B cell development, activation and differentiation

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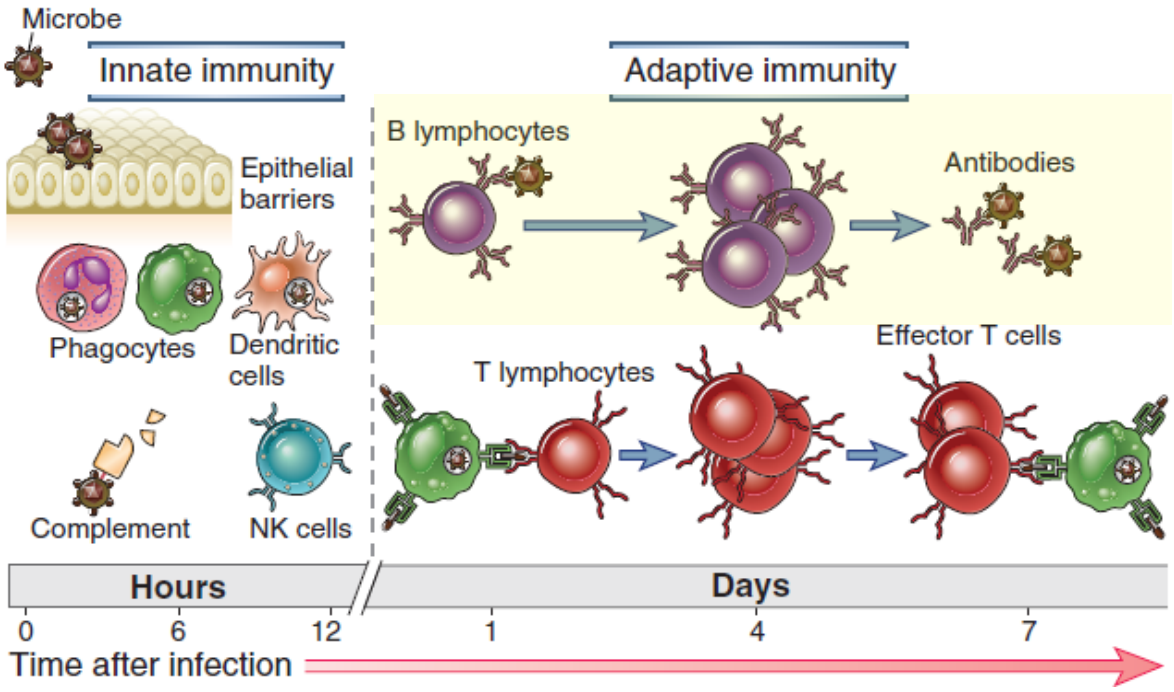
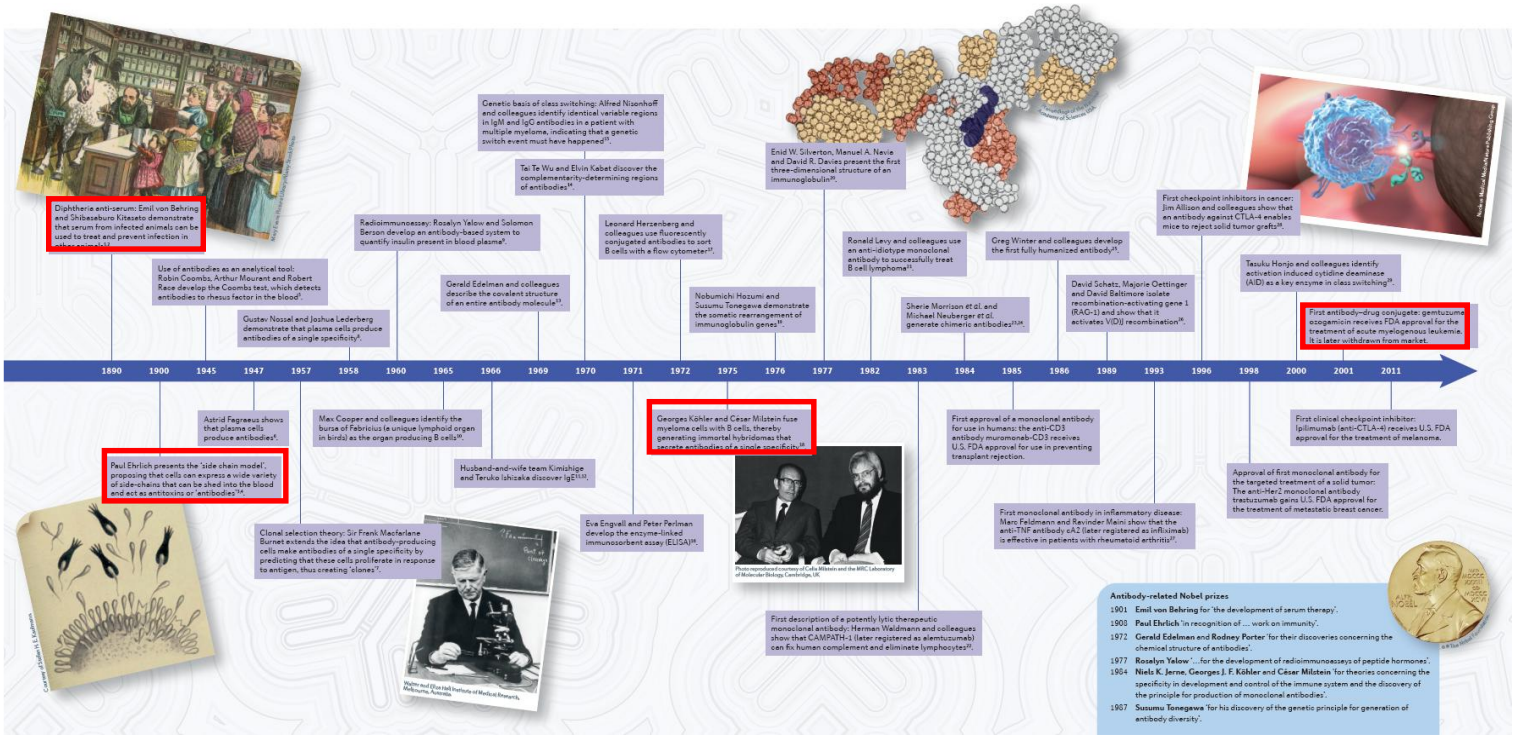
Overview

- B cell development and maturation
- B cell activation and differentiation
- B cell tolerance

