are important for commitment to the T lineage)

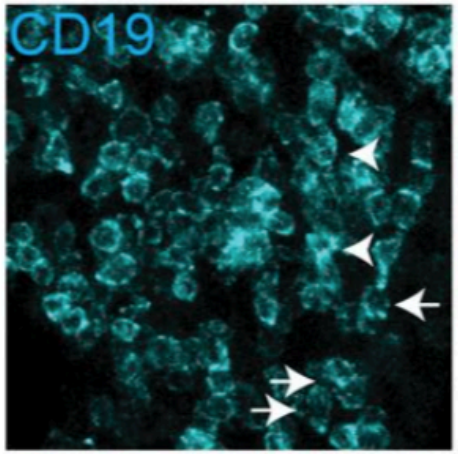
Theme 2: the bone marrow microenvironment

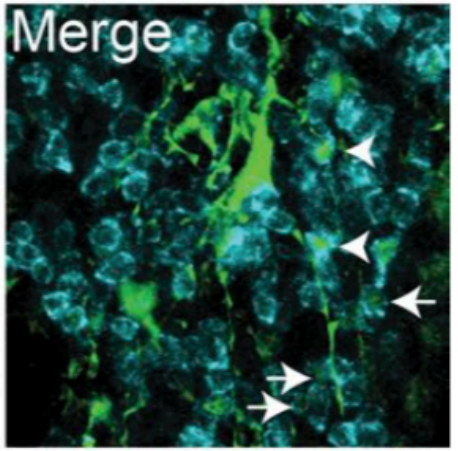
- Current model:
  - pro-B cells associate with CXCL12+IL-7+ stromal cells and attempt V(D)J recombination of IgH locus
  - pre-BCR transmits a signal that reduces adhesion and promotes motility, reducing the IL-7 signal
  - pre-BCR combines with the lower IL-7R signal to induce burst of pre-B proliferation

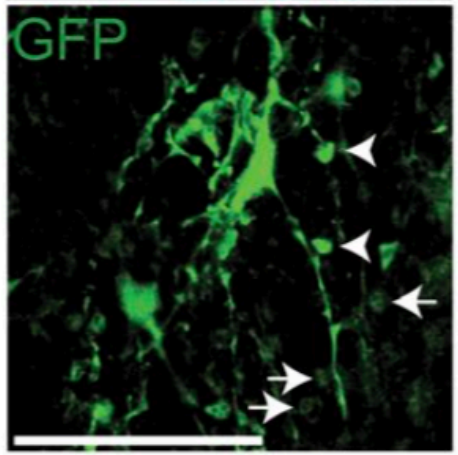
Early B cell development in the BM



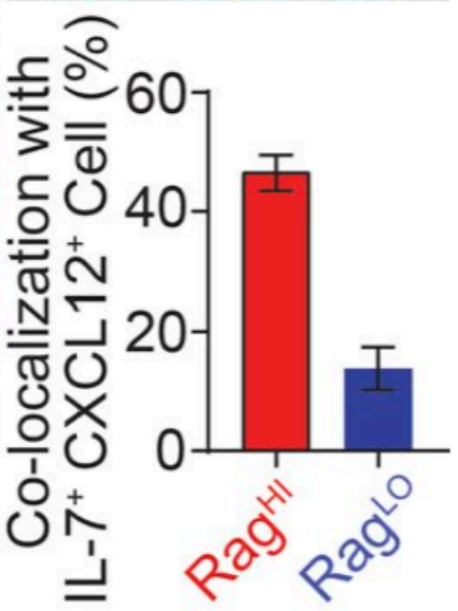
Rag-GFP







Il7-GFP



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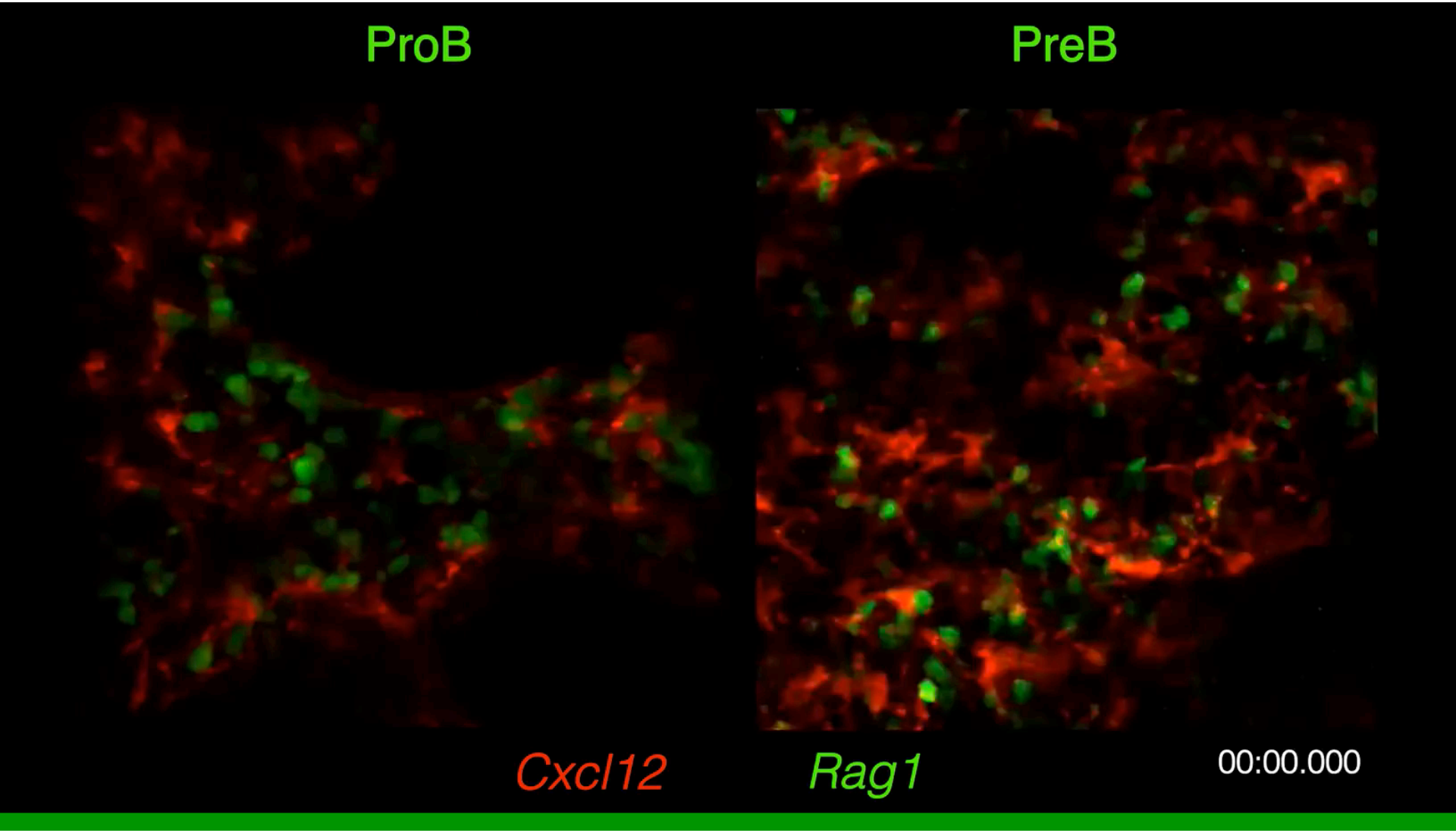
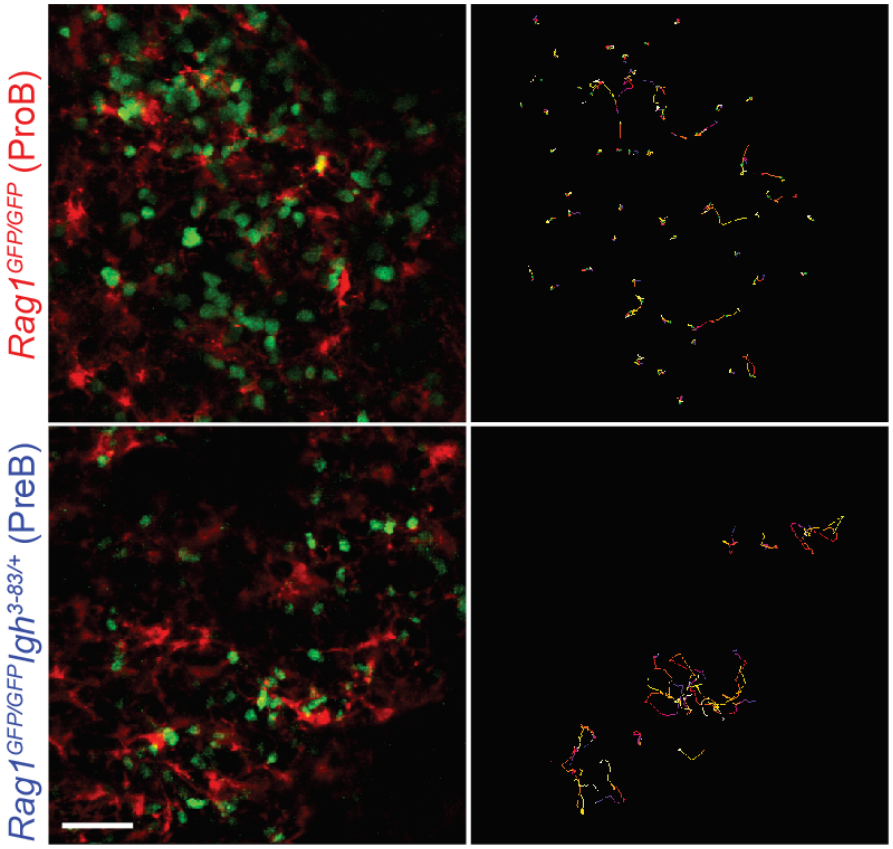
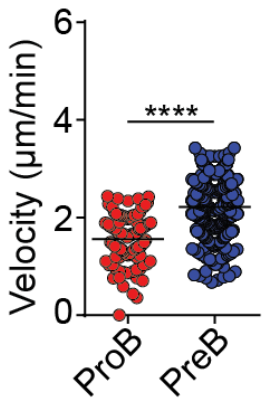
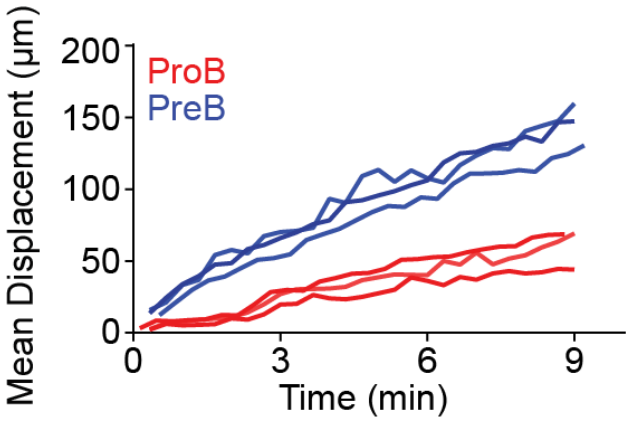
Early B cell development in the BM

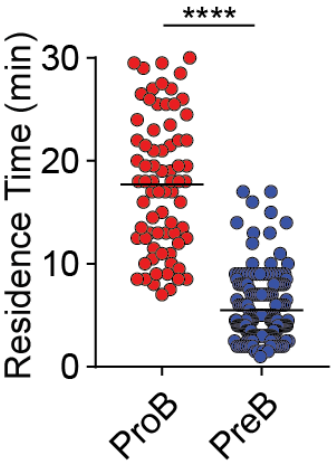


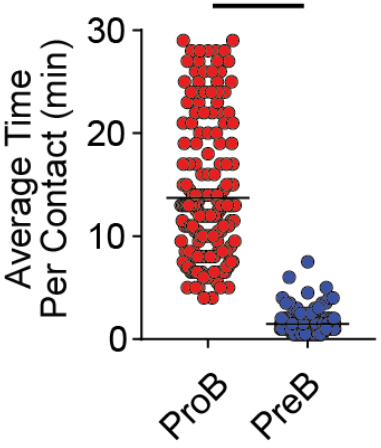
Motility parameters of proB and preB cells in vivo



