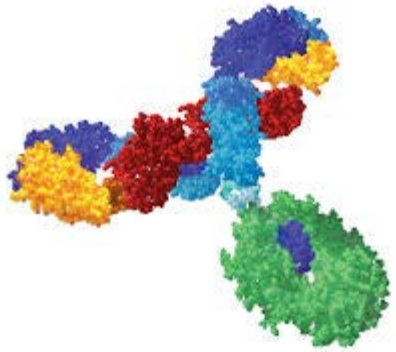
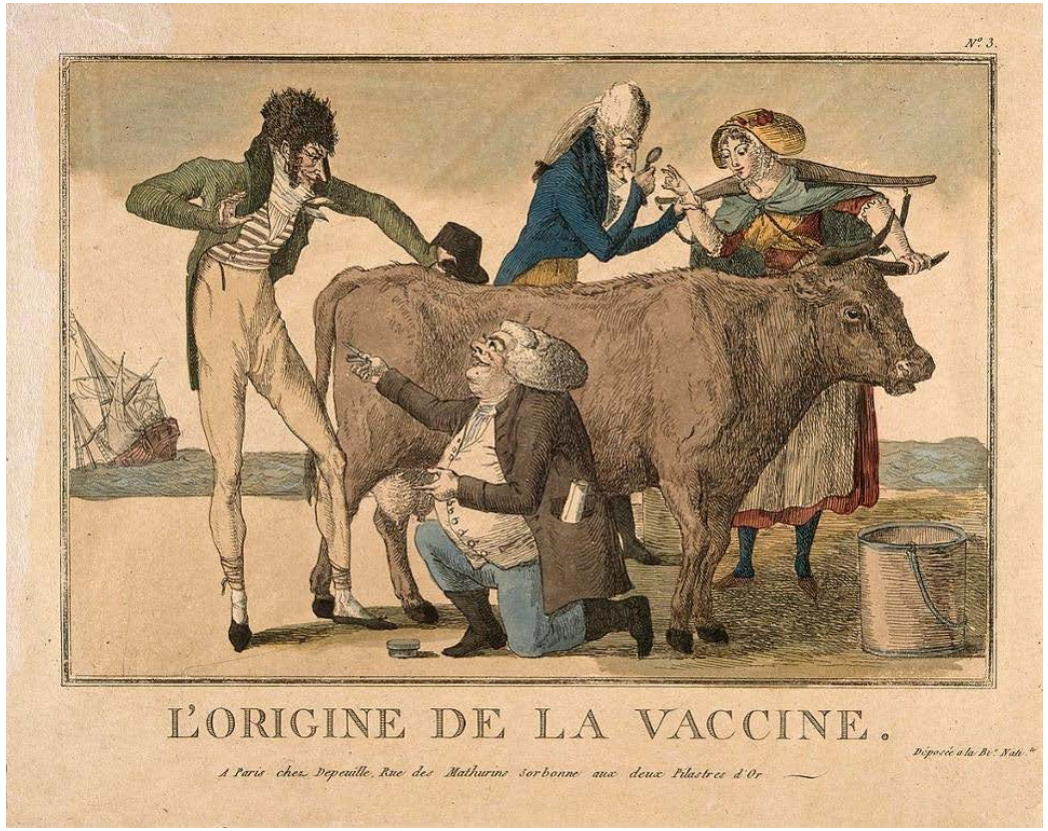