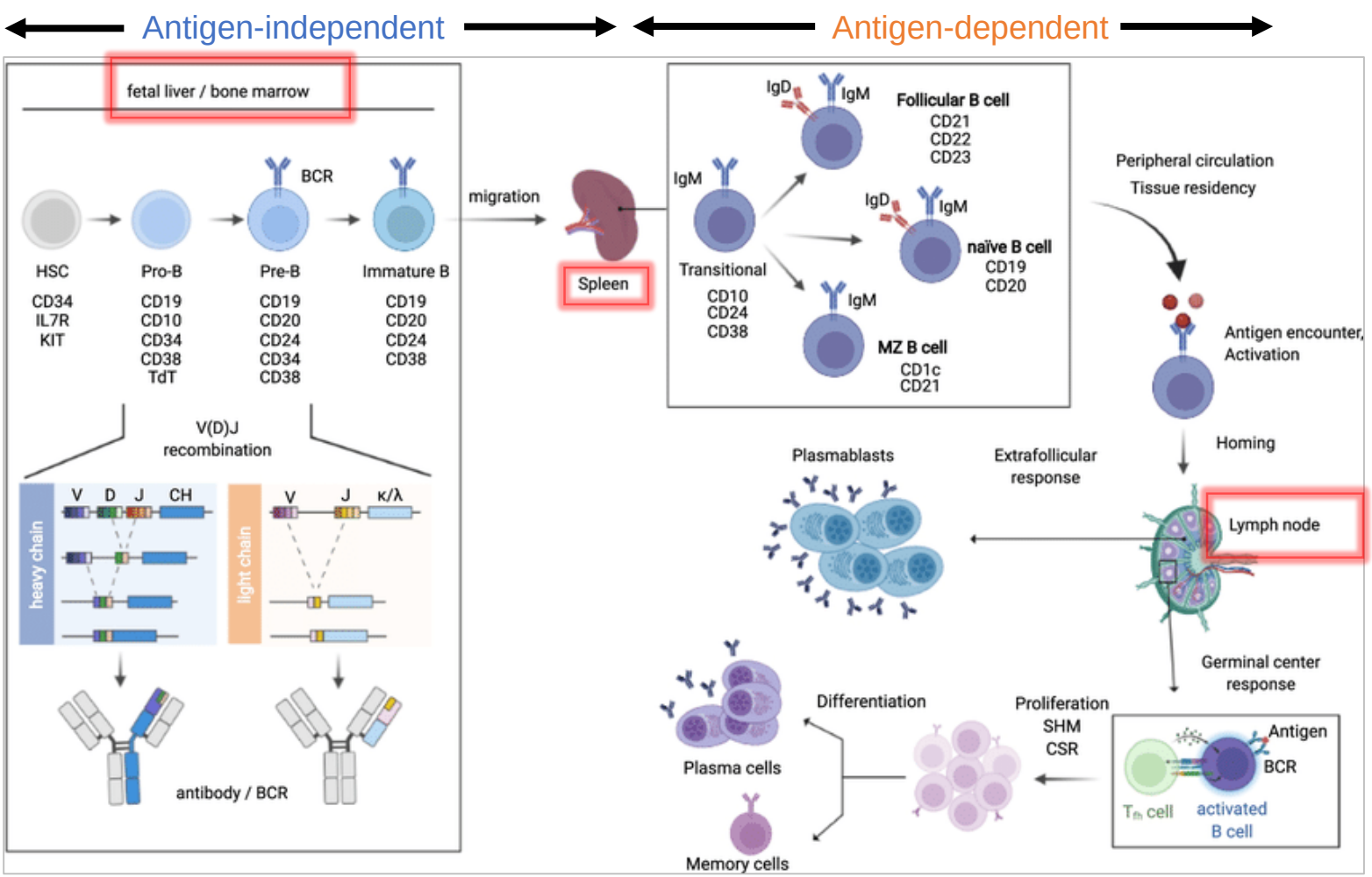


B cells: past – present - future



Where do B cells develop and mature

From hematopoietic stem cells to plasma cells

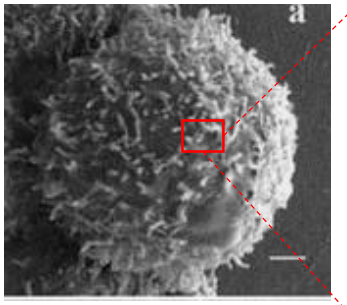


Schultheiss et al, Sem Immun 2022

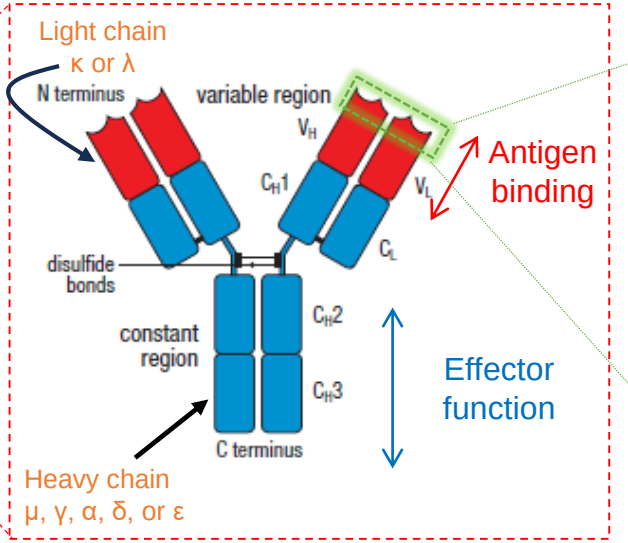
What makes a lymphocyte a B cell?

The immunoglobulin (B cell antigen receptor)

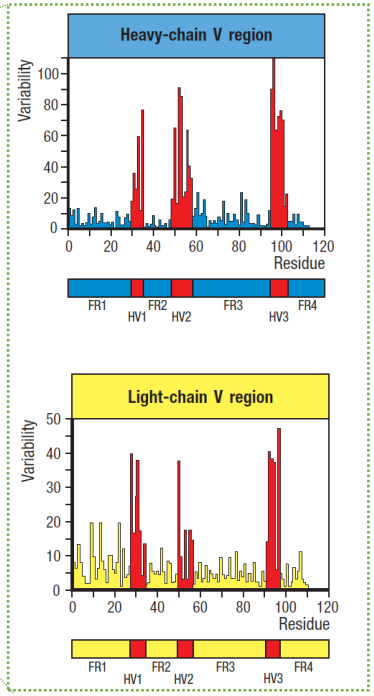
B cell



~1.5 x10^5 antigen receptors/B cell



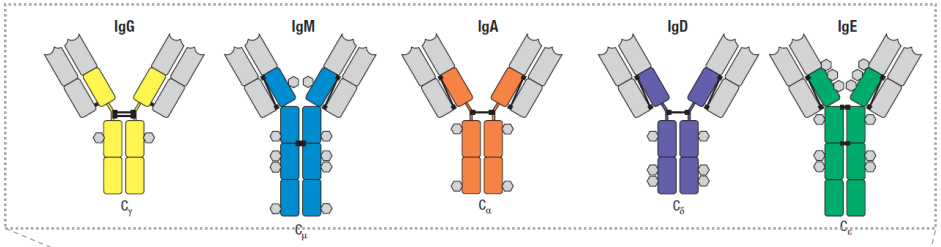
Hypervariable regions of the IgH and IgL chains determine the antigen binding site



Antibody classes and biological activities

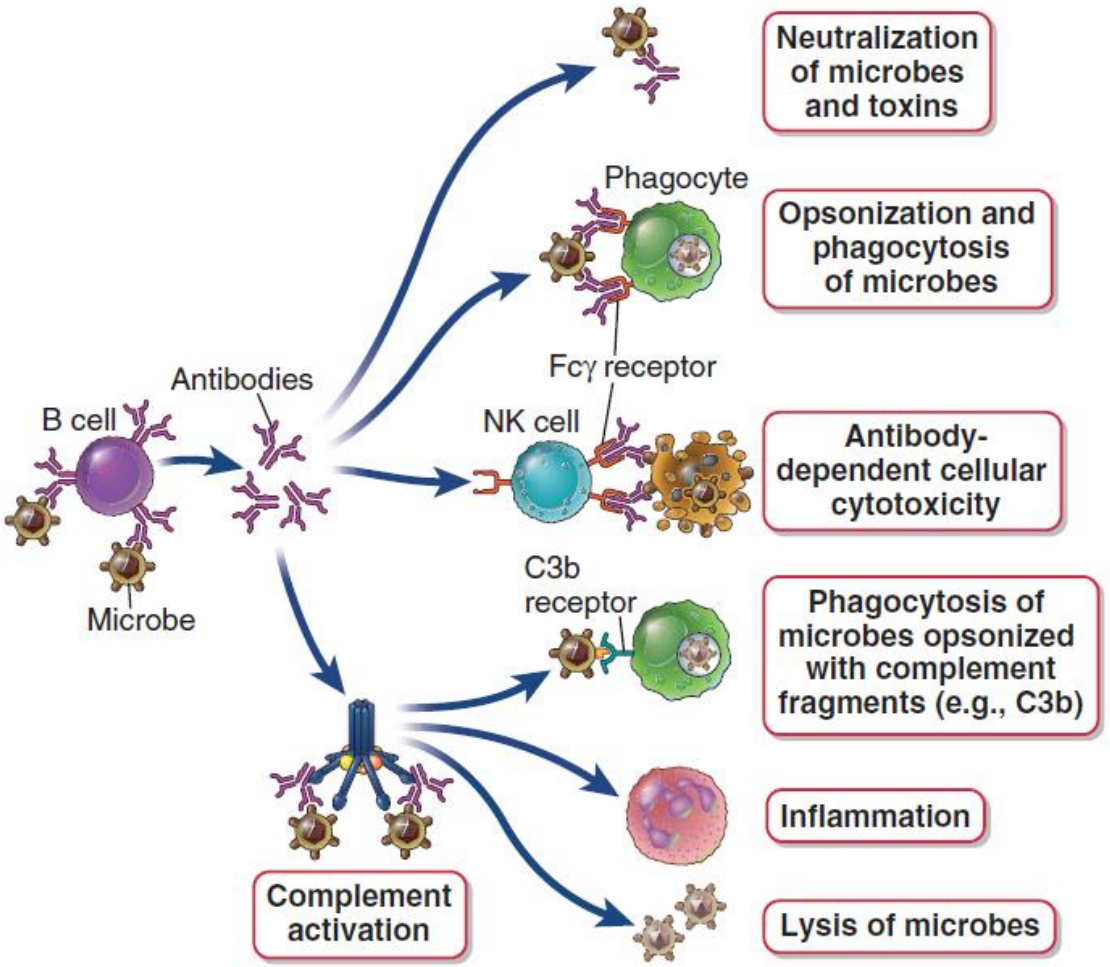
Different isotypes and subtypes of antibodies perform different effector functions.

- Different C regions of IgH chains
- 5 classes: IgM, IgG, IgA, IgD, IgE
- Subclasses: 4 for IgG (IgG1, IgG2, IgG3, IgG4), 2 for IgA (IgA1, IgA2)
- <u>All isotypes carry either kappa or lamda chains</u>



	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ε
Molecular weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2
Classical pathway of complement activation	++	+	+++	–	++++	–	–	–	–
Alternative pathway of complement activation	–	–	–	–	–	+	–	–	–
Placental transfer	+++	+	++	–/+	–	–	–	–	–
Binding to macrophage and phagocyte Fc receptors	+	–	+	–/+	–	+	+	–	+
High-affinity binding to mast cells and basophils	–	–	–	–	–	–	–	–	+++
Reactivity with staphylococcal Protein A	+	+	–/+	+	–	–	–	–	–

Beyond antigen binding: effector mechanisms of antibodies



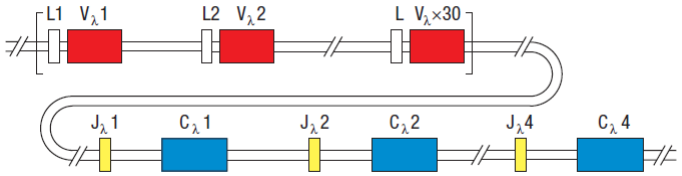
How are we protected against the great variety of pathogens?