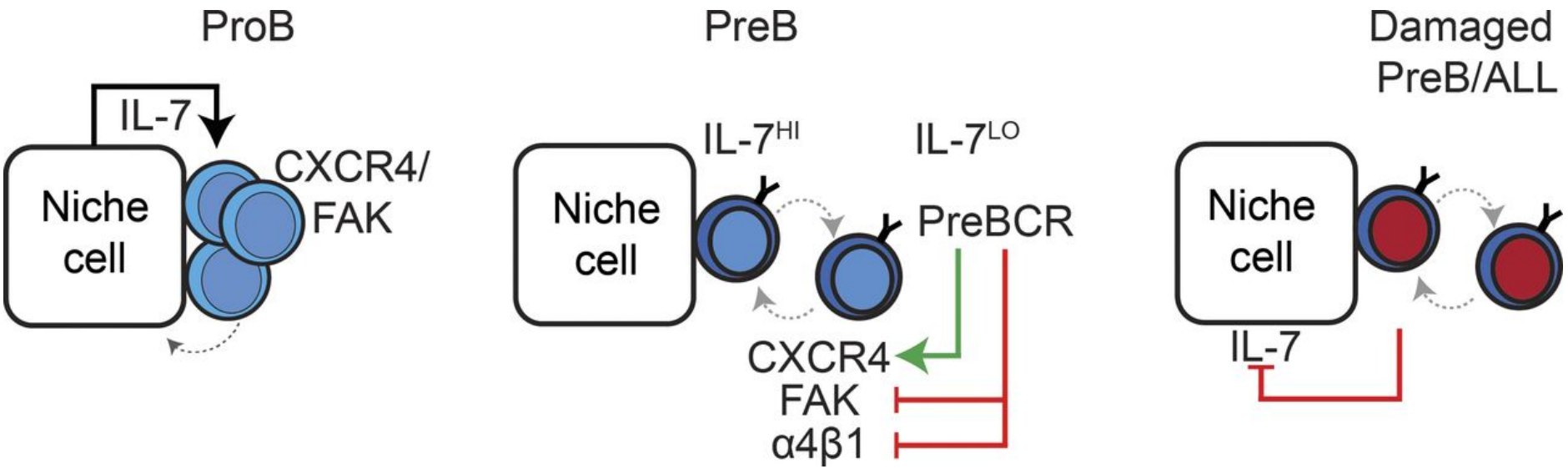




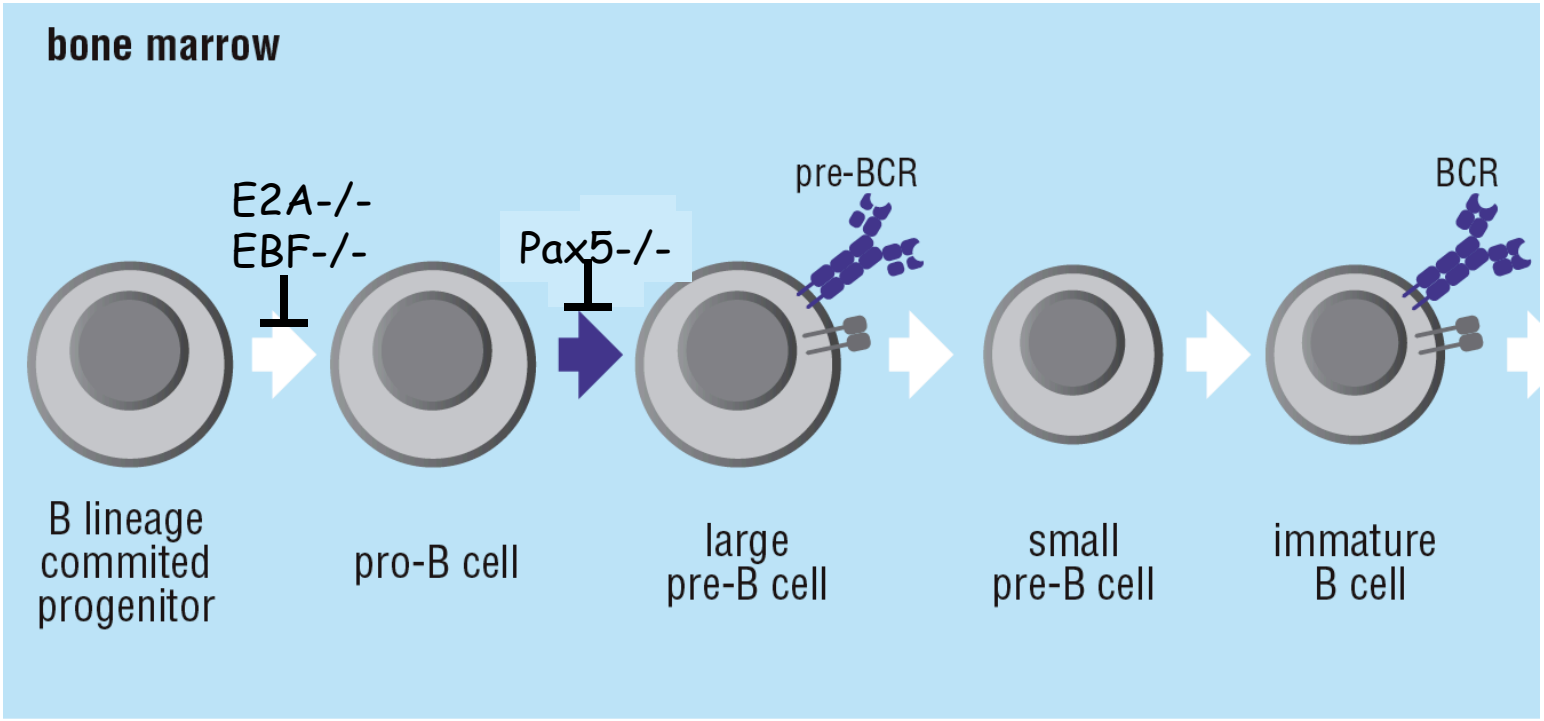




Early B cell development in the BM

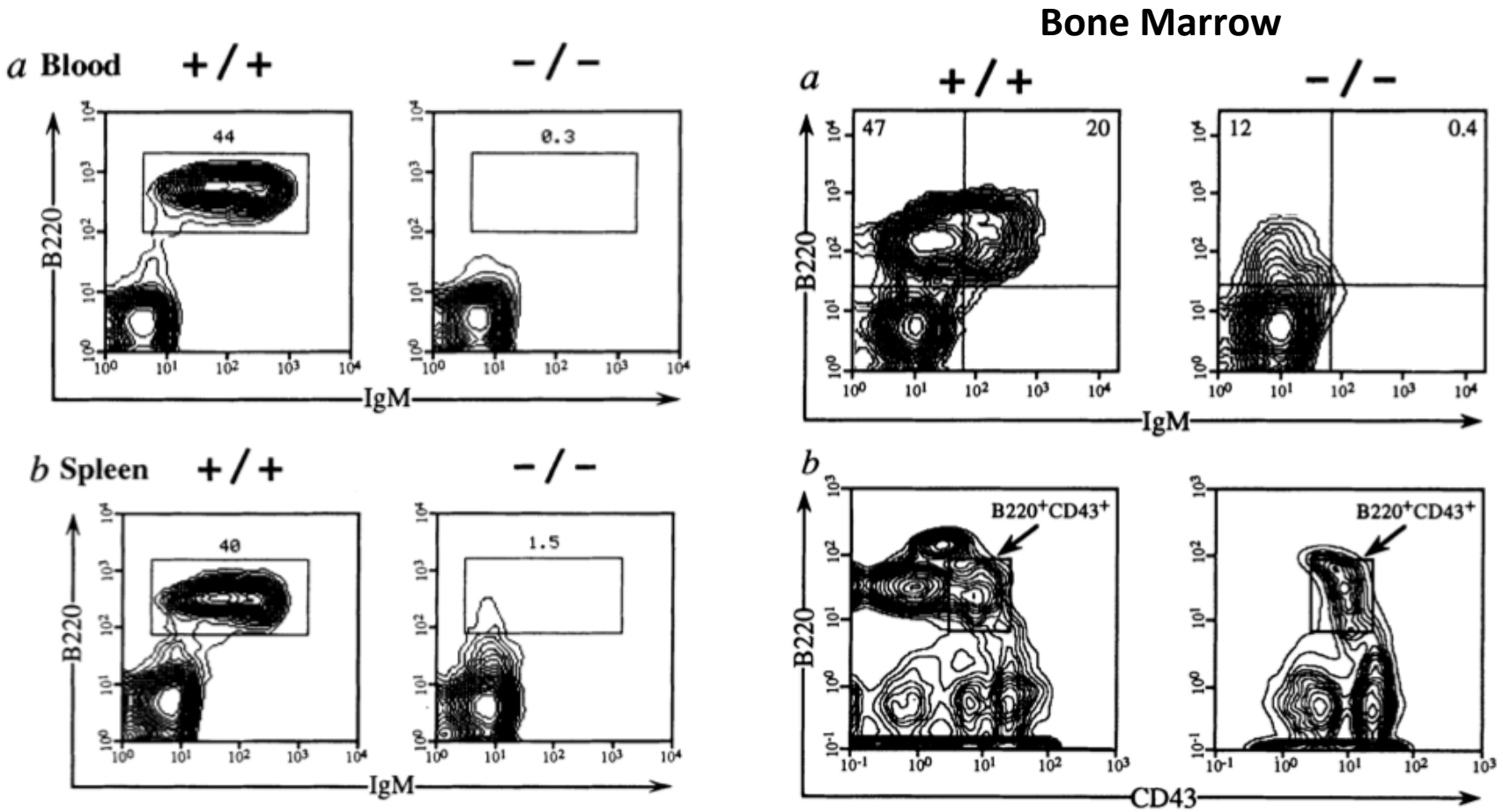


Theme 3: B lineage commitment: control by transcription factors

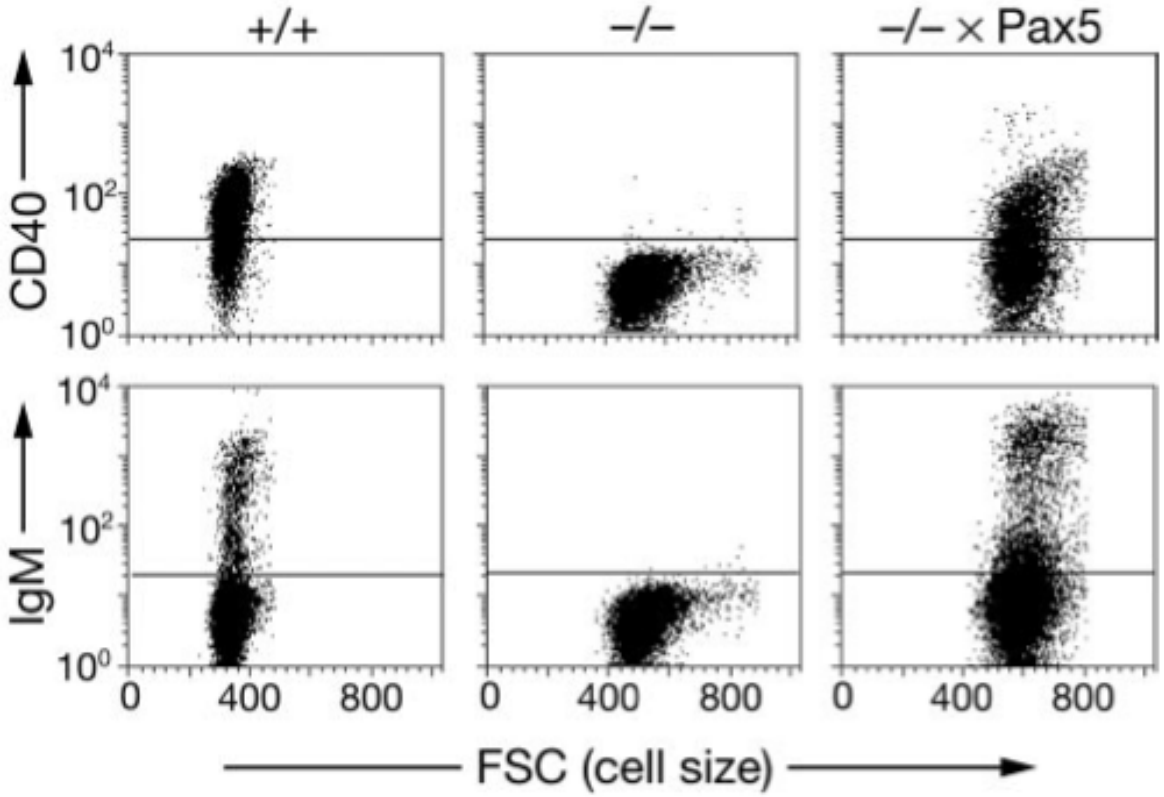


Knockouts of several transcription factors block B cell development at discrete stages