# Introduction to Antibody Structure/Function

Med Chem 528

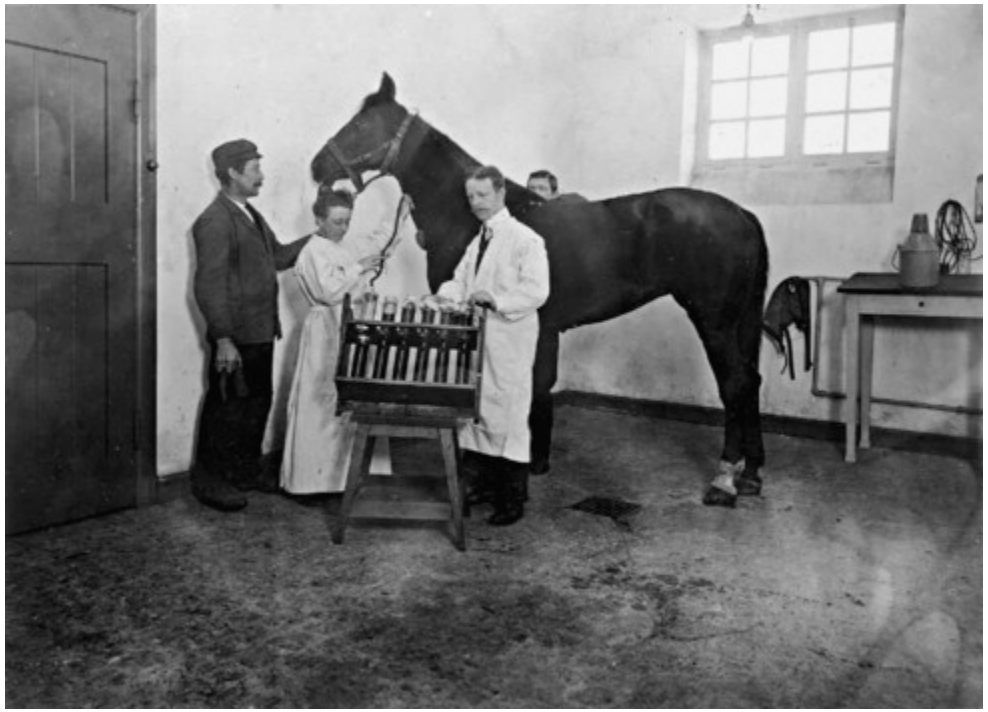
# Origins of antibodies

- Product of the adaptive immune system
  - B cells (antibody based immunity)
  - T cells (cell based immunity)
- Pre-exposure protects against subsequent infection.
  - Bacteria, viruses, toxins
- Plasma from exposed mice can confer protection to naïve mice
  - Infants protected by maternal antibodies
- Mediate most allergic responses



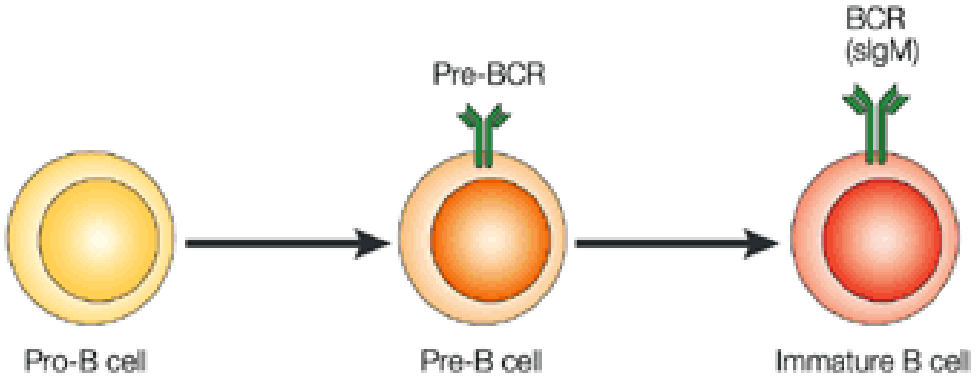
# Antibodies as medicine

- Antibodies/Immunoglobulins
  - Soluble glycoproteins
  - Product of the adaptive immune system
- Early uses (antitoxins)
- Passive therapy (Ebola 1976)
- Focus of modern biotherapeutics