The complexity of human Ig loci

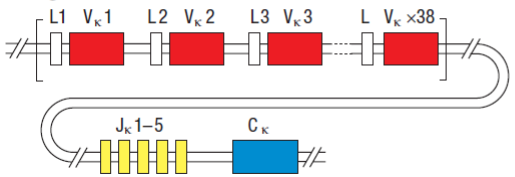
The germline organization of the heavy and light chains loci

Two genetic locus for light chains

λ light-chain locus (1050 kbp; chromosome 22)



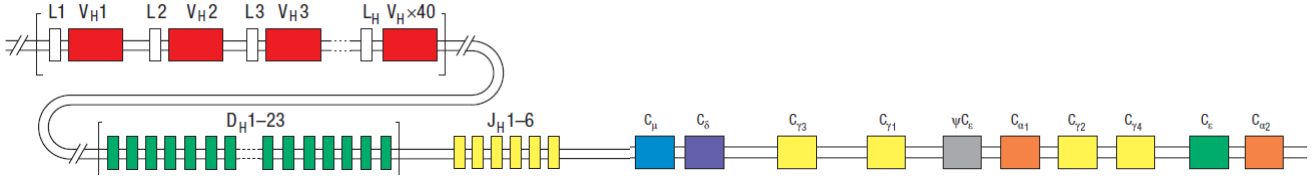
κ light-chain locus (1820 kbp; chromosome 2)



All antibody molecules share the same basic structural characteristics recarding their constant regions but display remarkable variability in the regions that bind antigens

One genetic locus for heavy chains

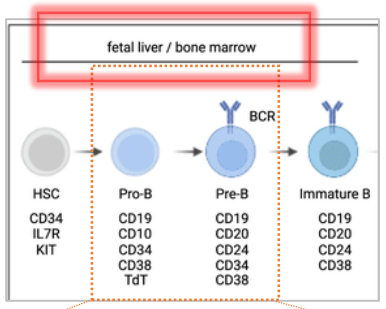
Heavy-chain locus (1250 kbp; chromosome 14)



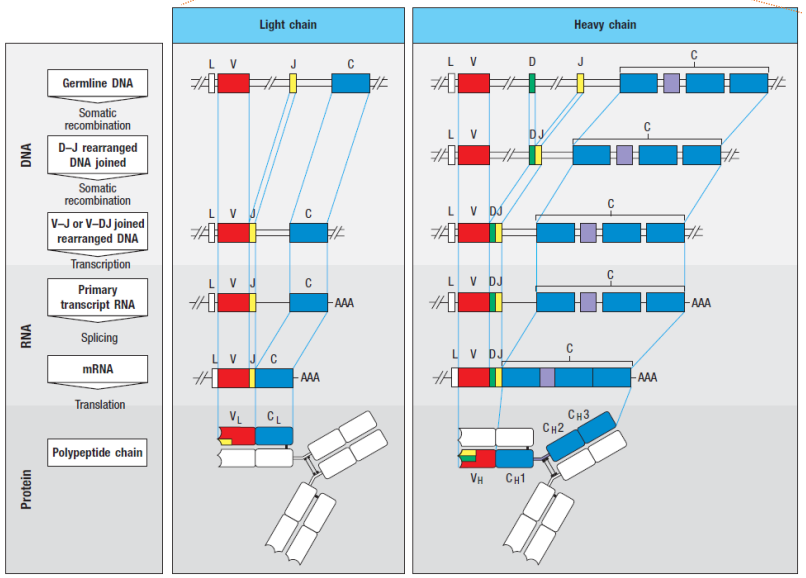
The V(D)J recombination

Susumu Tonegawa

Nobel Prize for Physiology or Medicine in 1987 for "his discovery of the genetic principle for generation of antibody diversity."



- The selection of the V, (D), J genetic segments is random (combinatorial diversity)
- The order of rearrangements is pre-defined: first the heavy chain, then the light chain
- The spontaneous addition/deletion of nucleotides before the joining of VDJ fragments increases diversity (junctional diversity)



Every B cell contains a unique rearranged DNA sequence for the heavy chain locus and a unique DNA sequence for the light chain locus

Generation of diversity in B cells

Mechanism	Immunoglobulin		
	Heavy Chain	κ	λ
Variable (V) segments	45	35	30
Diversity (D) segments	23	0	0
D segments read in all three reading frames	Rare	—	
N region diversification	V-D, D-J	None	
Joining (J) segments	6	5	4
Total potential repertoire with junctional diversity	~10^{11}		—

Combinatorial diversity: Different combinations of gene segments united by V(D)J recombination produce different antigen receptors.

45 V_H genes x 23 D genes x 6 J genes = 6210 possible recombinations

x

35 V_κ genes x 5 J genes = 175 possible recombinations (120 for the λ locus)

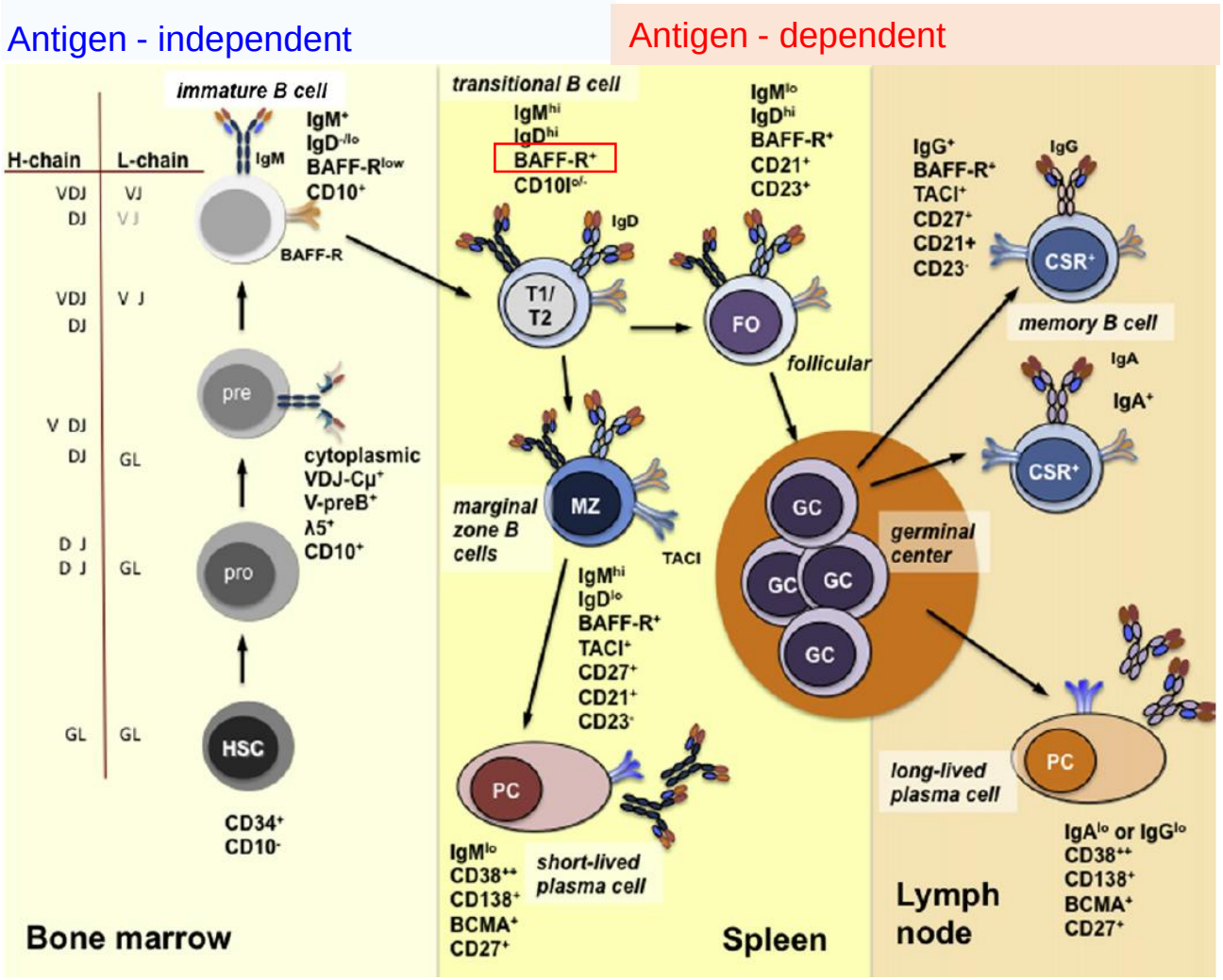
↓

10^6 possible antibody molecules

Junctional diversity: Removal or addition of nucleotides at the junctions of the V and D, D and J, or V and J segments at the time these segments are joined.

10^{11} potential antibody repertoire

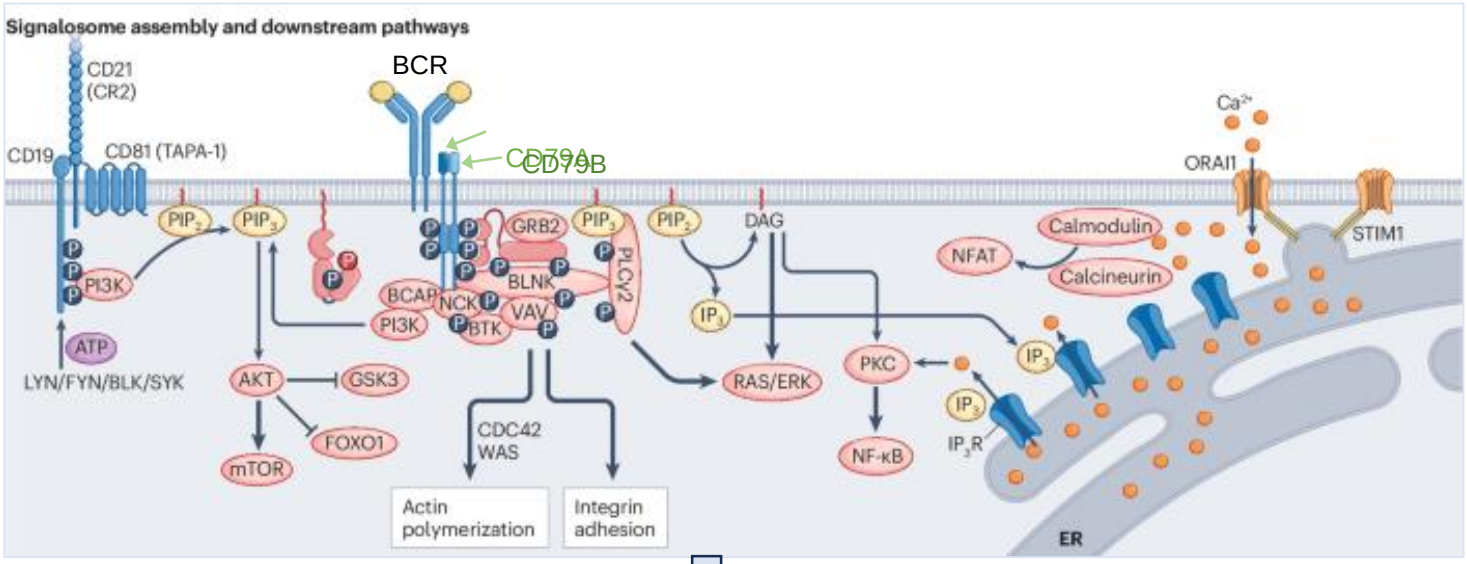
B cell development beyond the BM



Pieper K, Grimbacher B, Eibel H, JACI 2013

How are B cells activated and differentiated?