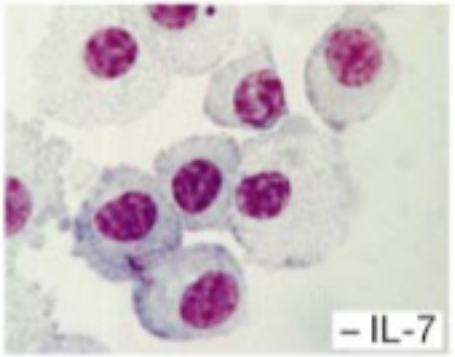
EBF requirement for B cell development



Lin & Grosschedl, Nature 1995



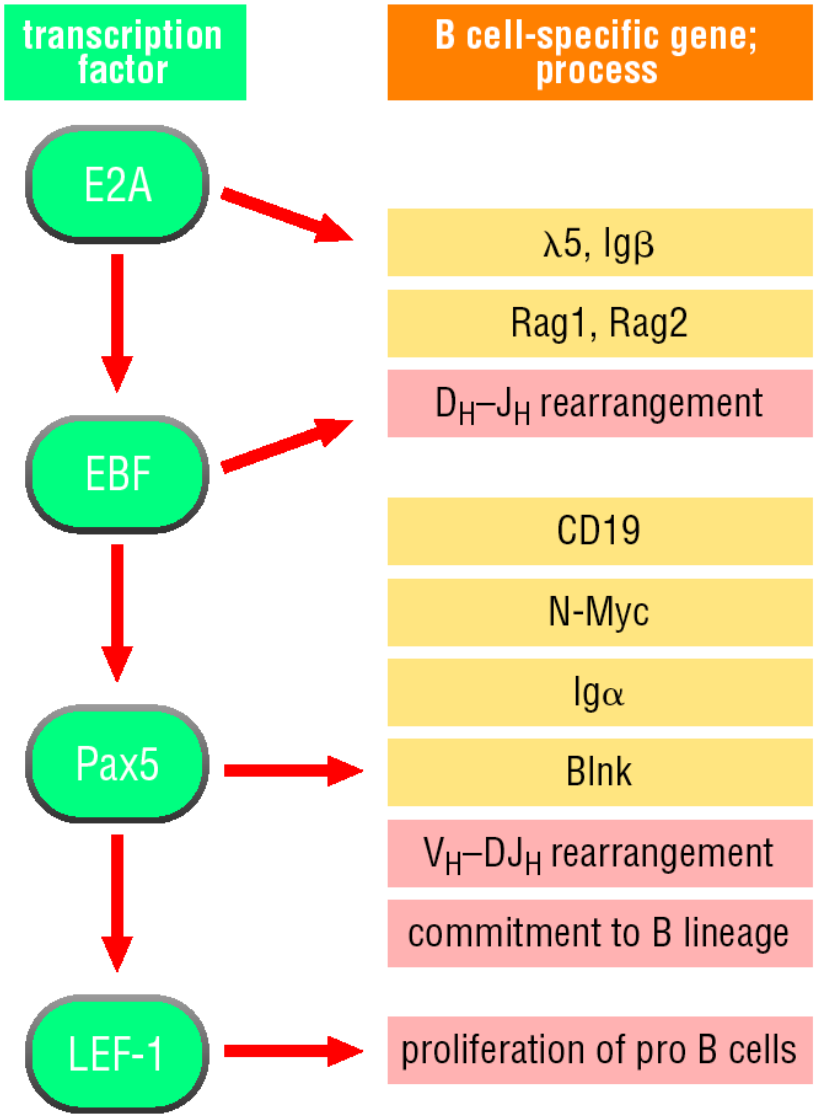
Pax5-/-



Pax5 is essential for in vitro differentiation of B lymphocytes. Wild-type and Pax5 −/− pro-B cells expressing a retroviral bcl2 gene were cultured in the absence of stromal cells and IL-7 for 3 days in medium alone followed by flow cytometric analysis. x Pax5 indicates transduction with Pax5 expressing retrovirus. FSC, forward scatter.

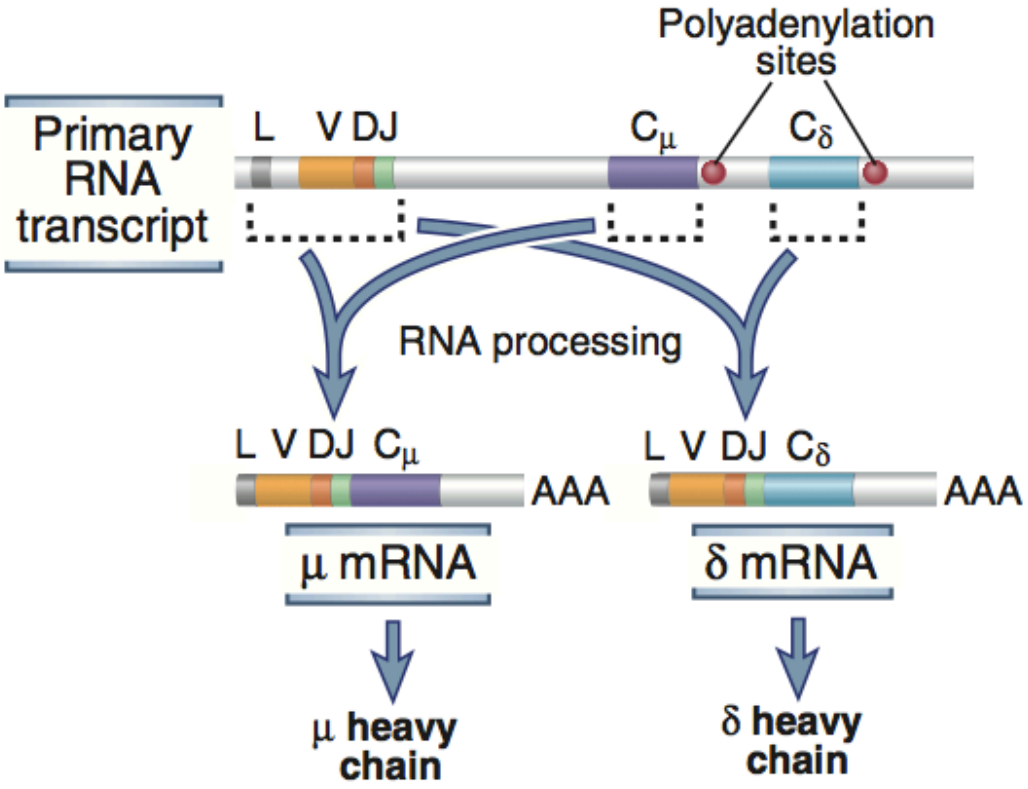
Nutt/Busslinger et al., Nature 1999

A hierarchy of transcription factors specifies B cell fate



- E2A and EBF are needed to turn on B cell specific genes including Pax5, which turns on additional B cell-specific genes
- Pax5 seems to act in two ways:
  - It promotes progression down the B cell lineage (expression of Igα, Blnk)
  - It shuts off genes needed to go down other lineages (M-CSF receptor, pre-Tα, Notch1) or associated with other lineages (myeloperoxidase, perforin, etc.)
- Pax5 controls the transcription of its target genes by recruiting chromatin-remodeling, histone-modifying, and basal transcription factor complexes.

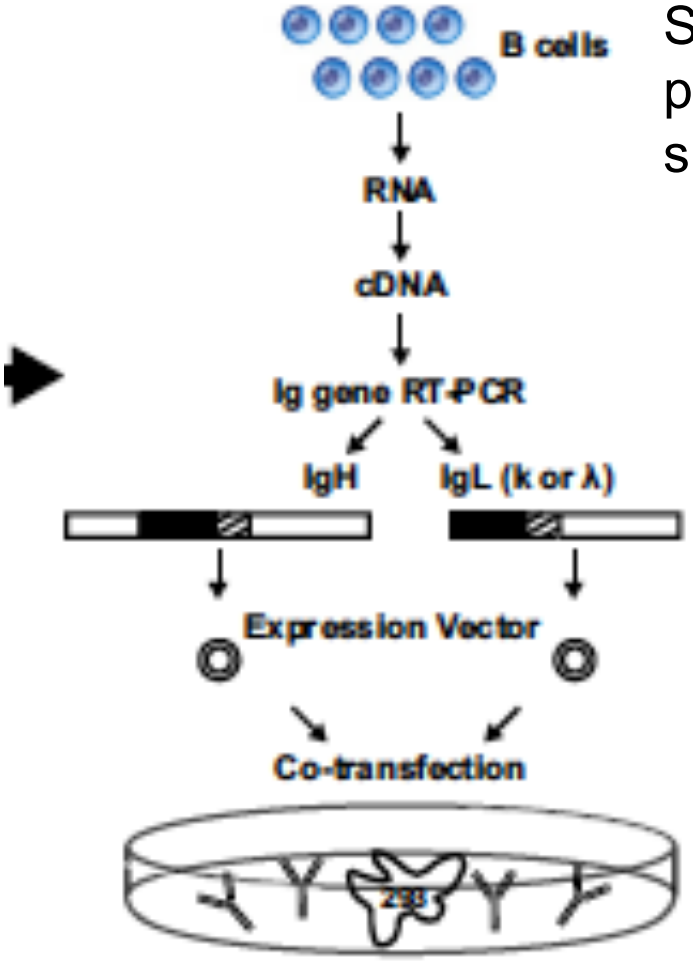
IgM and IgD co-expression by follicular B cells



Alternative processing of a primary RNA transcript results in the formation of a μ or δ mRNA. Dashed lines indicate the H chain segments that are joined by RNA splicing.

Theme 4: Tolerance of B cells

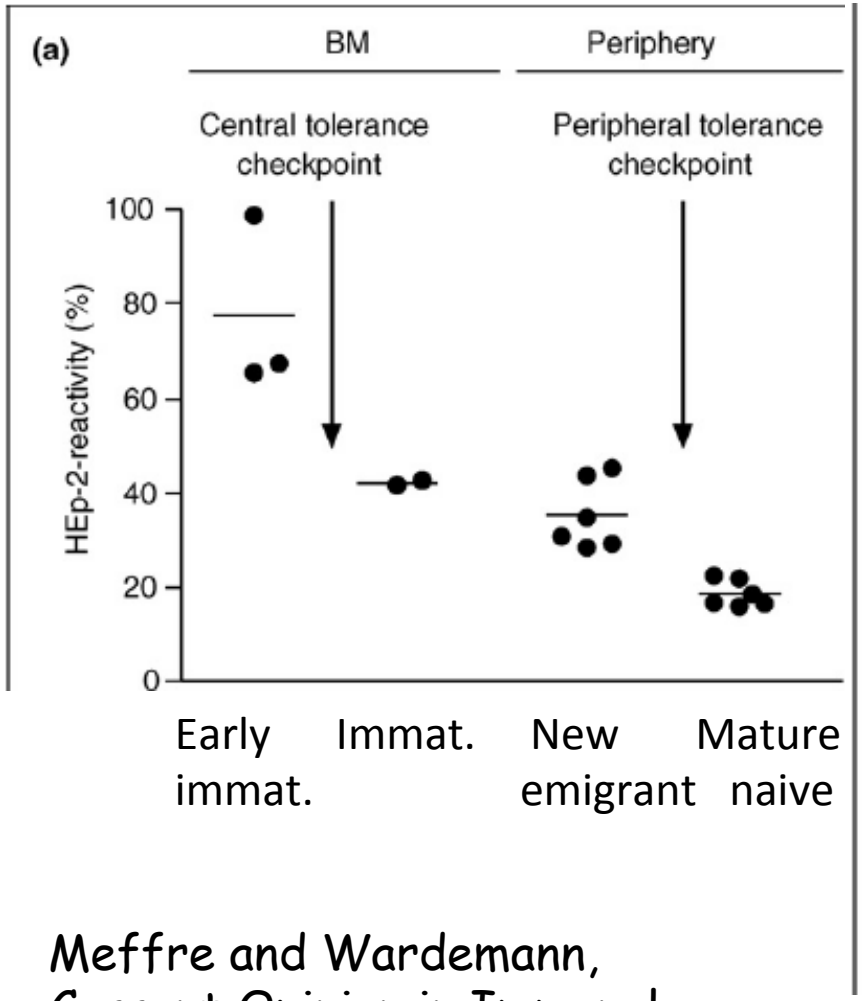
Method for studying the self-reactivity of human B cells