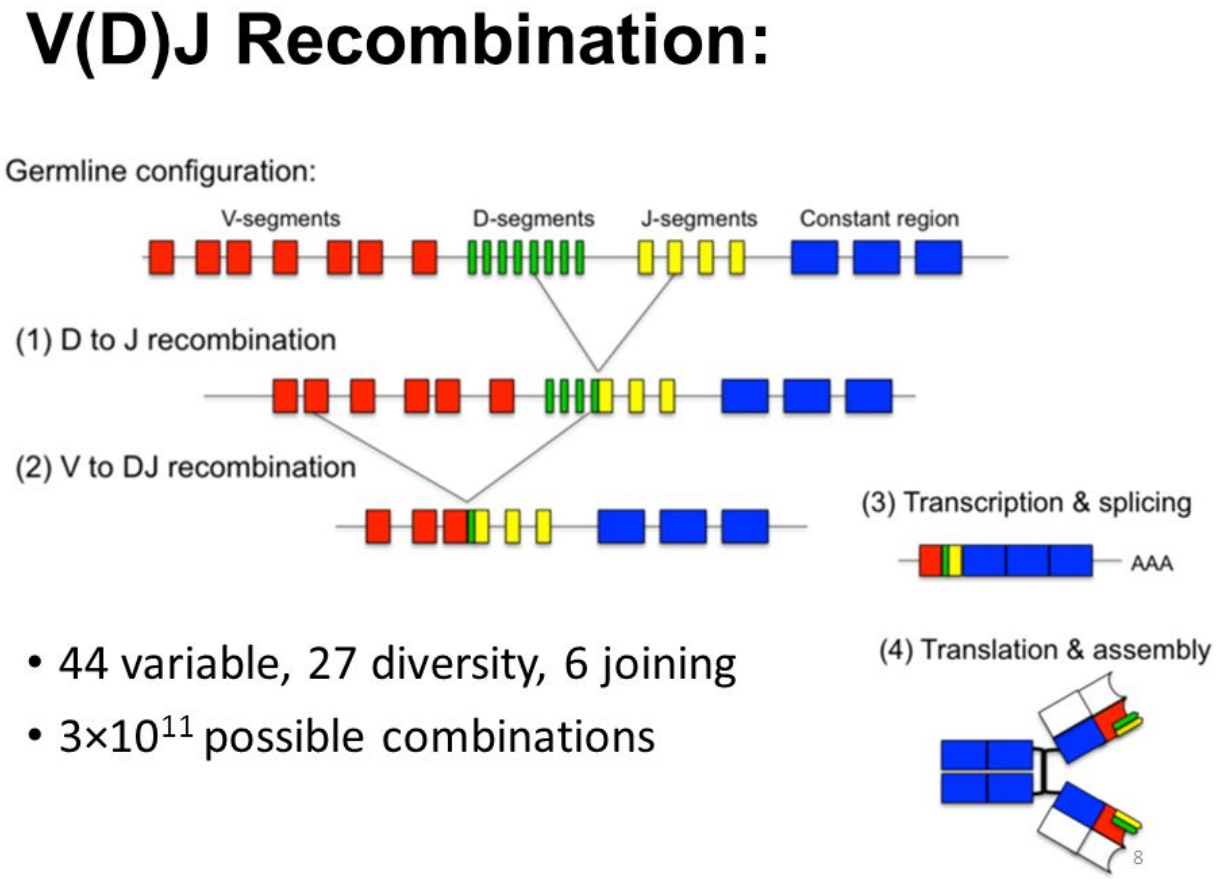
# B cells mature to make antibodies

- Originate in **B**one barrow
  - Assemble heavy/light chain
  - Make intact B-cell receptor (BRC)
- Clonal deletion
  - Prevent self-reactivity
- Activated by antigen encounter
  - Otherwise it only survives for days
- Recruited for affinity maturation



# Antibodies are loosely encoded in DNA

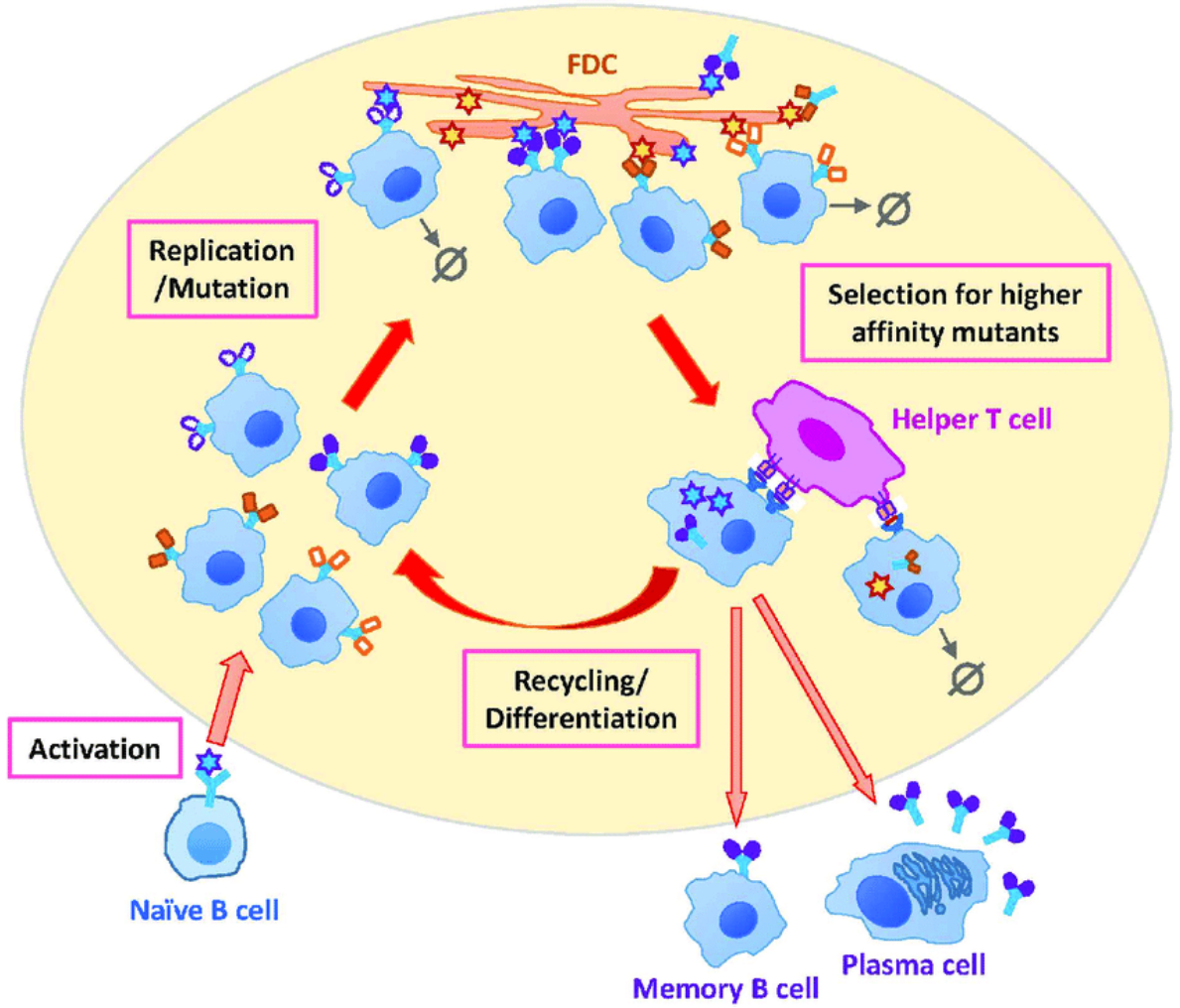
- First assembled as B-cell receptors (BRCs)
- Randomly assembled from V, D, and J gene segments
- Heavy chain VDJ recombination
  - 2/3$^{rd}$ of all have codons out-of-frame
- Light chain recombination
  - Kappa
  - Lambda
  - Overall only ~1/4$^{th}$ of all cells end up with a functional BCR
- "Germline antibody"
  - Original antibody sequence on B-cells