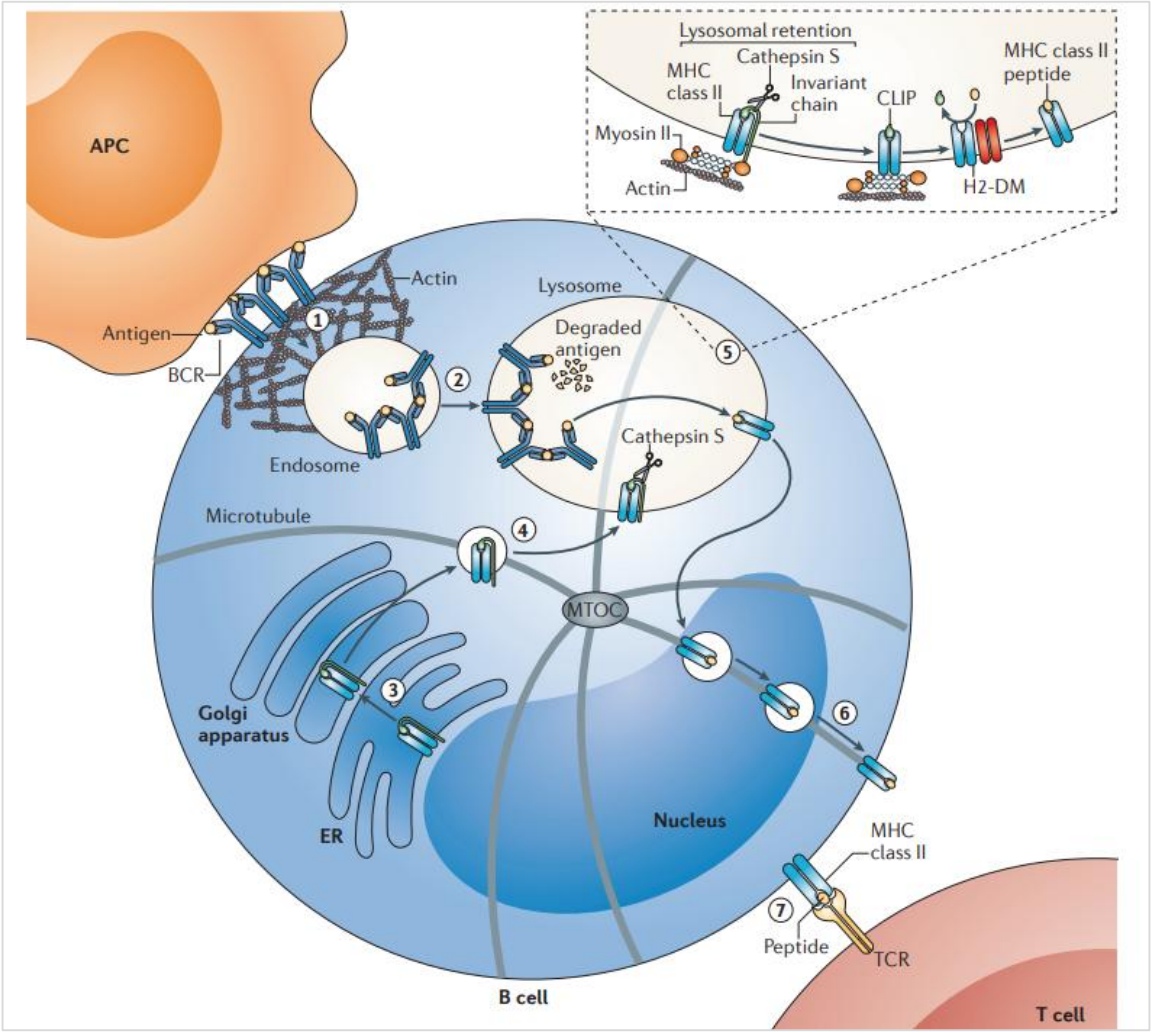
Antigen recognition by the B cell receptor triggers signaling pathways for B cell activation and differentiation



Degn et al. *Nat Rev Immunol* 2025

- ✓ activation
- ✓ proliferation
- ✓ differentiation
- ✓ migration

B cells as professional antigen presenting cells



Yuseff et al, Nat Rev Imm 2013

Recirculation of naïve B cells and their differentiation into antibody-secreting and memory B cells

Naïve follicular B cells scan follicles for antigens

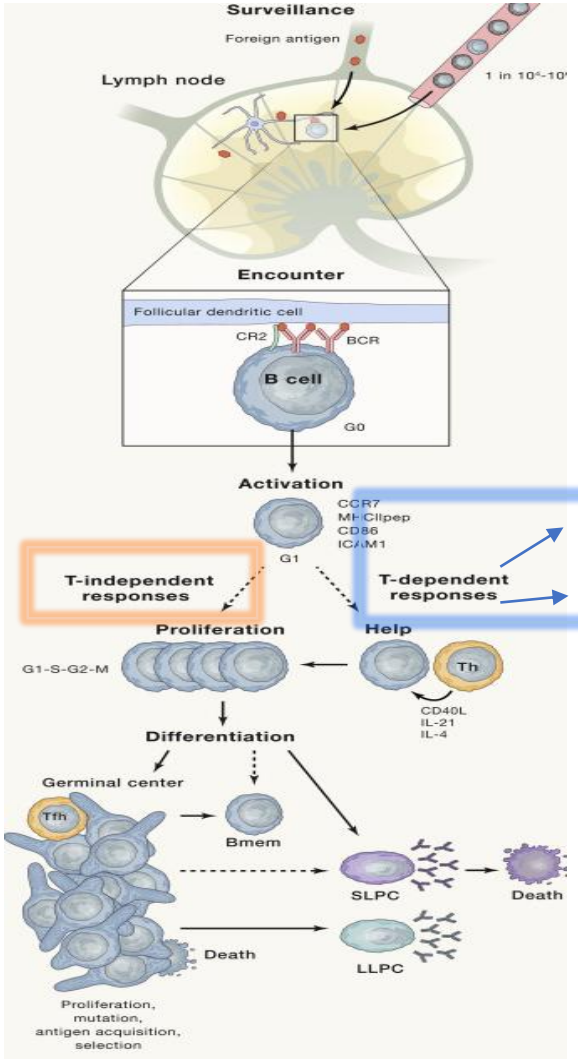
Recognition of antigen on follicular dendritic cells results on B cell activation

3 pathways of differentiation:

- T cell dependent [germinal center (GC) reactions)]
- T cell independent
- Extrafollicular pathway (EF)

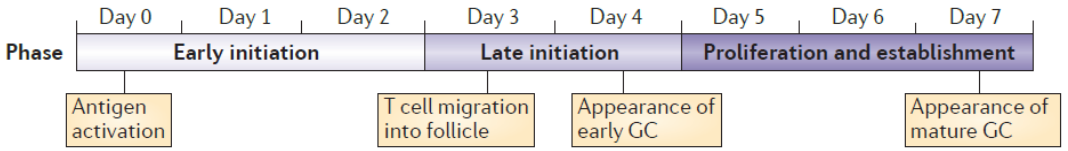
All pathways differ regarding their ability to generate memory B cells and plasma cells

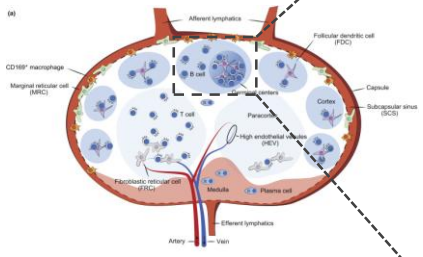
	T-I (EF)	T-D (EF)	T-D (GC)
Clonal expansion	+	++	+++
Affinity maturation	+/–	+	+++
Class switching	+/–	+++	+++
Memory formation	+/–	+	+++
LLPC development	–	?	+++
Time to Ab	3 d	3-5 d	7+ d