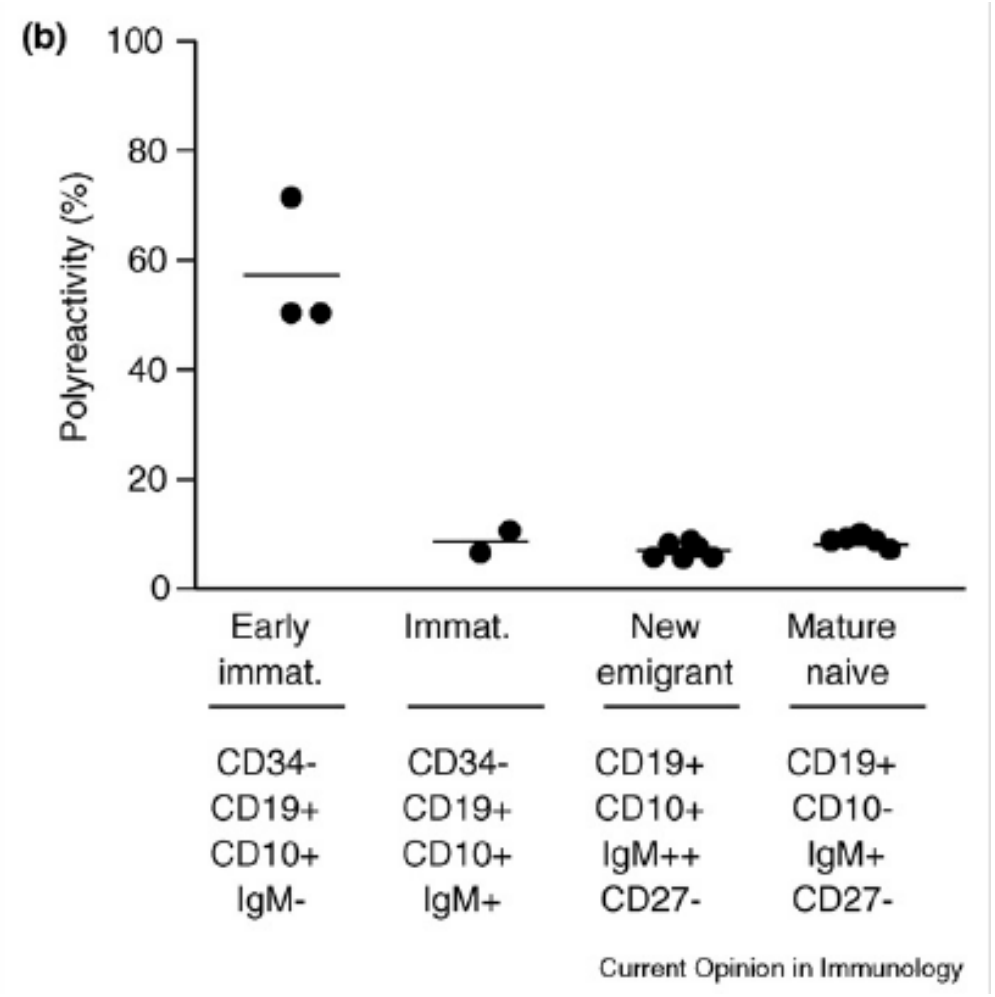


Sorted based on phenotype and/or specificity, etc.

Test antibodies for properties, self reactivity, etc.

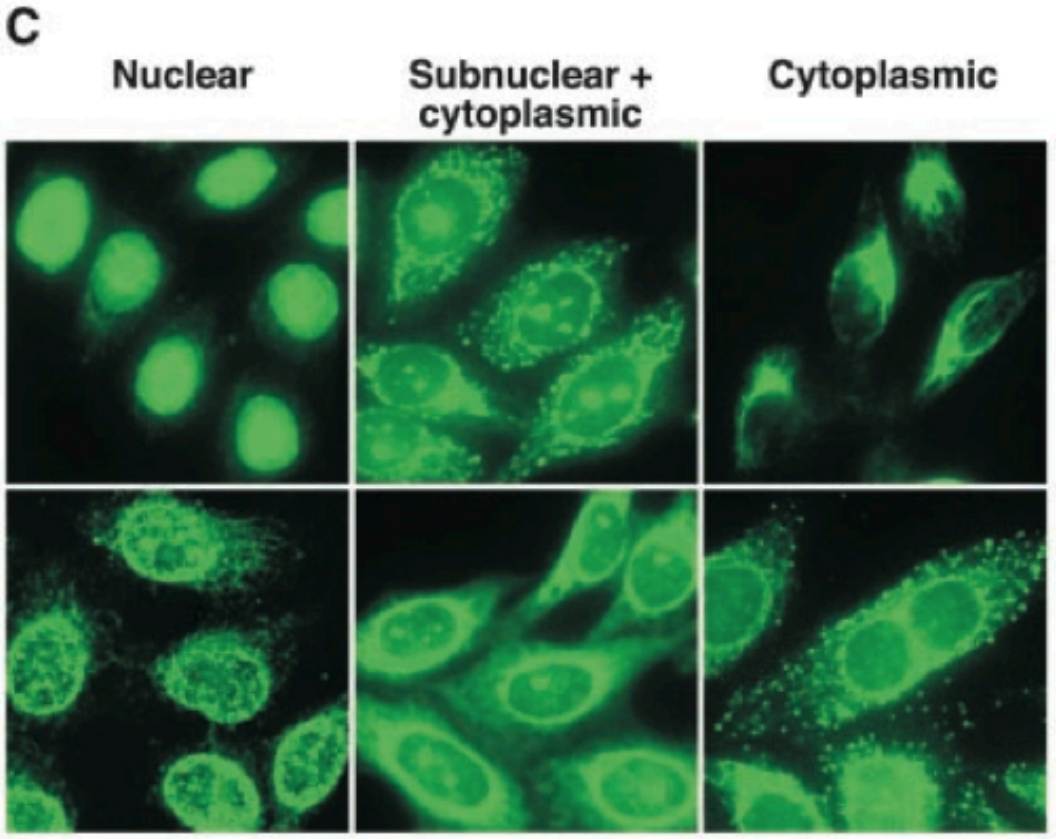
The primary repertoire of B cells includes many self-reactive cells

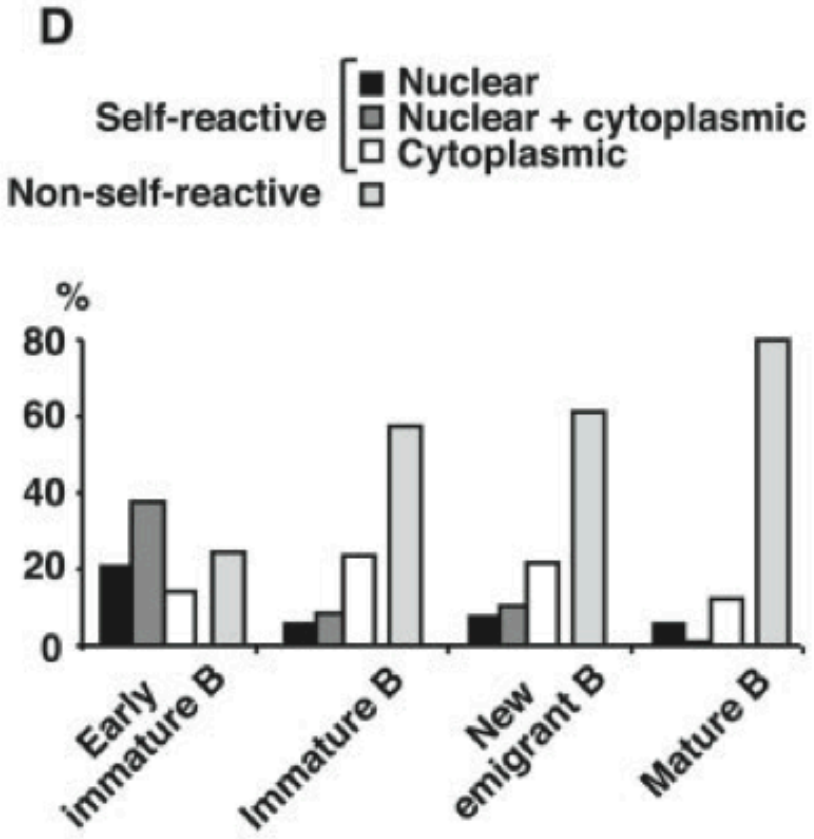




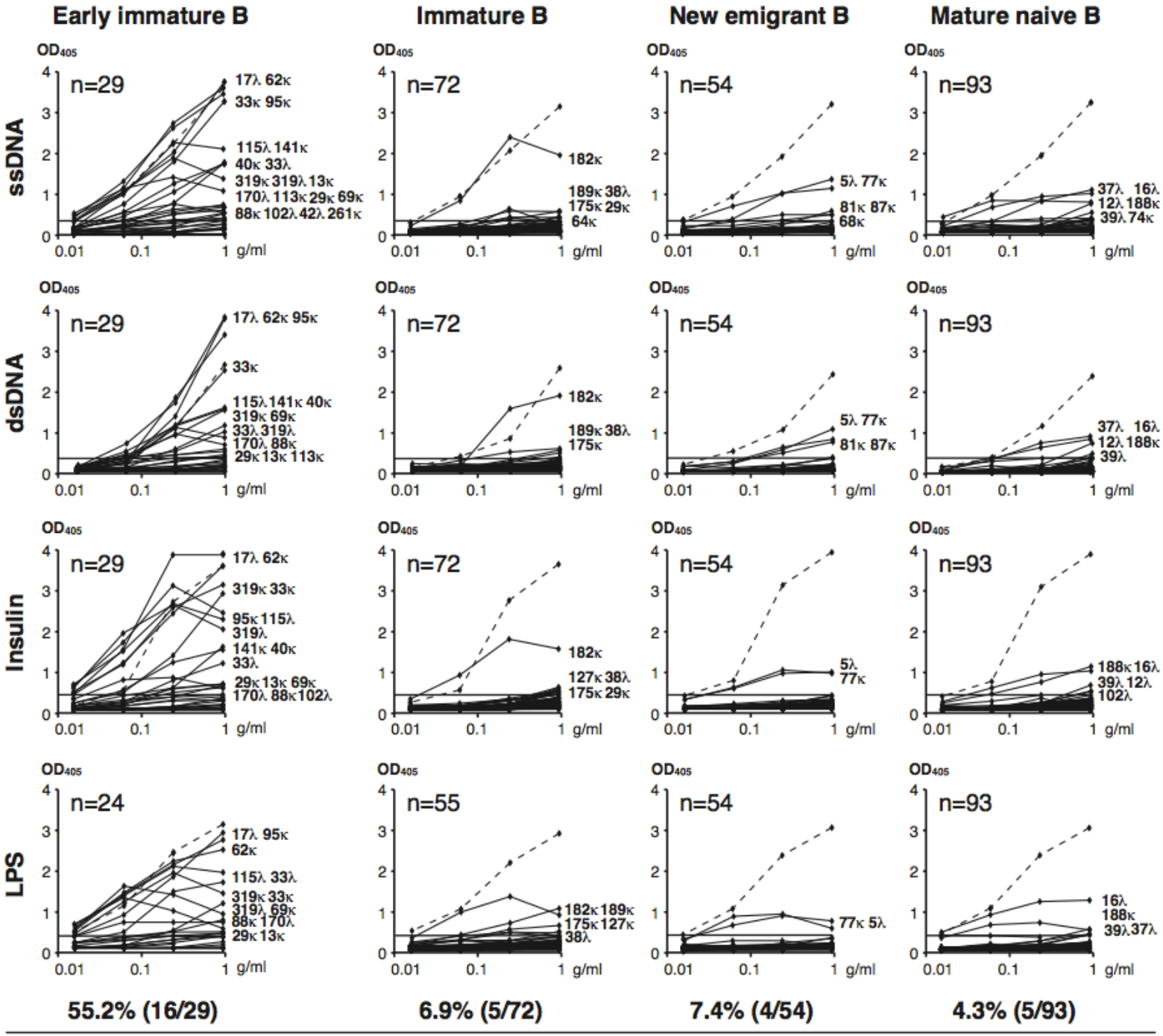
Meffre and Wardemann,
Current Opinion in Immunology
20:632, 2008