Janeway, Immunology 2001

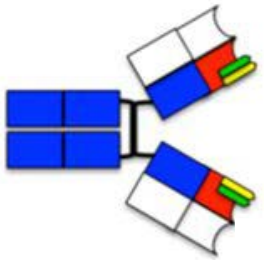
# B cells mature to make antibodies

- Affinity maturation
  - Germinal centers
    - Lymph nodes & spleen
  - Mixing of:
    - B-cells
    - T-cells
    - Follicular dendritic cells (FDC)
- B-cells compete for antigen
- Somatic hypermutation
- Winners form plasma cells
  - Antibody factories
- Also form memory B-cells
  - Rapid response to subsequent infections

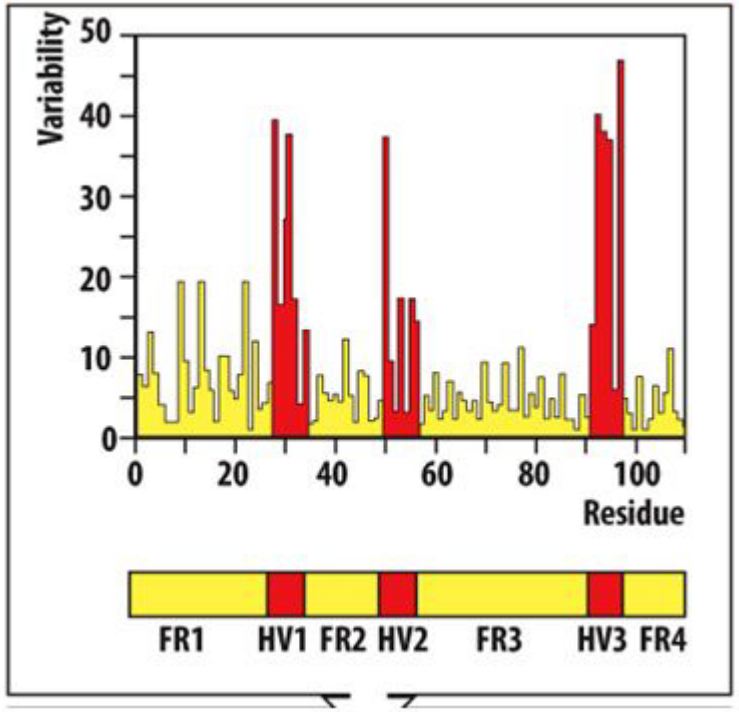



Wang, S. 2017 *PLoS Comp Biol*

# Results in huge sequence diversity

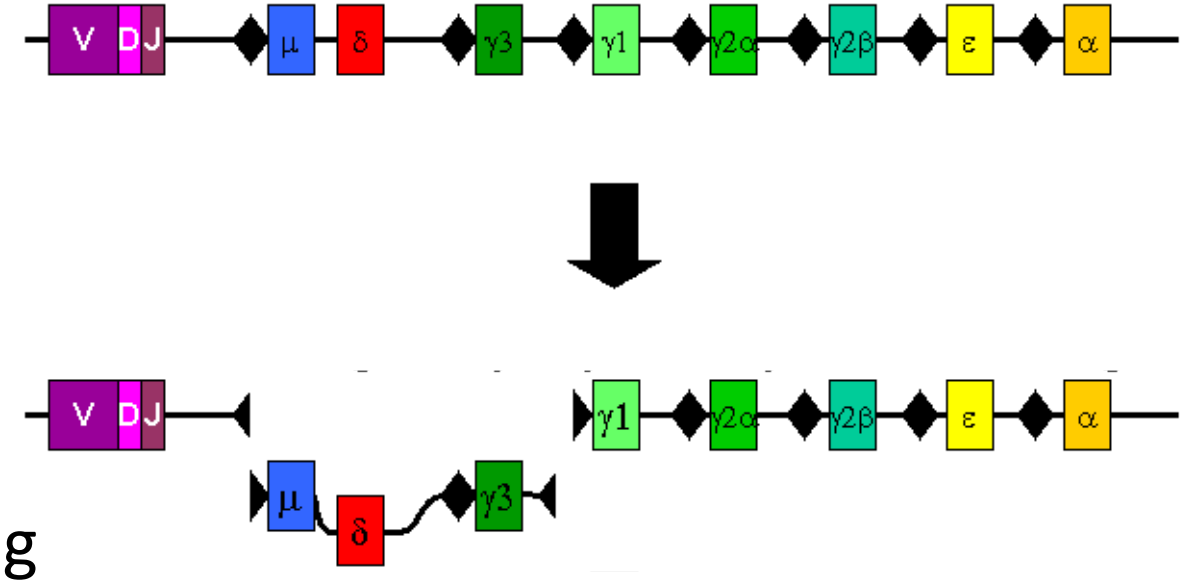


- Extensive sequence diversity
  - Extensive paratope repertoire
- Mutations can also occur outside of the VDJ variable regions
  - Likely not tolerated
  - Non-functional BCR
- Can make an antibody against nearly anything
  - Proteins
  - Carbohydrates
  - Small molecules (haptens)
- High binding affinity ($K_D$ sub nM)
- High binding specificity



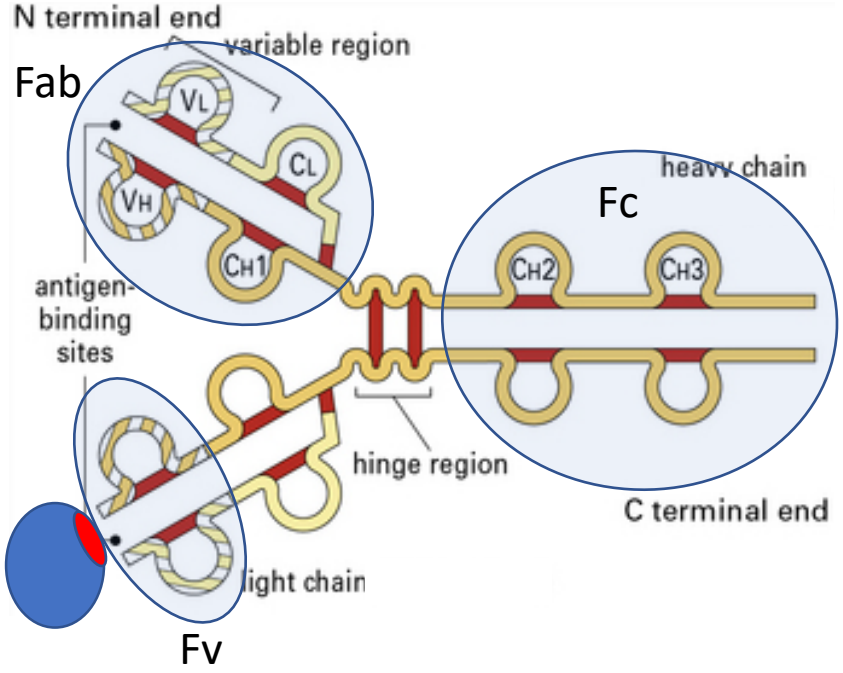
# There are several classes of immunoglobulins