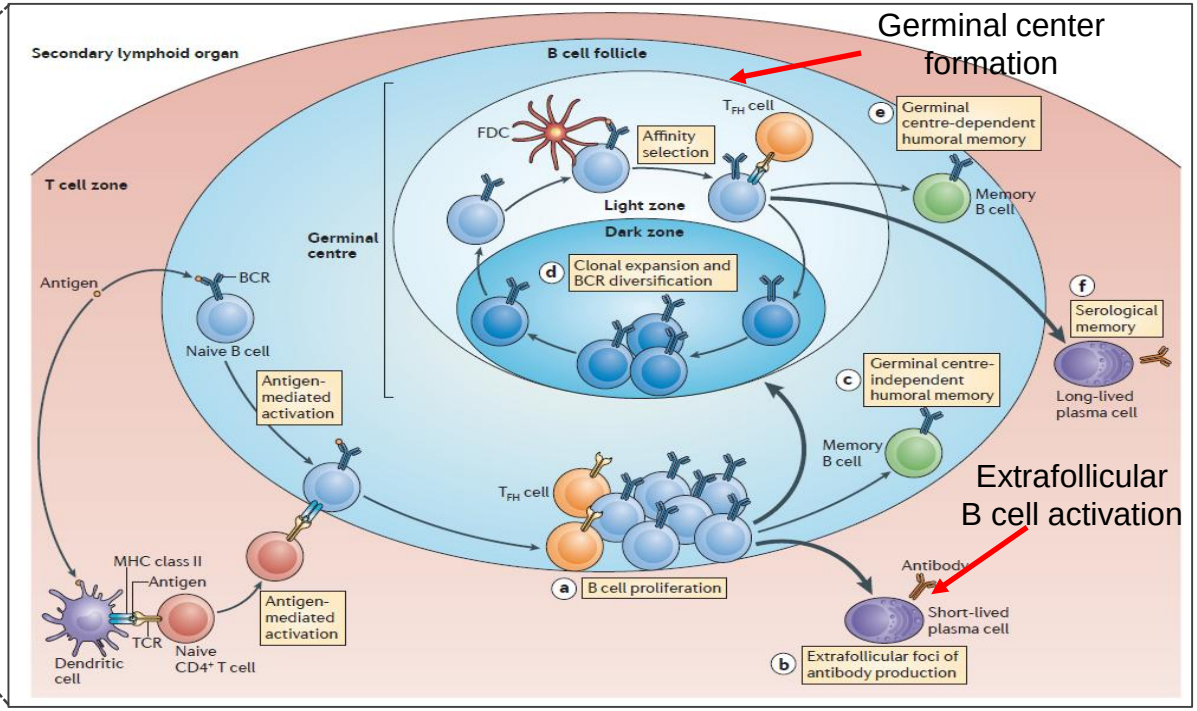


Cyster and Allen C, Cell 2019

The generation of long-term immunity: the T cell-dependent antibody responses

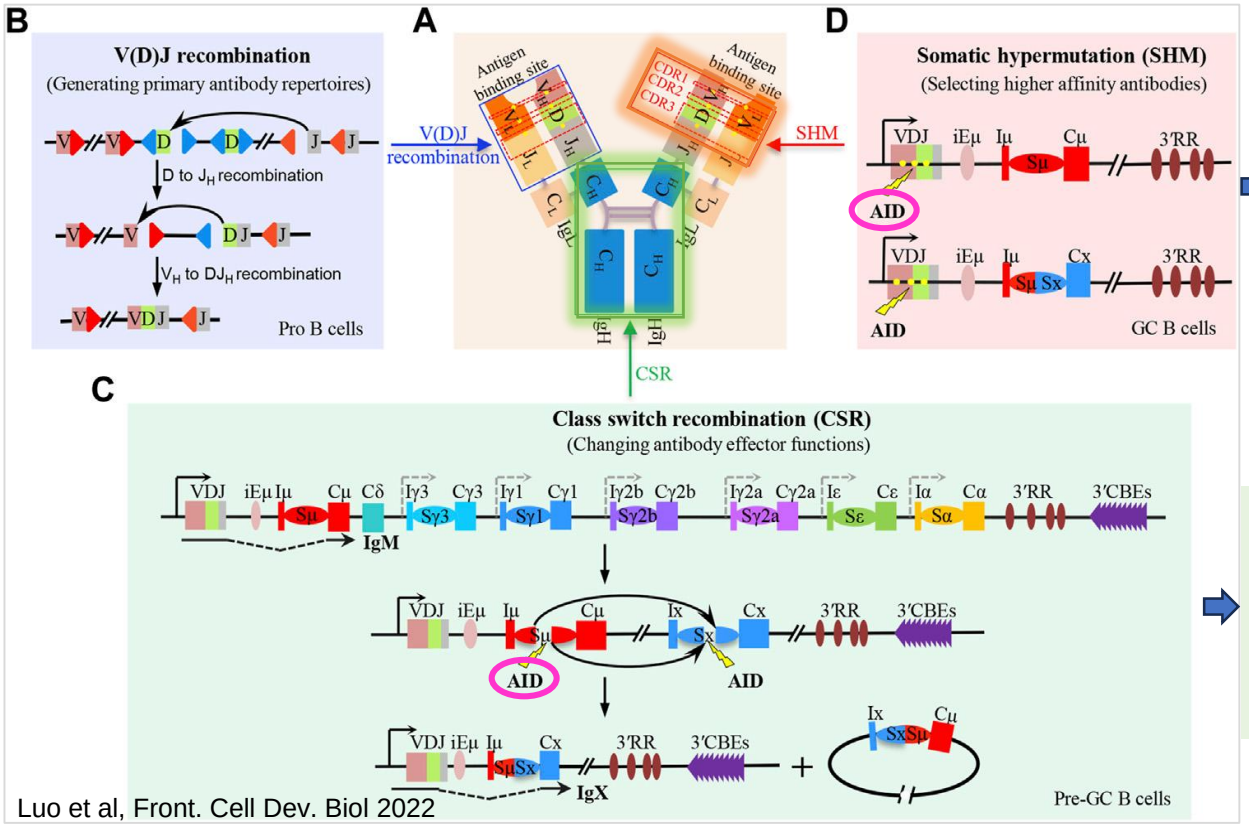






The primary antibody repertoire is diversified by two processes that modify the rearranged immunoglobulin gene

The mechanisms of somatic hypermutation and class-switch recombination



mutations into the heavy-chain and light-chain V regions → ↑ affinity for the antigen

Mutations in the the initial μ heavy chain C regions are replaced by heavy-chain C regions of another isotype → new effector functions, SAME antigen specificity

Luo et al, Front. Cell Dev. Biol 2022

Extrafollicular B cell activation versus follicular/germinal center reaction

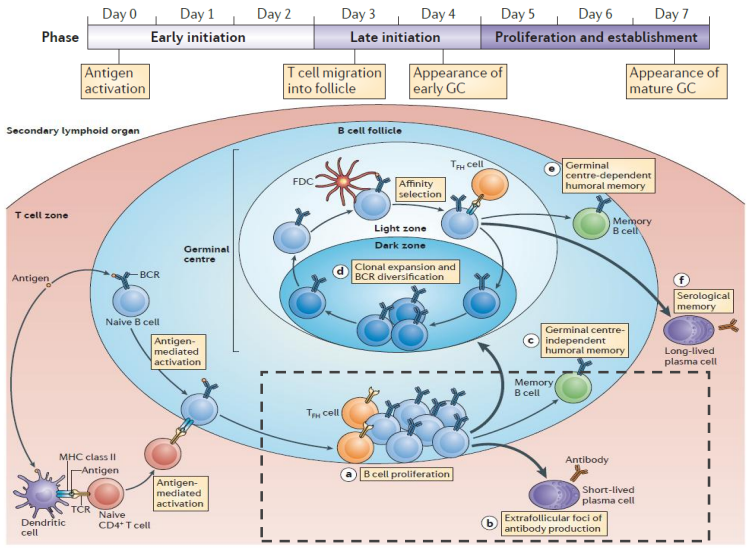
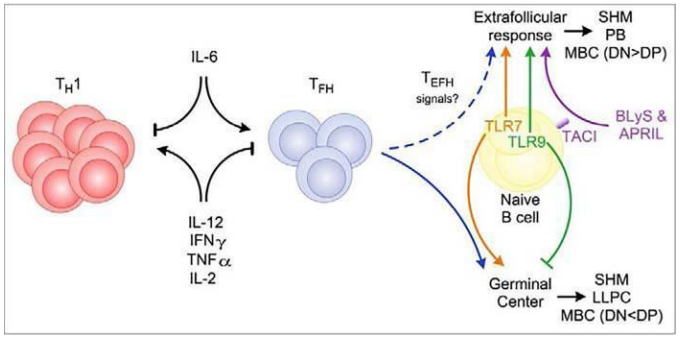


TABLE 11–1 Extrafollicular and Germinal Center B Cell Responses

Feature	Follicular/Germinal Center	Extrafollicular
Localization	Secondary follicles	Medullary cords of lymph nodes and at junctions between T cell zone and red pulp of spleen
CD40 signals	Required	Required
Specialized T cell help	T_{FH} cells in germinal center	Extrafollicular T helper cells
AID expression	Yes	Yes
Class switching	Yes	Yes
Somatic hypermutation	High rate	Low rate
Antibody affinity	High	Low
Terminally differentiated B cells	Long-lived plasma cells and memory cells	Short-lived plasma cells (life span of ~3 days)
Fate of plasma cells	Bone marrow or local MALT	Most die by apoptosis in secondary lymphoid tissues where they were produced
B cell transcription factors	Bcl-6	Blimp-1

The effect of cytokines on dominating EF or GC responses

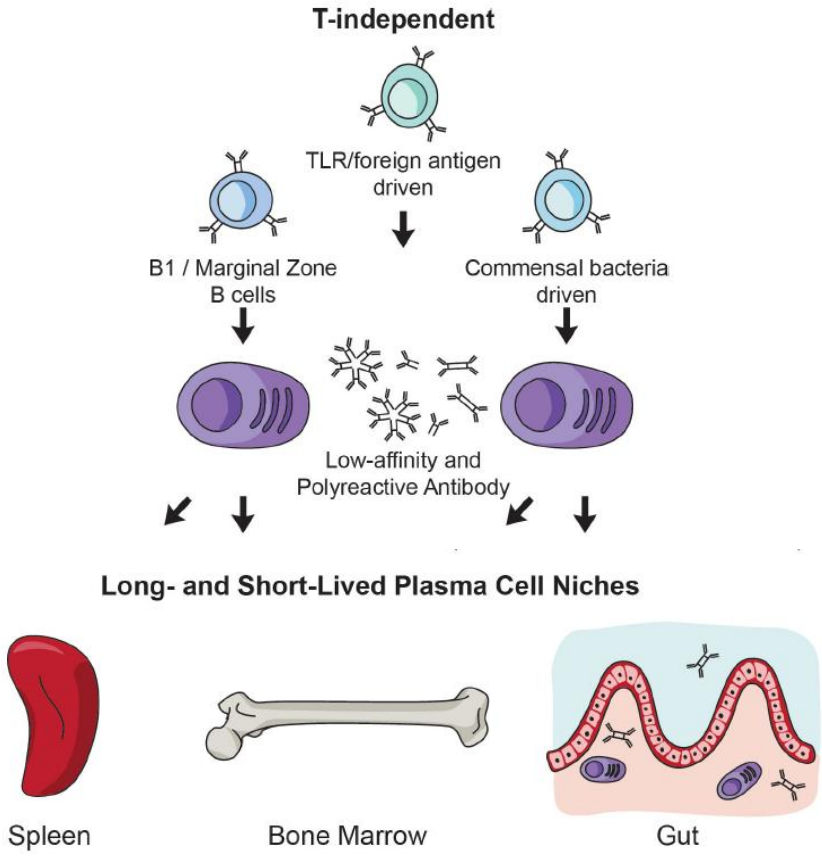


Elsner R and Shlomchik M, Immunity 2019

Do memory B cells exist in the absence of T cells?