Autoreactivty of antibodies assessed by HEp-2 cell immunofluorescence assay

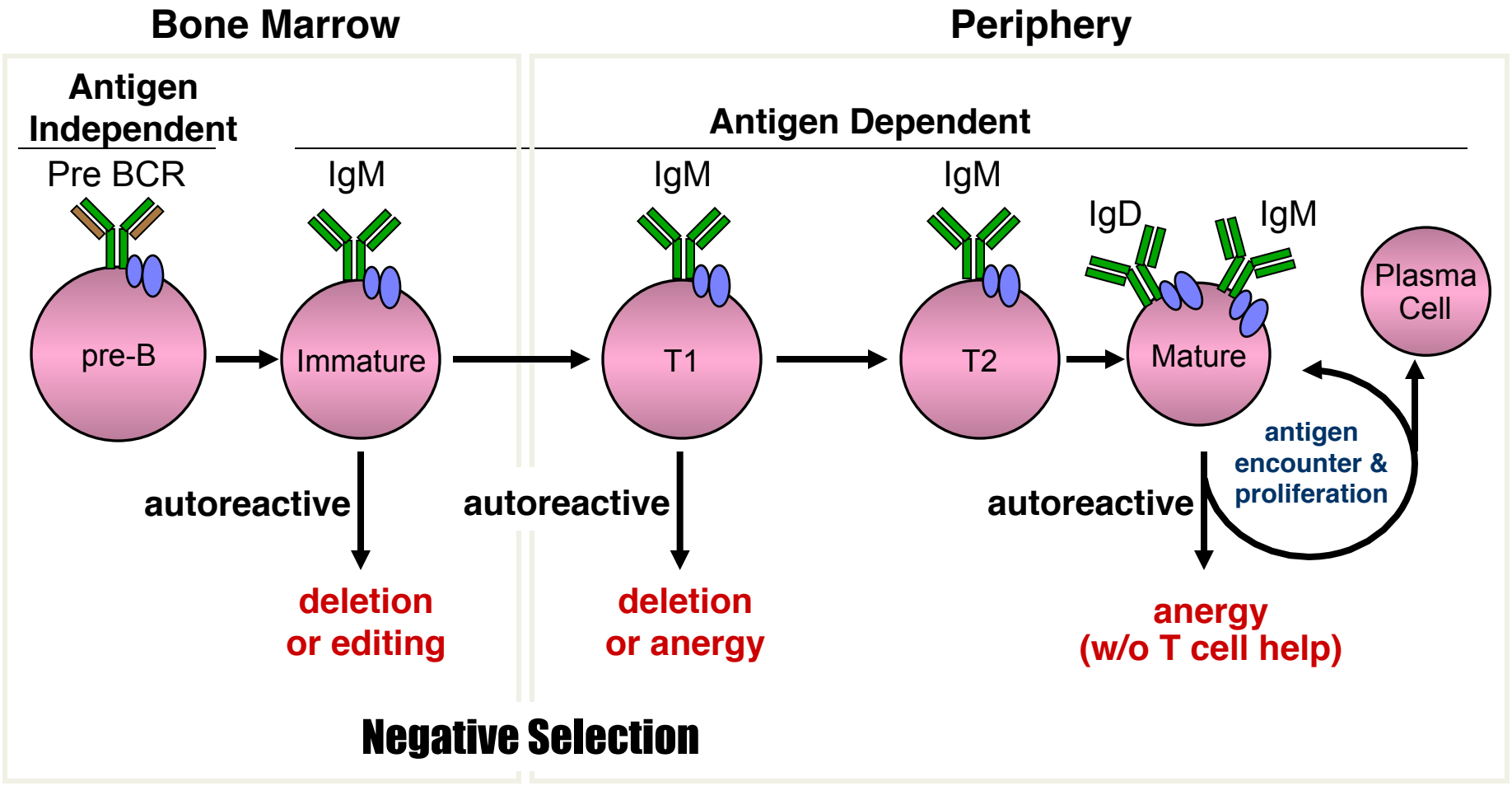




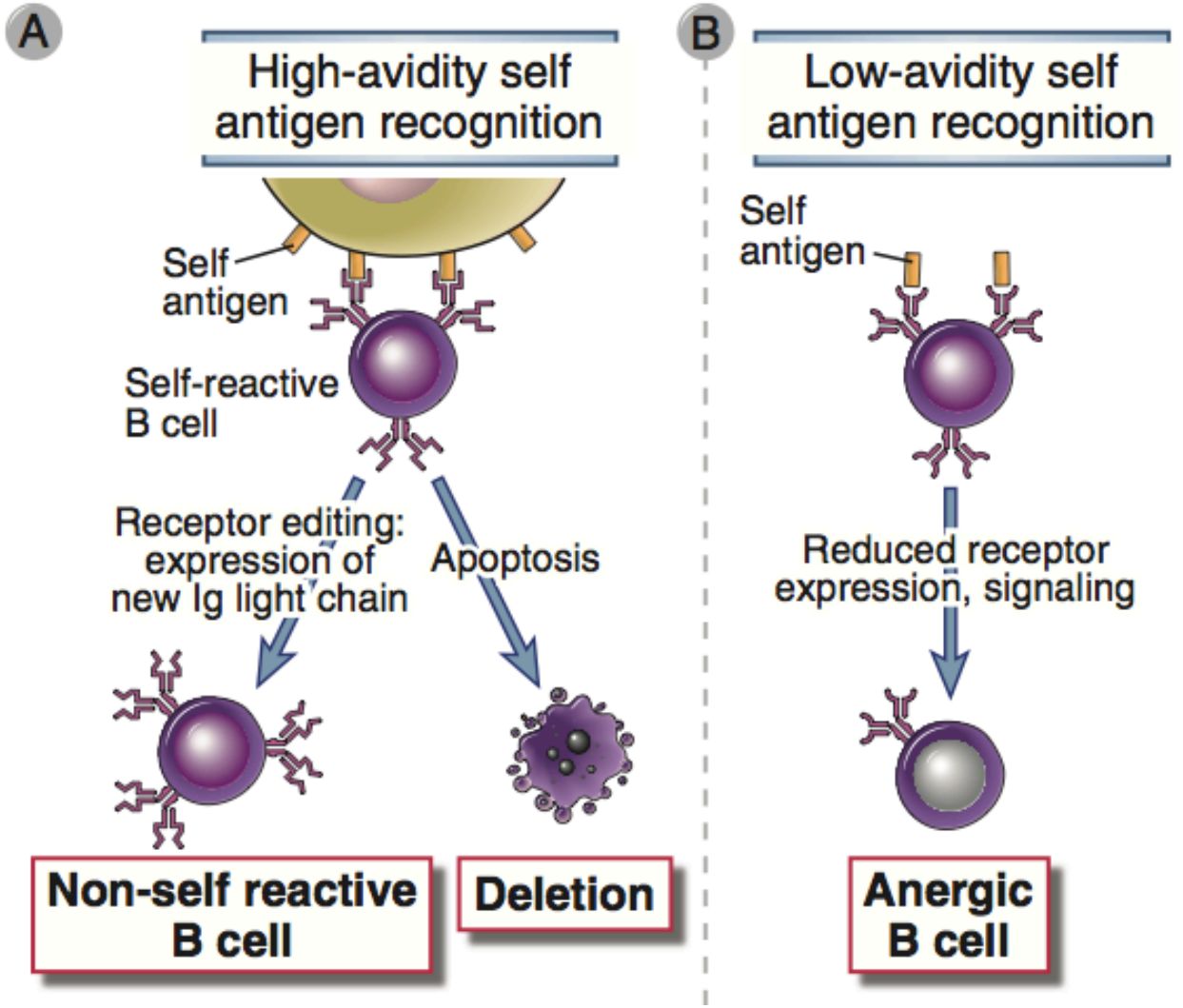
Wardemann et al., Science 2003


Early immature B
Immature B
New emigrant B
Mature naive B
ssDNA
dsDNA
Insulin
LPS
OD405
g/ml
0.01
0.1
1
n=29
17λ 62κ
33κ 95κ
115λ 141κ
40κ 33λ
319κ 319λ 13κ
170λ 113κ 29κ 69κ
88κ 102λ 42λ 261κ
n=72
182κ
189κ 38λ
175κ 29κ
64κ
n=54
5λ 77κ
81κ 87κ
68κ
n=93
37λ 16λ
12λ 188κ
39λ 74κ
17λ 62κ 95κ
33κ
115λ 141κ 40κ
319κ 69κ
33λ 319λ
170λ 88κ
29κ 13κ 113κ
182κ
189κ 38λ
175κ
5λ 77κ
81κ 87κ
37λ 16λ
12λ 188κ
39λ
17λ 62κ
319κ 33κ
95κ 115λ
319λ
141κ 40κ
33λ
29κ 13κ 69κ
170λ 88κ 102λ
182κ
127κ 38λ
175κ 29κ
5λ
77κ
188κ 16λ
39λ 12λ
102λ
n=24
17λ 95κ
62κ
115λ 33λ
319κ 33κ
319λ 69κ
88κ 170λ
29κ 13κ
n=55
182κ 189κ
175κ 127κ
38λ
77κ 5λ
16λ
188κ
39λ 37λ
55.2% (16/29)
6.9% (5/72)
7.4% (4/54)
4.3% (5/93)
Polyreactive

Theme 4: Fate of self-reactive B cells



Central tolerance in B cells



- Immature B cells that recognize self antigens in the BM with high avidity (e.g., multivalent arrays of antigens on cells) die by apoptosis (**deletion**) or change the specificity of their antigen receptors (**receptor editing**).
- Weak recognition of self antigens in the bone marrow may lead to **anergy** (functional inactivation) of the B cells.

- DNA, membrane antigen (e.g. MHC class I)
- soluble antigen (e.g. transgenic hen egg lysozyme (HEL) autoantigen)

Central B Cell Tolerance

- ***Receptor editing.*** If immature B cells recognize self antigens in the BM displayed in multivalent form (e.g., on cell surfaces), many BCRs on each B cell are cross-linked, thus delivering strong signals to the cells. This re-activates their *RAG1* and *RAG2* genes and initiate a new round of VJ recombination in the Ig κ Lc locus.

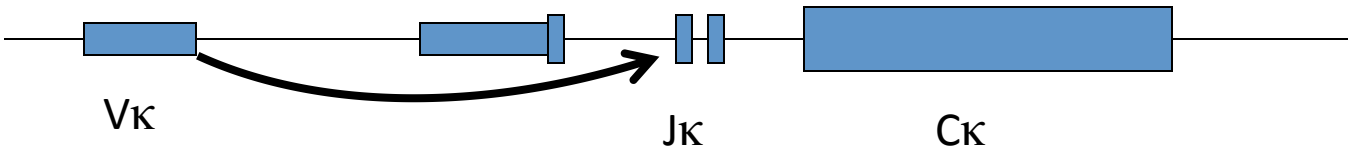
- A Vκ segment upstream of the already rearranged VκJκ unit is joined to a downstream Jκ. As a result, the previously rearranged VκJκ exon in the self-reactive immature B cell is deleted, and a new Ig Lc is expressed, thus creating a BCR with a new specificity.

- If the edited κ Lc rearrangement is non-productive, rearrangement may proceed at the κ locus on the other chromosome, and if that is non-productive, rearrangements at the λ Lc loci may follow. A B cell expressing a λ Lc is frequently a cell that has undergone receptor editing.