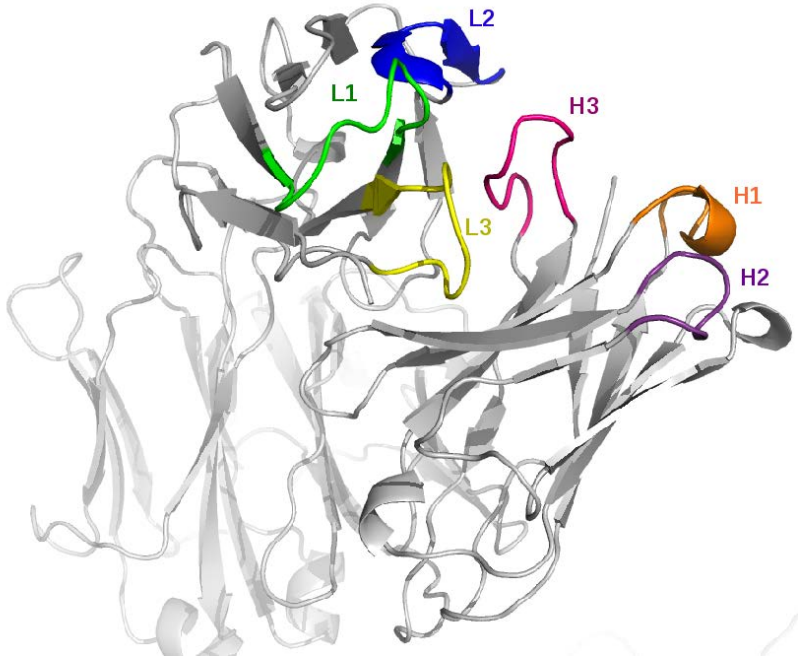
- Many Ig constant regions on the heavy chain gene
  - IgM, IgD, IgG3, IgG1, IgG2, IgG4, IgE, IgA
- All BCRs/antibodies start as IgM
  - Also IgD during development
- B-cells “class switch” to form other types of Igs.
  - Each have various functions
- Type of antigen influences class switching
  - Toxin/bacteria/virus etc.



# Anatomy of IgG1

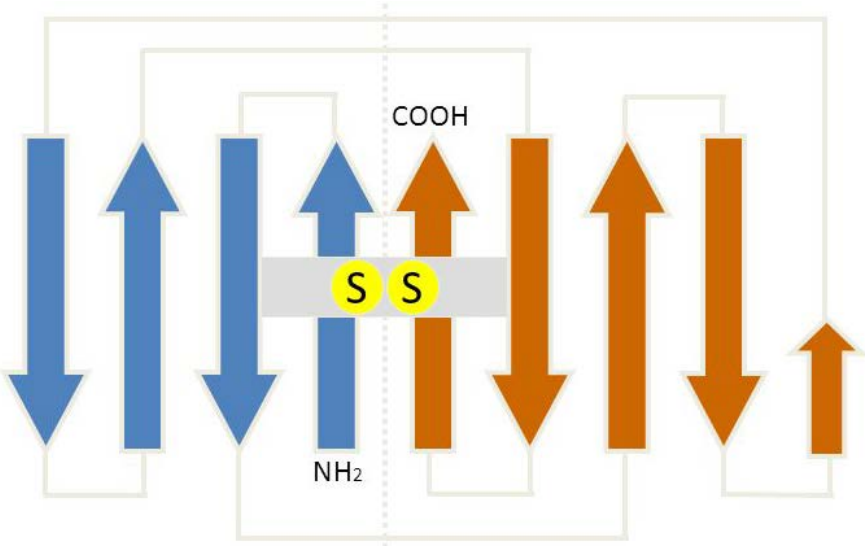
- Structure of antibodies (IgG1)
  - 2x heavy chains (~450 residues)
  - 2x light chains (~212 residues)
  - Disulfide bonds (inter & intra chain)
- Common terms:
  - Antigen
  - Epitope
  - Paratope
  - Fab
  - Fc
  - Fv
  - Complementarity determining regions (CDRs)
  - Framework regions