The T cell-independent antibody responses

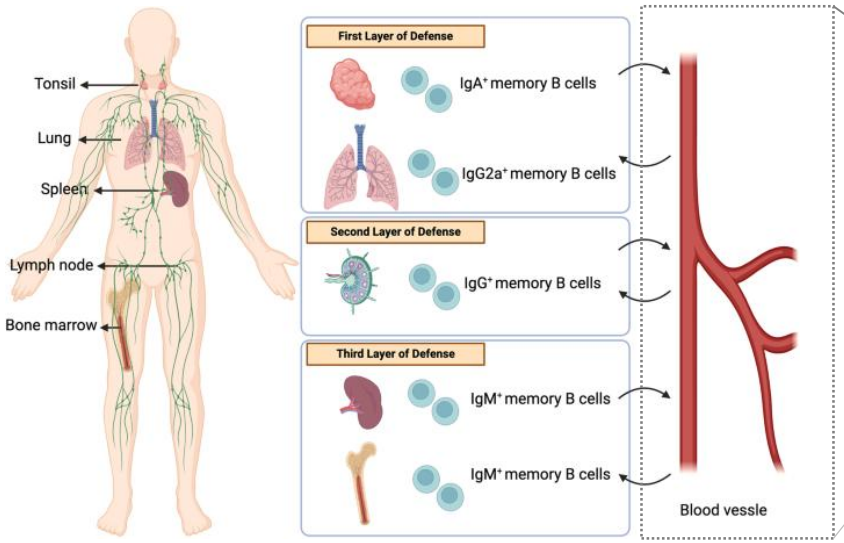
- **Antigen:** polysaccharide and/or lipopolysaccharide structures
- **Immunoglobulin:** mainly IgM antibodies (low affinity) with broad reactivity/polyreactivity, IgA
- **Class switching** mediated by cytokines of nonlymphoid origin (BAFF, APRIL, TGF-β)
- Rapid response
- Germinal centers can be formed → collapse earlier
- Synergistic function of TLRs
- Polysaccharide vaccines (pneumococcal vaccine) can induce quite long-lived protection



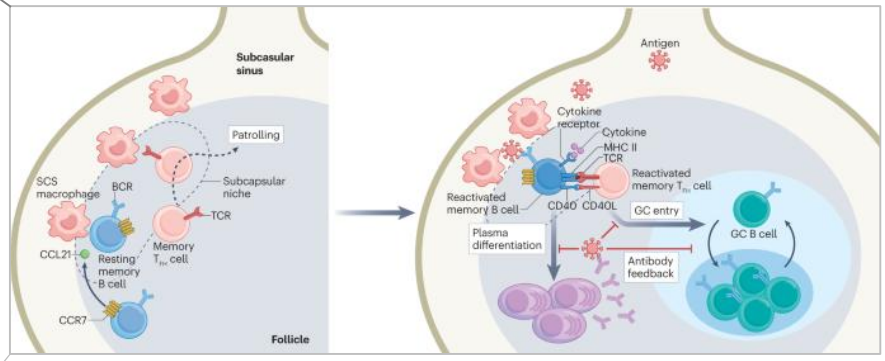
Allman D et al, Immun Rev 2019

Heterogeneity in the memory B cell pool offers multilayered defense upon reinfection.

Memory B cells are spread through the lymphoid tissues or they enter blood stream

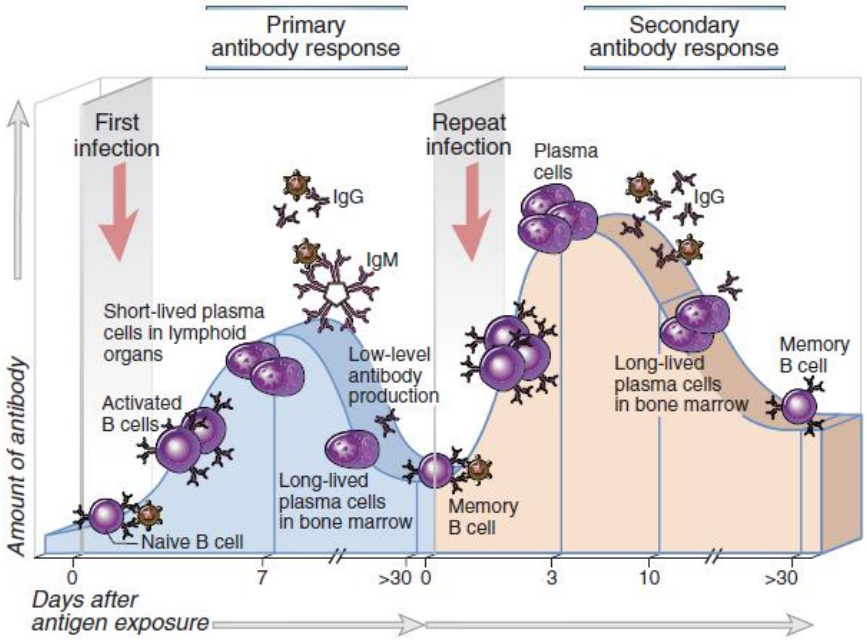


Re-activation upon antigen encountering → rapid differentiation into antibody-secreting cells and production of high-affinity antibodies

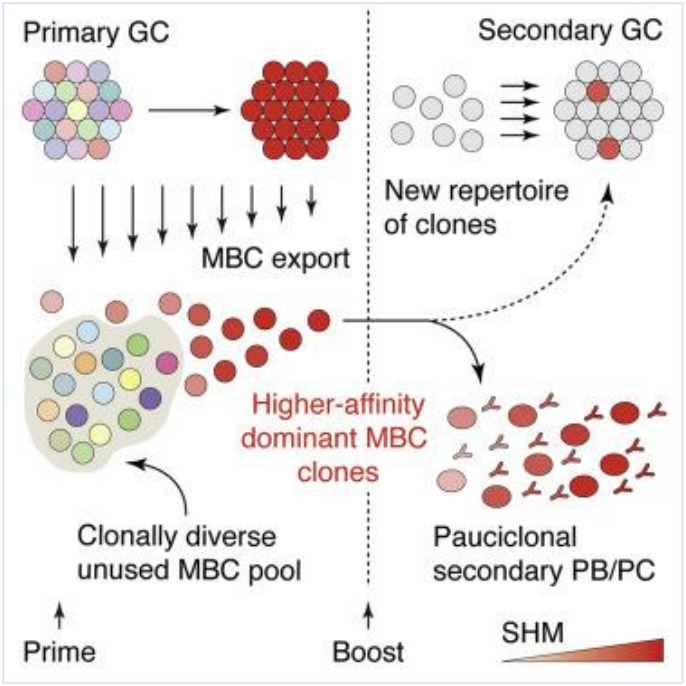


Primary and secondary humoral immune responses

"old view"



"new view"



Both naïve and memory B cells contribute to the generation of a wide repertoire of antibodies that can effectively protect throughout life

Plasma cells: the mediators of long-term immunity

Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors

Xiaocong Yu[1]*, Tshidi Tsibane[2]*, Patricia A. McGraw[1], Frances S. House[1], Christopher J. Keefer[1], Mark D. Hicar[1], Terrence M. Tumpey[3], Claudia Pappas[2,3], Lucy A. Perrone[3], Osvaldo Martinez[2], James Stevens[3,4], Ian A. Wilson[4], Patricia V. Aguilar[2], Eric L. Altschuler[5], Christopher F. Basler[2] & James E. Crowe Jr[1]

survivors to the 1918 pandemic virus. Here we show that of the 32 individuals tested that were born in or before 1915, each showed seroreactivity with the 1918 virus, nearly 90 years after the pandemic. Seven of the eight donor samples tested had circulating B cells that secreted antibodies that bound the 1918 HA. We isolated B cells from subjects and generated five monoclonal antibodies that showed potent neutralizing activity against 1918 virus from three separate donors. These antibodies also cross-reacted with the genetically similar HA of a 1930 swine H1N1 influenza strain, but did not cross-react with HAs of more contemporary human influenza viruses. The antibody genes had an unusually high degree of somatic mutation. The antibodies bound to the 1918 HA protein with high affinity, had exceptional virus-neutralizing potency and protected mice from lethal infection. Isolation of viruses that escaped inhibition suggested that the antibodies recognize classical antigenic sites on the HA surface. Thus, these studies demonstrate that survivors of the 1918 influenza pandemic possess highly functional, virus-neutralizing antibodies to this uniquely virulent virus, and that humans can sustain circulating B memory cells to viruses for many decades after exposure—well into the tenth decade of life.