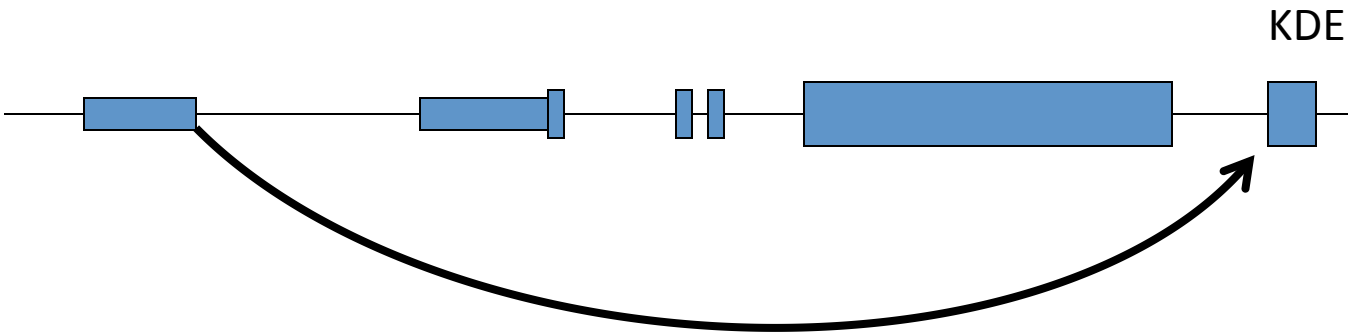
- ***Deletion.*** If editing fails, the immature B cells may die by apoptosis. The mechanisms of deletion are not well defined.

Receptor Editing Mechanisms

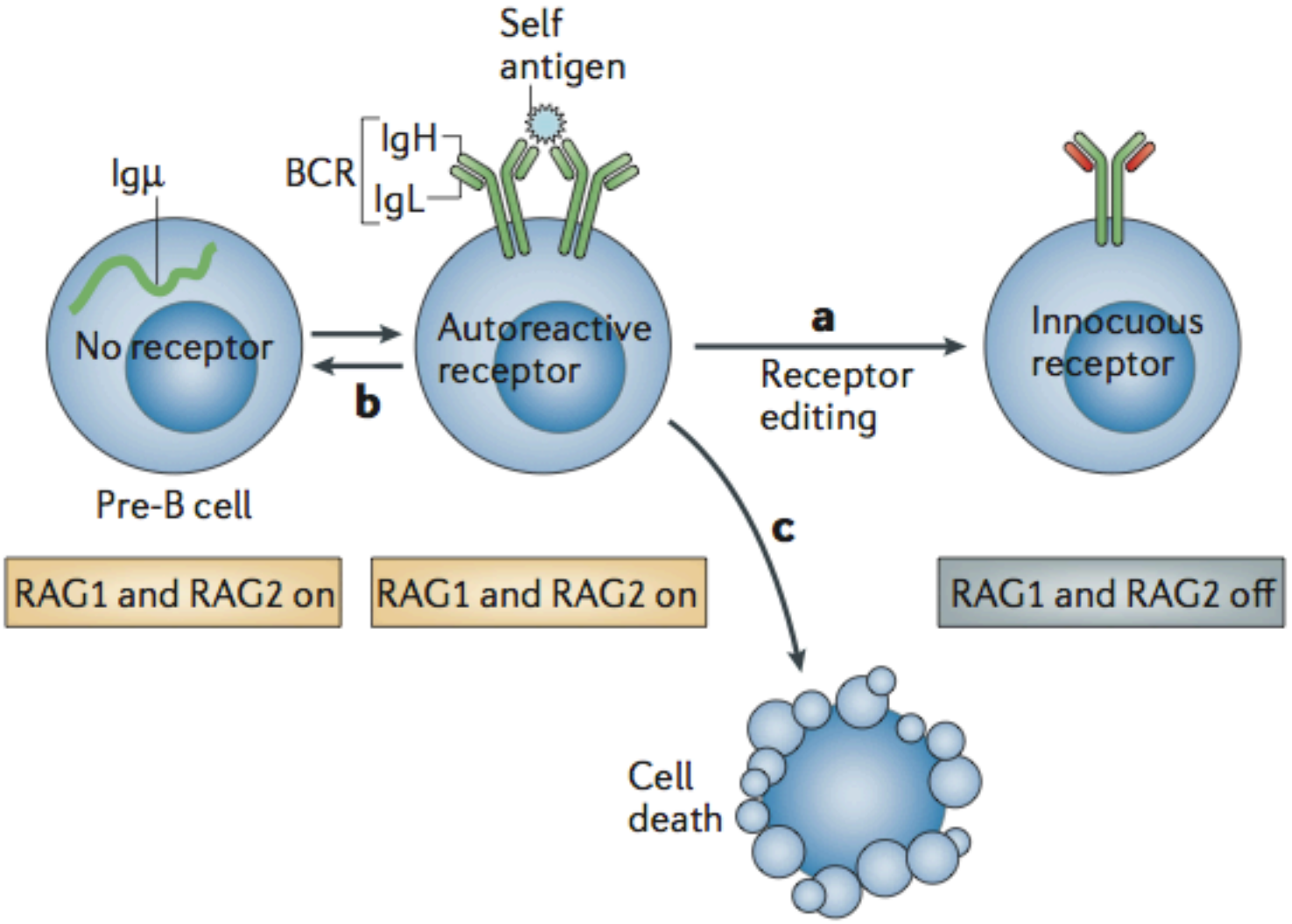
1. Upstream Vκ can rearrange to downstream Jκ



2. Upstream Vκ can rearrange to KDE (κ deleting element) deleting Cκ; this would be followed by a rearrangement of another light chain allele



Receptor editing occurs in the bone marrow



Nemazee 2006 *Nat Rev Immunol*

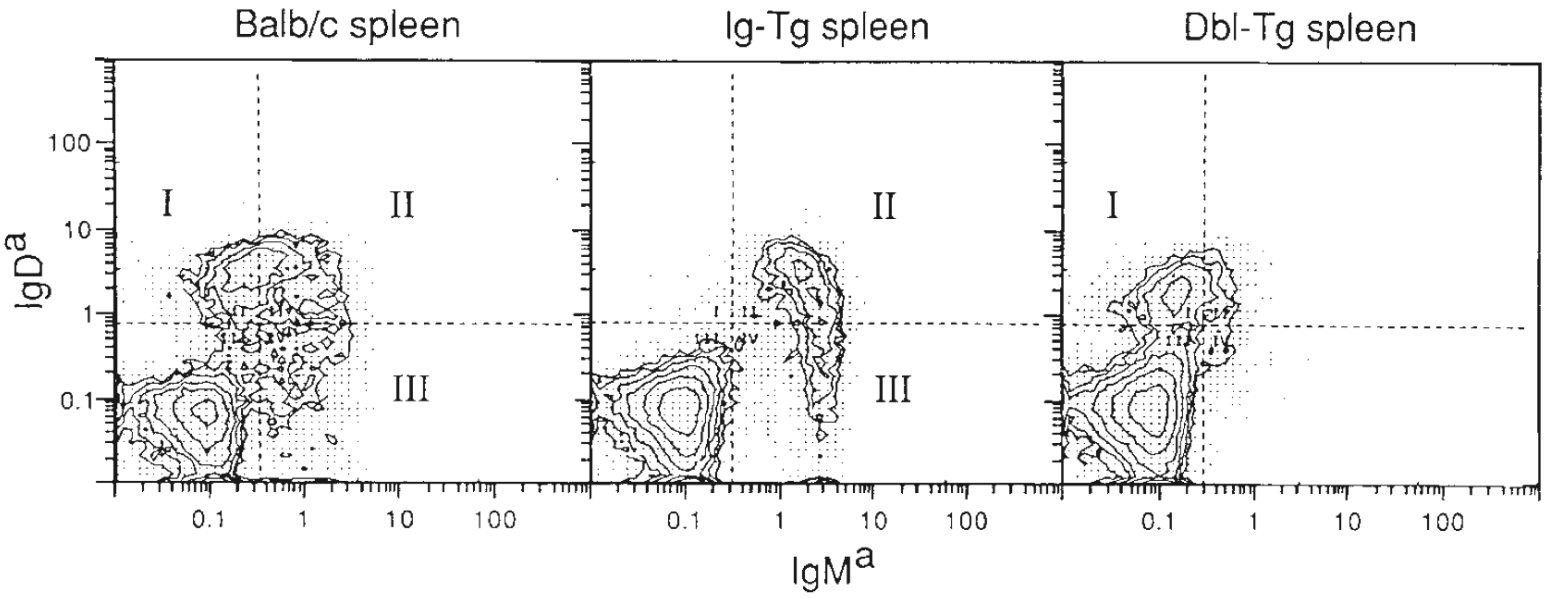
Peripheral tolerance in B cells

- ***Anergy.*** Some self-reactive B cells that are repeatedly stimulated by self antigens become unresponsive to further activation. These cells require high levels of the growth factor BAFF and cannot compete efficiently with less BAFF–dependent normal naive B cells for survival.
  - If anergic B cells are totally inactivated and slowly lost, why not just delete them outright? Evidence is emerging in support of a concept called '**clonal redemption**' where these cells can enter germinal centers (GCs) in response to foreign antigen, mutate away from self and contribute to production of protective antibodies.

- **Peripheral generation of self-reactive B cells in GCs**: The high rate of somatic mutation of Ig genes that occurs in germinal centers has the risk of generating self-reactive B cells. Typically these cells will die because they fail to receive necessary helper T cell-derived survival signals. Fas ligand on activated T cells can also contribute to killing some autoreactive B cells by engagement of Fas.

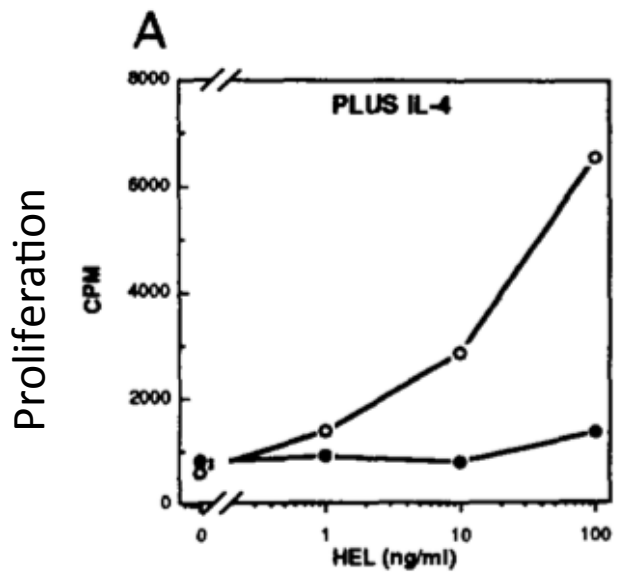
Mouse model of B cell anergy

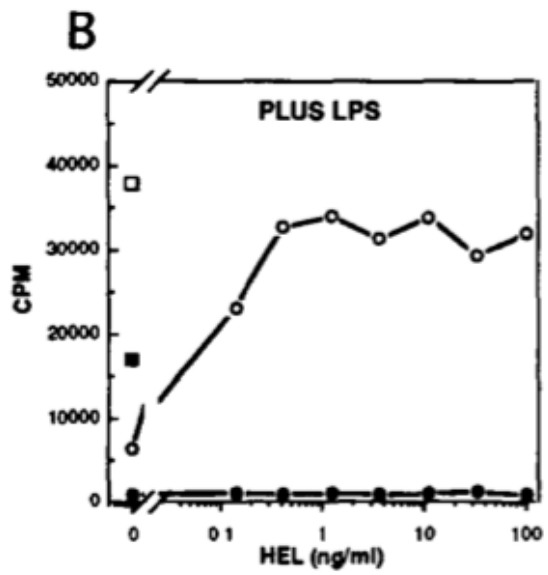
- anti-hen egg lysozyme (HEL) Ig-transgenic x soluble HEL-transgenic (Double-Tg)
- modulation of sIgM contributes to anergic state