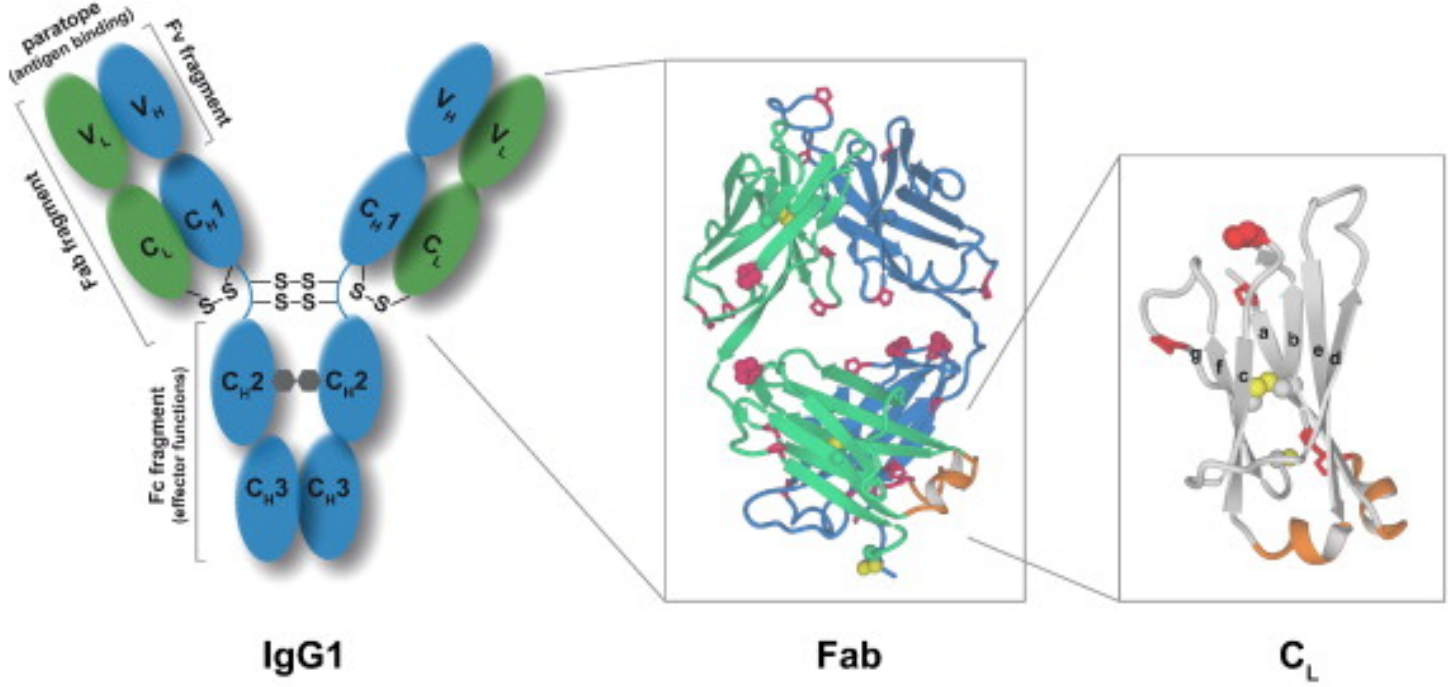


# Structure of Ig domains

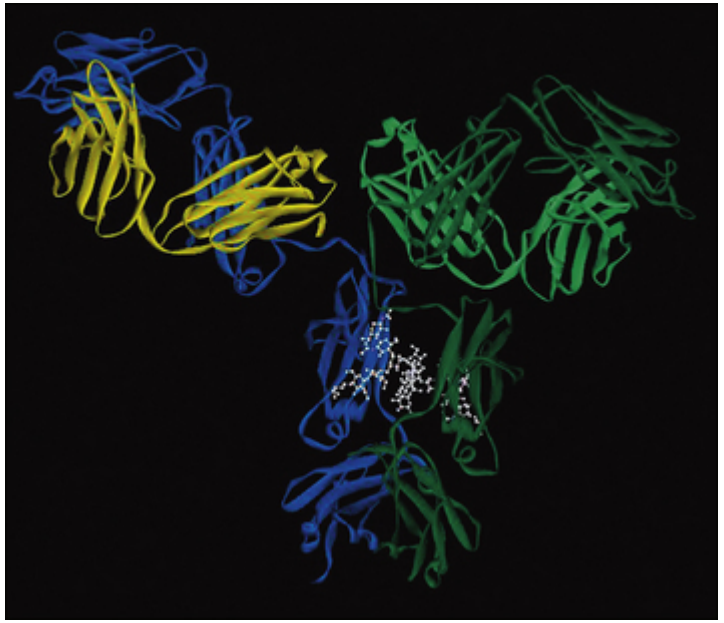
- Immunoglobulin fold
  - Beta strands
  - Central disulfide
- Interdomain disulfides
  - Link light & heavy
    - $C_H1 - C_L$
  - Two heavy chains
    - Hinge region
- Ig domains dimerize
  - Non-covalent interactions