	Naive B cell	Plasmablast	Short-lived plasma cell	Long-lived plasma cell
Blimp-1	-	++	++	+++
Lifespan	++	+	+	+++
Proliferation	-	++	-	-
Location	BM, SLO, blood	SLO, Blood	SLO	BM, LP
Isotype	IgM/D	All isotypes	IgM>IgG	IgG>IgA>IgM
Ab secretion rate	-	++	++	++++
Glucose uptake	-	++	++	+++
Autophagy	+	++	++	+++
mTORC1 activity	-	+	+	+++
UPR	-	+++	+++	+++

Tellier and Nutt Eur. J. Immunol. 2019. 49

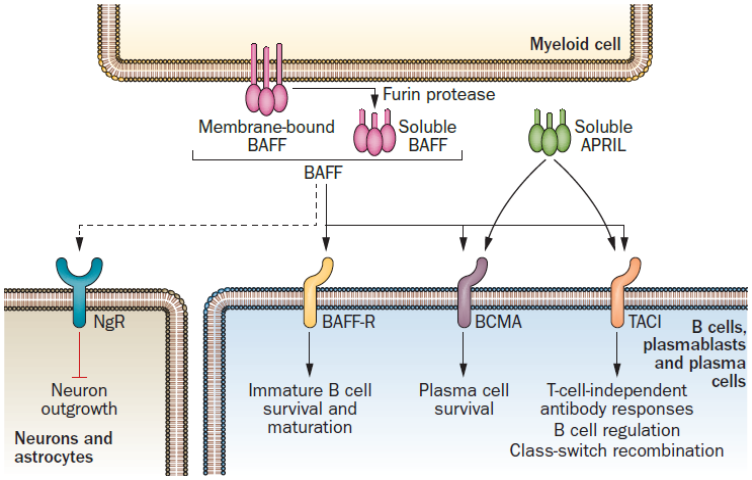
Respond to B cell depletion and immunosuppression

Refractory → chronicity of autoimmune refractoriness

How B cells survive in the absence of an antigen?

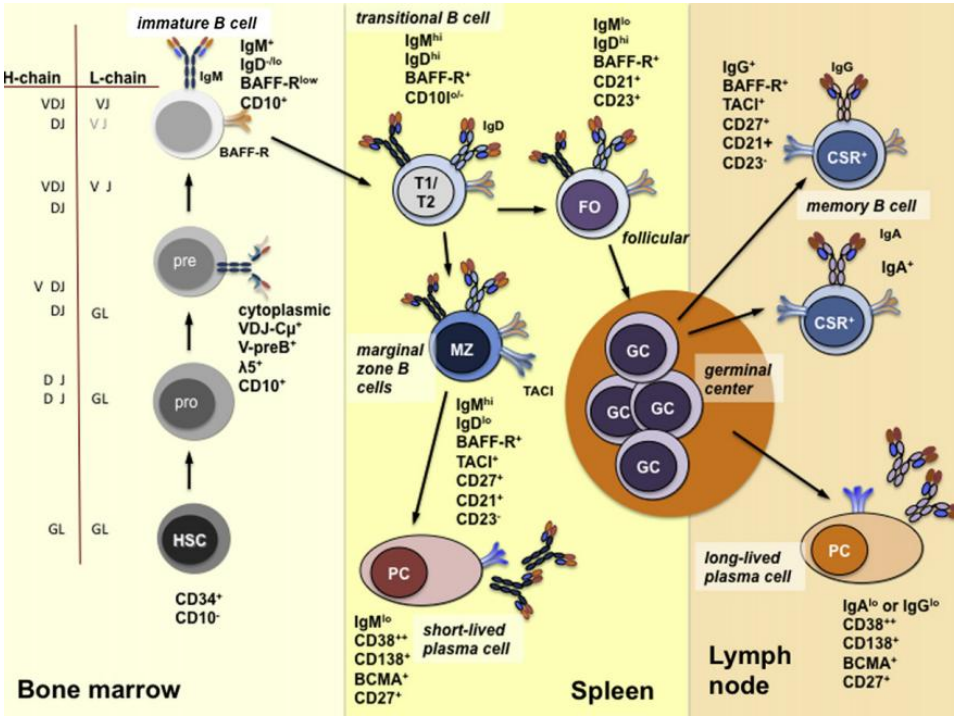
The role of the BAFF/APRIL system

2 cytokines – 3 shared receptors



Vincent F et al, Nat Rev Rheum 2017

Distinct expression pattern of BAFF/APRIL receptors correlates with their function



Pieper et al, JACI 2013

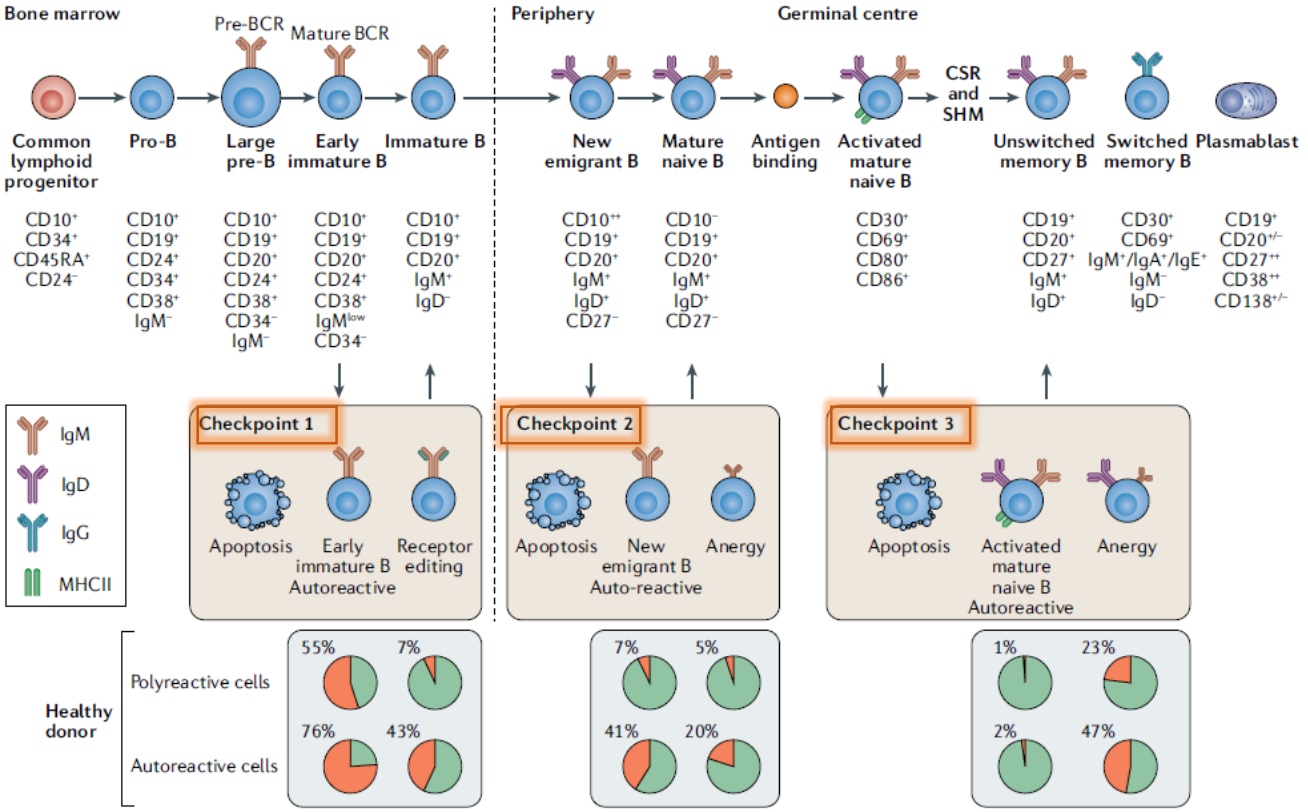
Memory B cells do not depend on BAFF/APRIL for their survival → resistant to anti-BAFF treatment

How B cells do not respond to self antigens?

Eliminating self-reactive B cells: mechanisms of tolerance

More than half of the nascent B cells in initially express autoreactive antibodies

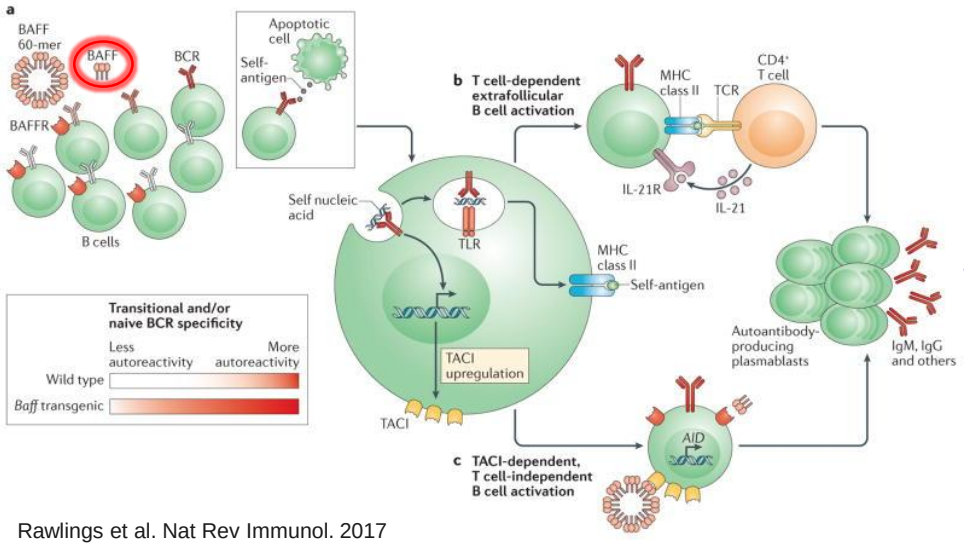
→ most of these autoantibodies are removed from the repertoire before maturation into naïve B cells.