Goodnow et al., *Nature* 1989

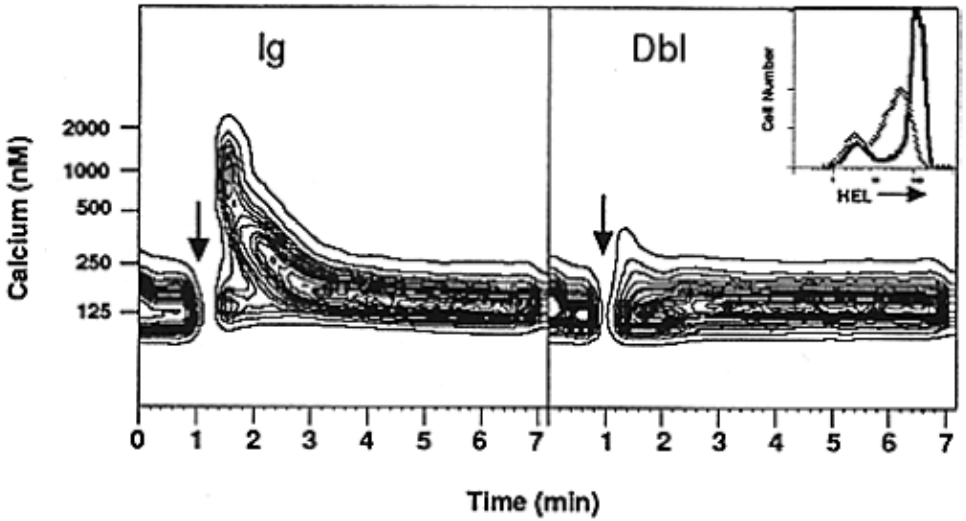
Block in BCR signaling in anergic B cells

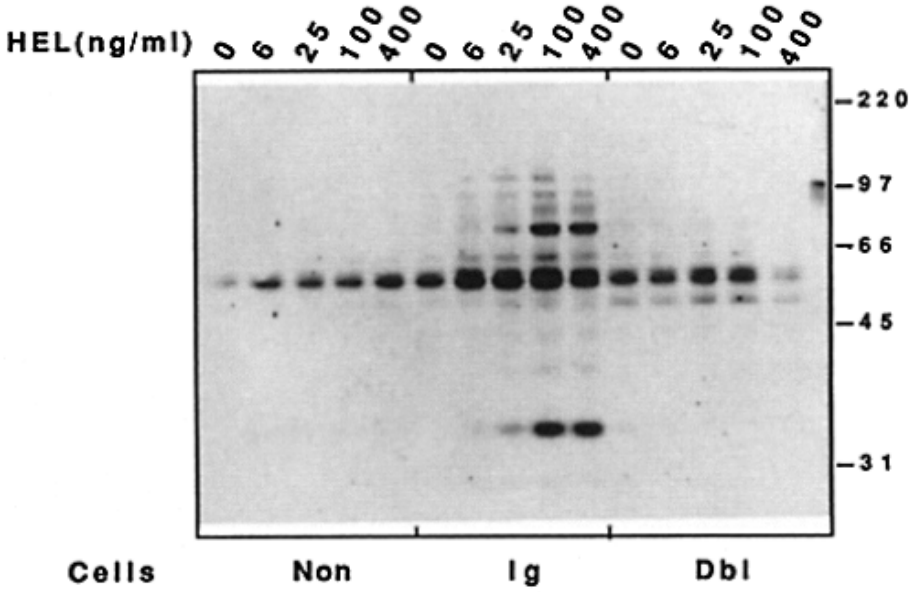




Ig-tg B cells

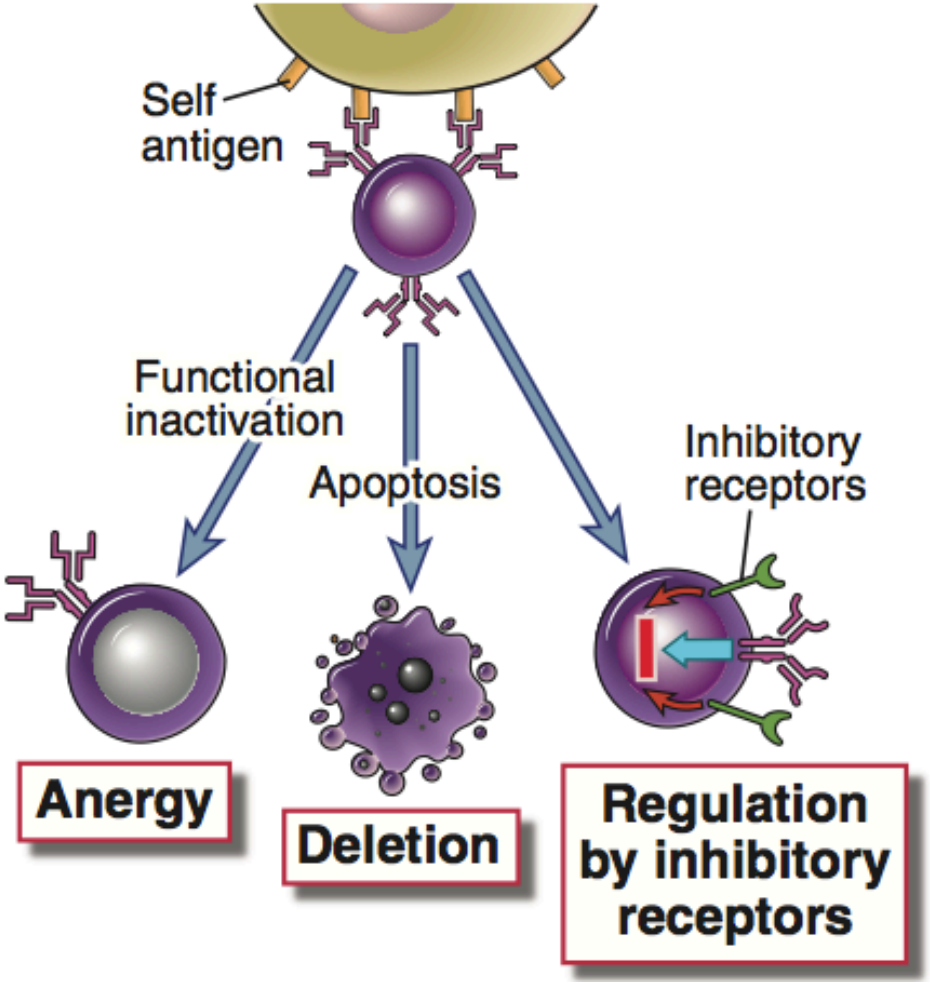
Ig/sHEL Dbl-tg B cells





Cooke et al., *JEM* 1994

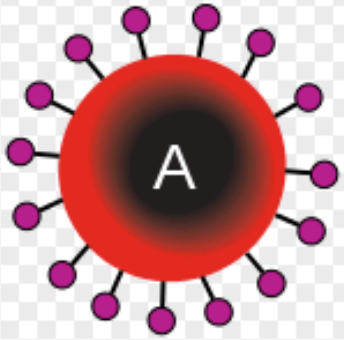
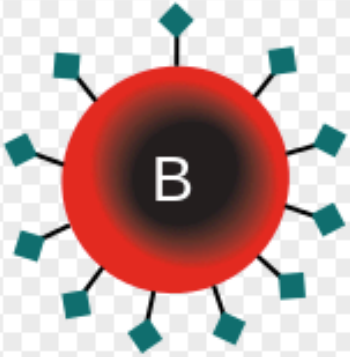
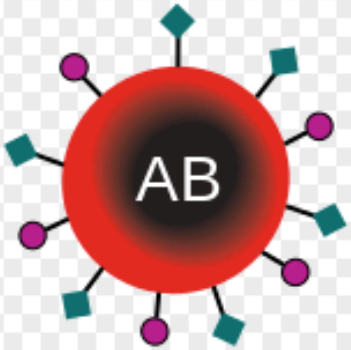
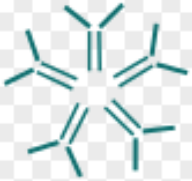
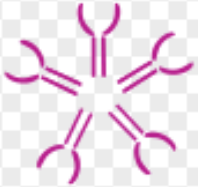
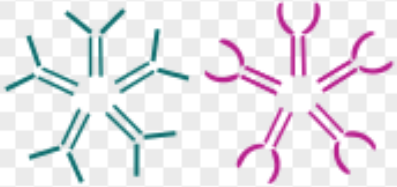
Peripheral tolerance in B cells



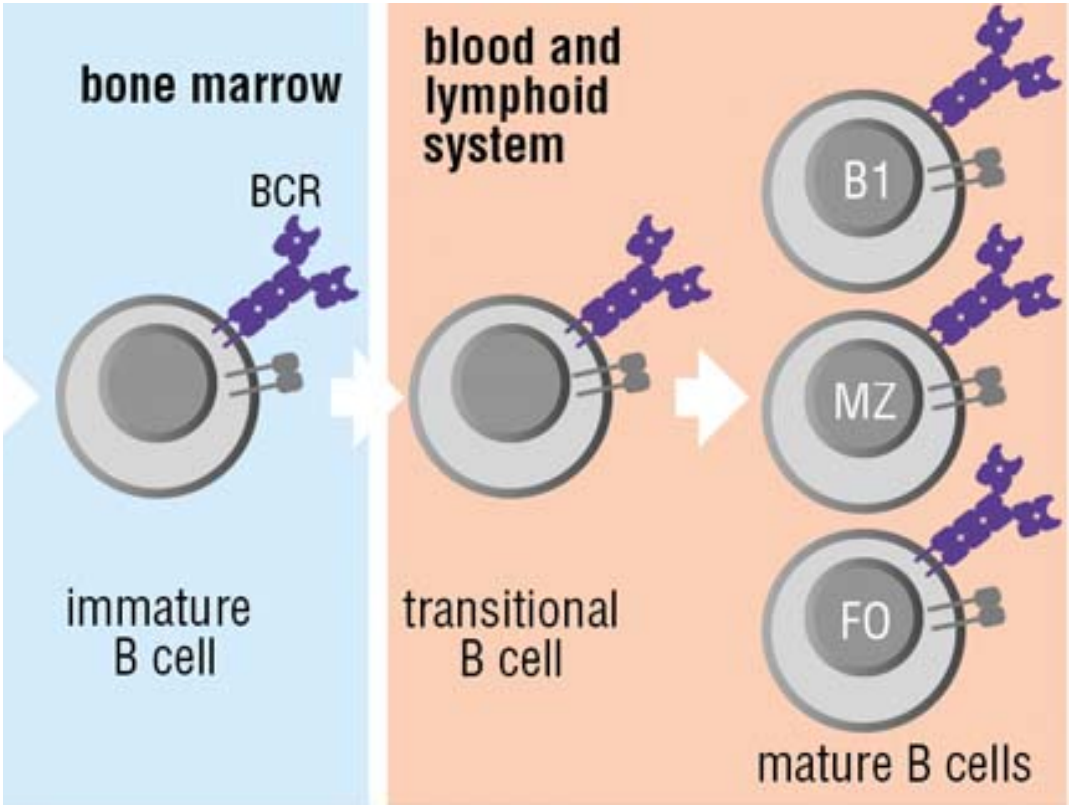
- B cells that encounter self antigens in peripheral tissues become anergic or die by apoptosis. In some situations, recognition of self antigens may trigger inhibitory receptors that prevent B cell activation.

Questions

- What are some autoantibody-mediated diseases?
- What types of genetic defects or variants might underlie these diseases?
- Do you think there is a connection between B cell tolerance and blood group antigens?