Sun et al. Nat Rev Neurol. 2020

T cell-dependent and T cell-independent extrafollicular activation pathways in autoimmunity

Excess of BAFF promotes the survival and selection of immature B cells from the BM to the periphery



Rawlings et al. Nat Rev Immunol. 2017

Antibody-dependent functions

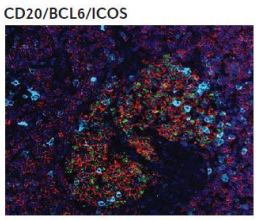
- Myeloid cell activation
- Complement activation
- Antibody-depend cell lysis

Antibody-independent functions

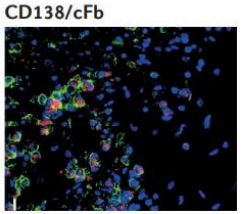
- Inflammatory cytokines production
- Antigen presentation
- T cell costimulation
- T cell polarization

Ectopic germinal centres sustain antigen-specific and disease-specific autoimmune responses

Salivary glands in Sjogren

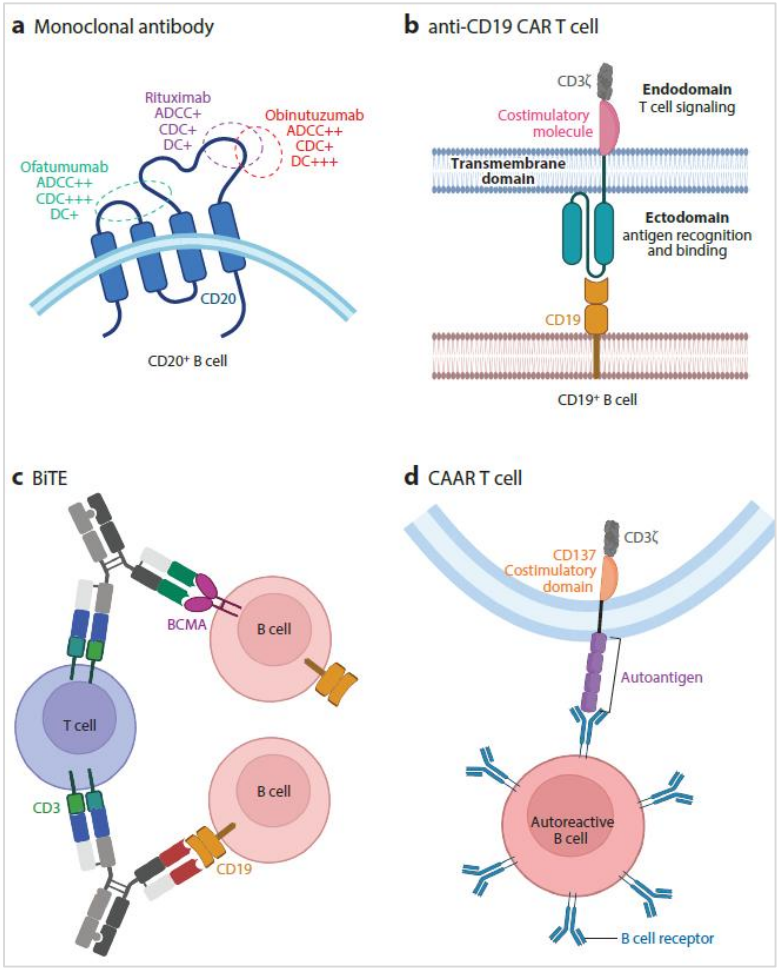


Synovium in RA



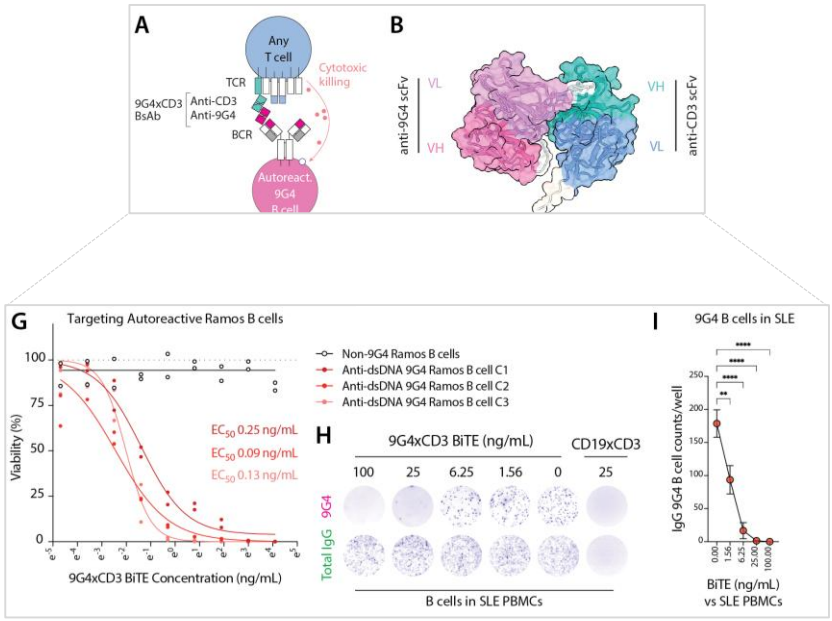
Bombardieri et al, Nat Rev Reum 2017

Targeting B cells in autoimmunity



Abeles et al, Annu. Rev. Immunol. 2024

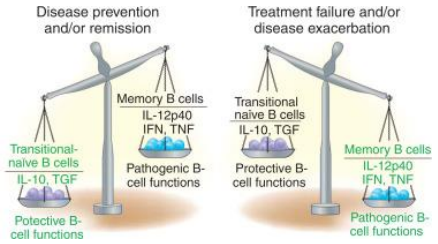
BiTE targeting the 9G4 BCR idiotype → precision immunotherapy for the selective depletion of autoreactive B cells in SLE



Liu et al, Arthritis Rheumatol. 2024

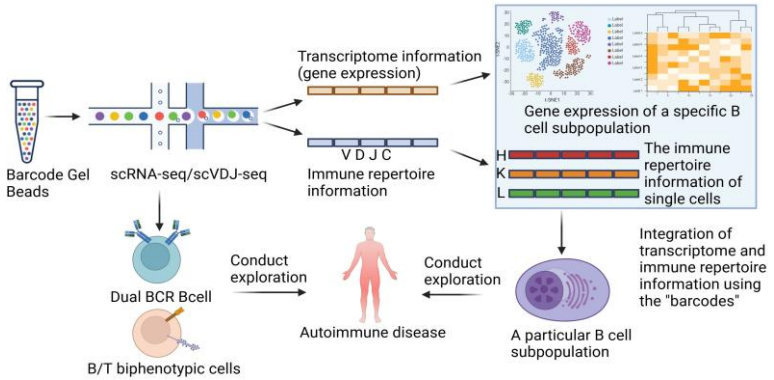
Decoding the BCR repertoire for dissecting the immunopathological mechanisms of B cells in autoimmune disease

Relative balance between pathologic/normal B cells determines disease course and response to treatment.



Manjarrez-Orduño et al. J Invest Dermatol. 2009

Identification of new therapeutic targets



Quan et al, Cells 2025

Open question: Would the BCR repertoire of B cells localized in the secondary lymphoid organs or the inflamed tissues be the same with the ones in the circulation?

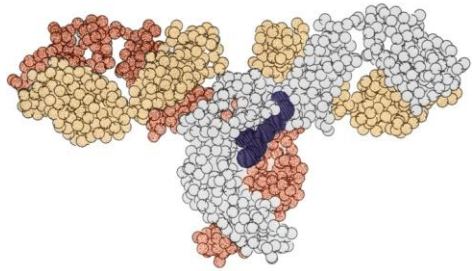
19:30-20:30
Pharma industry / Biotech perspectives in immunology
Chair: DT Boumpas

- CART cells in autoimmune diseases (Cristopher Sjowall, Bristol Myers Squib)
- Novel B cell directed therapies (Roberta Manfroni, Zenas BioPharma)
- Immunology pipeline (A. Moulis, Medical Director, AbbVie)

"I have many ideas, but often they don't work. In discoveries, the most important thing is to do the experiment."

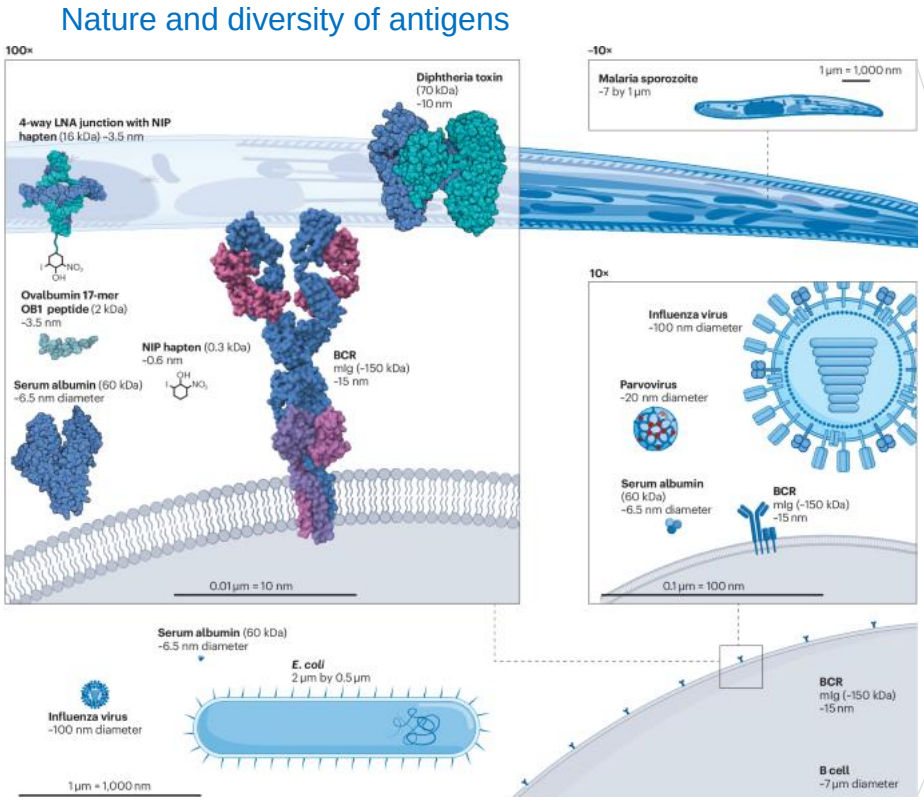
Georges J.F. Köhler

Nobel Prize in Physiology or Medicine 1984 for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies



Thank you

Antigen receptors can bind antigens through their CDRs that are complementary to the size and shape of the antigen



Degn, S.E., Tolar, P. Nat Rev Immunol 2025

Antigen – Antibody: Lock and key