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	 A and B antigens	None

Theme 5: Three types of mature B cells



B1, marginal zone, and follicular B cells

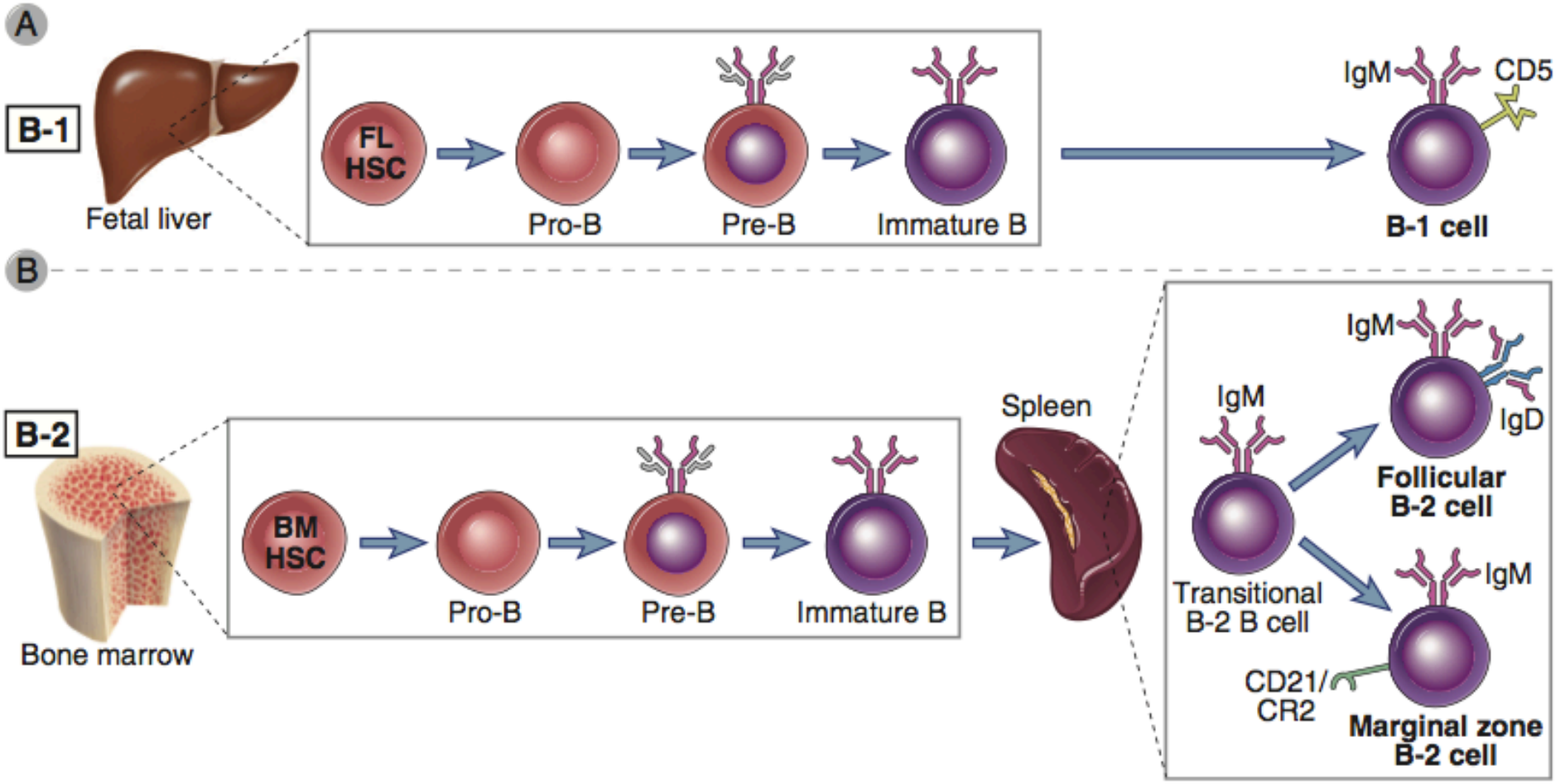
Three types of mature B cells

- **Recirculating follicular B cells** (aka "conventional B cells", B2 cells): circulate between LN follicles and blood: size of population determined by BAFF levels

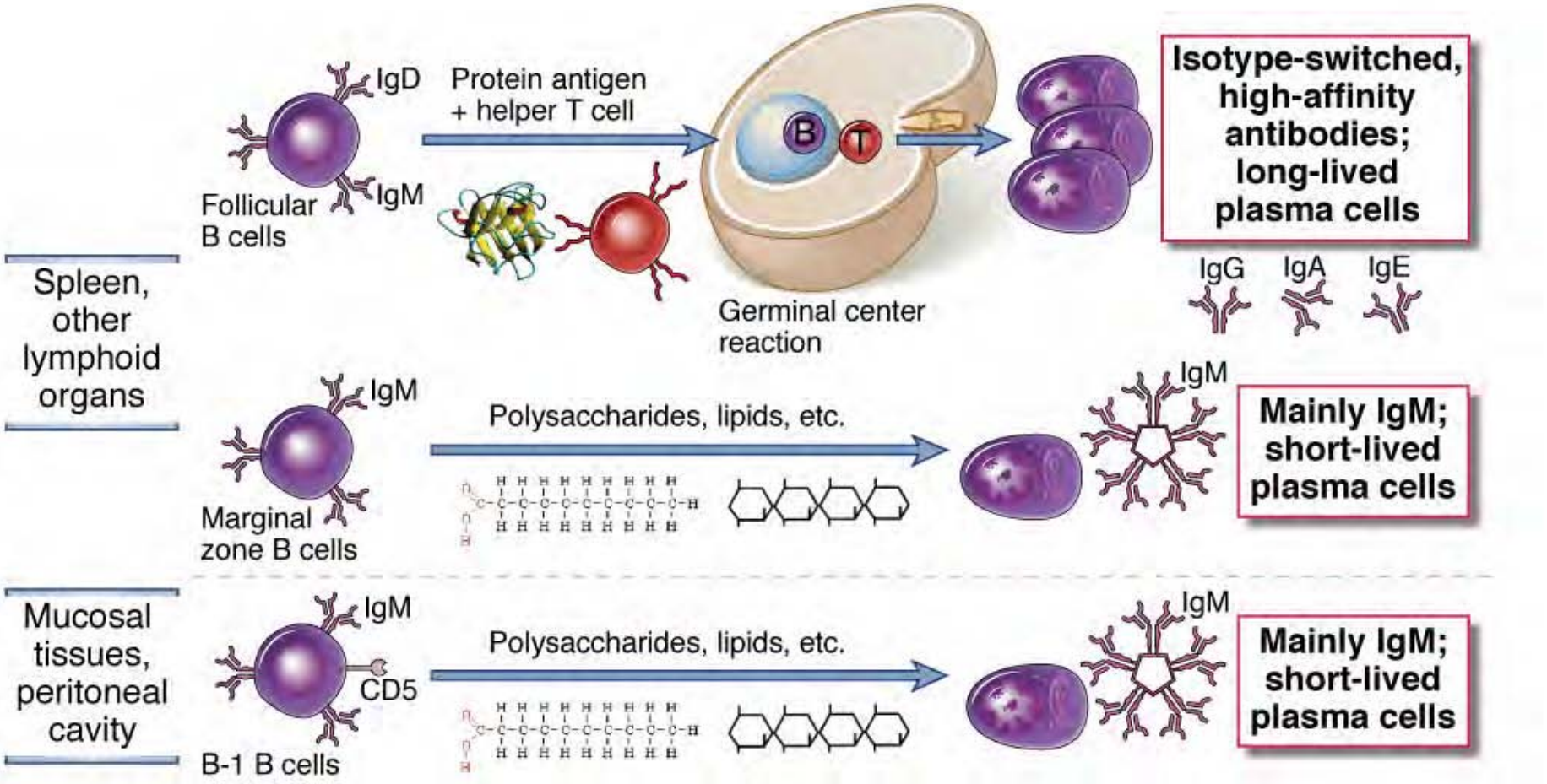
- **Marginal zone B cells**: reside in marginal zone (MZ) of spleen where they can respond to particulate antigen in blood (bacteria, etc.); also dependent on BAFF for survival. Dependent on Notch signaling

- **B1 B cells**: prominent in peritoneal and pleural cavities, present in spleen. Produce "natural antibody" and also respond to T-independent antigens. (less dependent on BAFF)

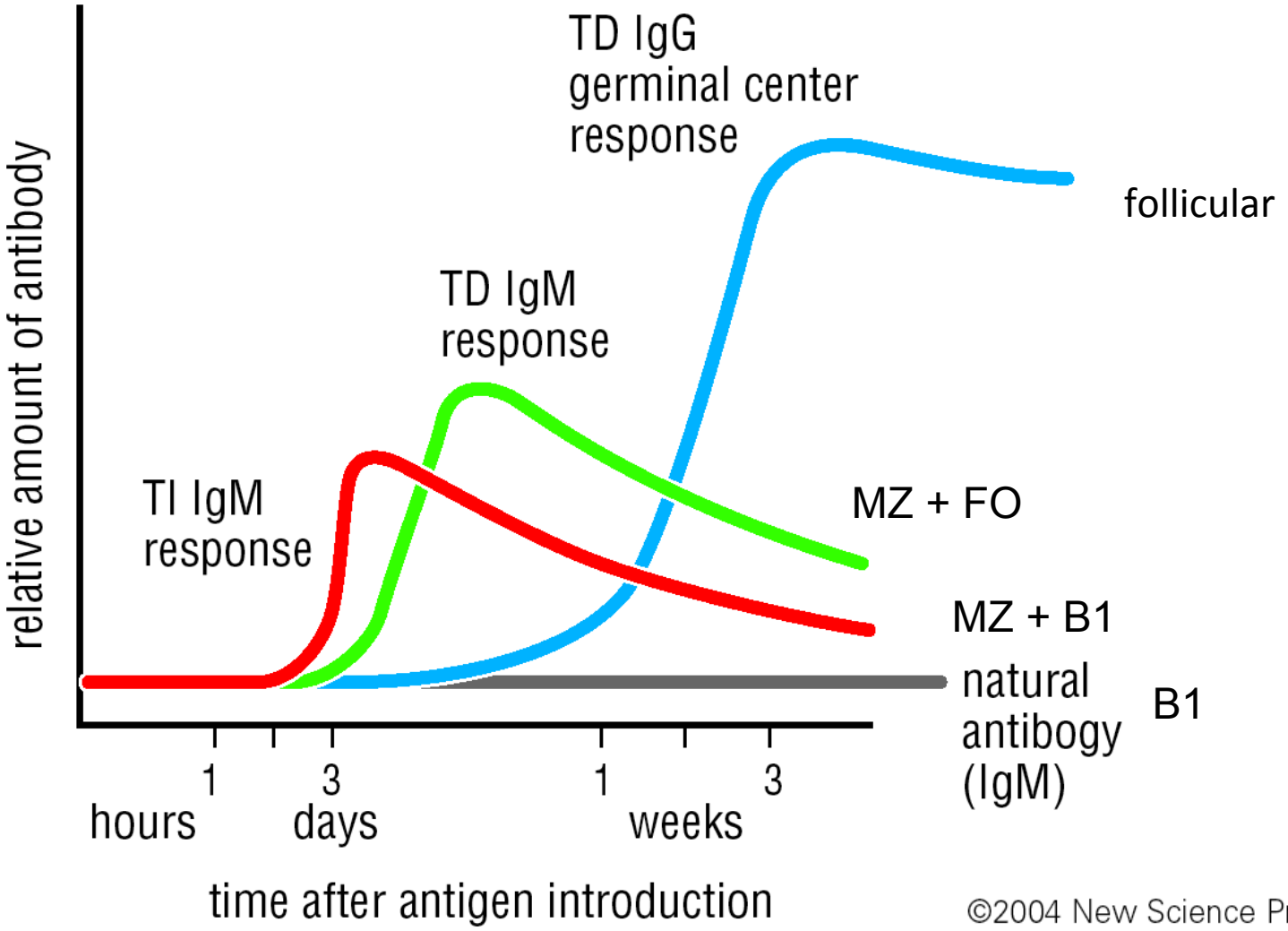
Three types of mature B cells



Different B cell subsets preferentially mediate T-dependent or T-independent responses



Biological roles of three types of B cells



Questions?

Reading List

B cell development and tolerance

- Mikkola I, Heavey B, Horcher M, Busslinger M. (2002). Reversion of B cell commitment upon loss of Pax5 expression. Science 297:110-113.
- Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. (2003). Predominant autoantibody production by early human B cell precursors. Science 301:1374-7.
- von Boehmer, H., Melchers, F., (2010). Checkpoints in lymphocyte development and autoimmune disease. Nat. Immunol. 11: 14-20.
- Fistonich C,…, Periera P. (2018) Cell circuits between B cell progenitors and IL-7+ mesenchymal progenitor cells control B cell development. J. Exp. Med. 215, 2586-99.