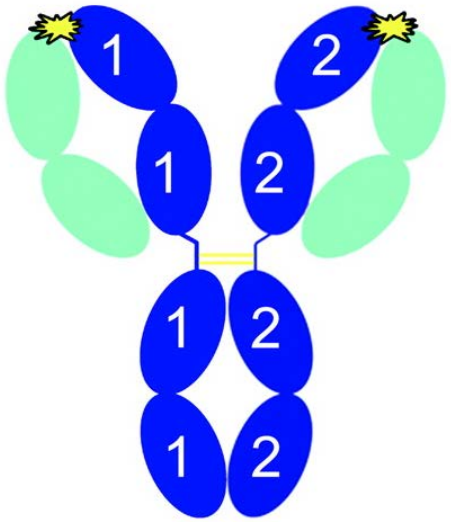

Feige and Buchner, *Biochimica et Biophisica Acta,* 2014

# Domain arrangements in IgGs

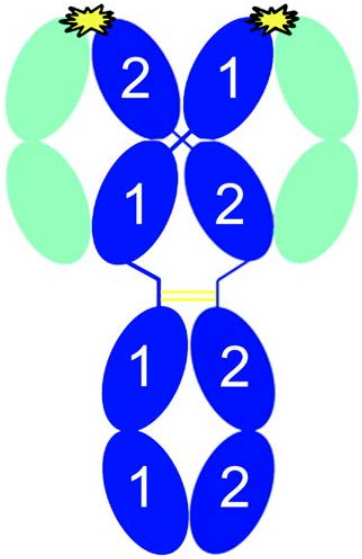


- Nearly all available structures are just Fab or Fc
  - Very few intact IgG structures
- Always a Y-shape?
  - Most IgGs have flexible Fabs
- Can form domain swaps (2G12)
- Probably more to the story
  - Some evidence of Fab-Fc interactions
  - IgG serum concentration ~ 10-20 mg/mL
  - Inhibit Fc interactions with free IgG

Typical IgG



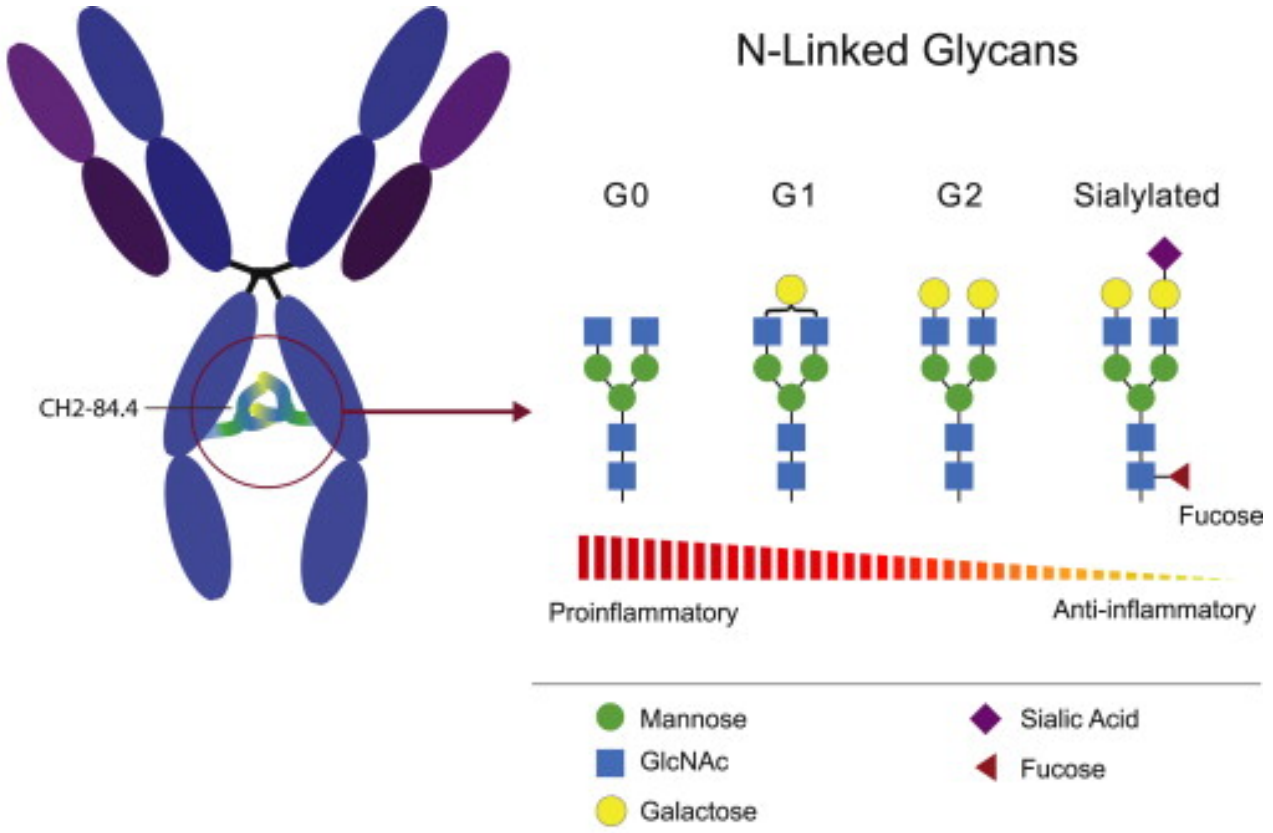

IgG “2G12”




West A.P., et al, *JVI* 2009

# Glycosylation of IgGs

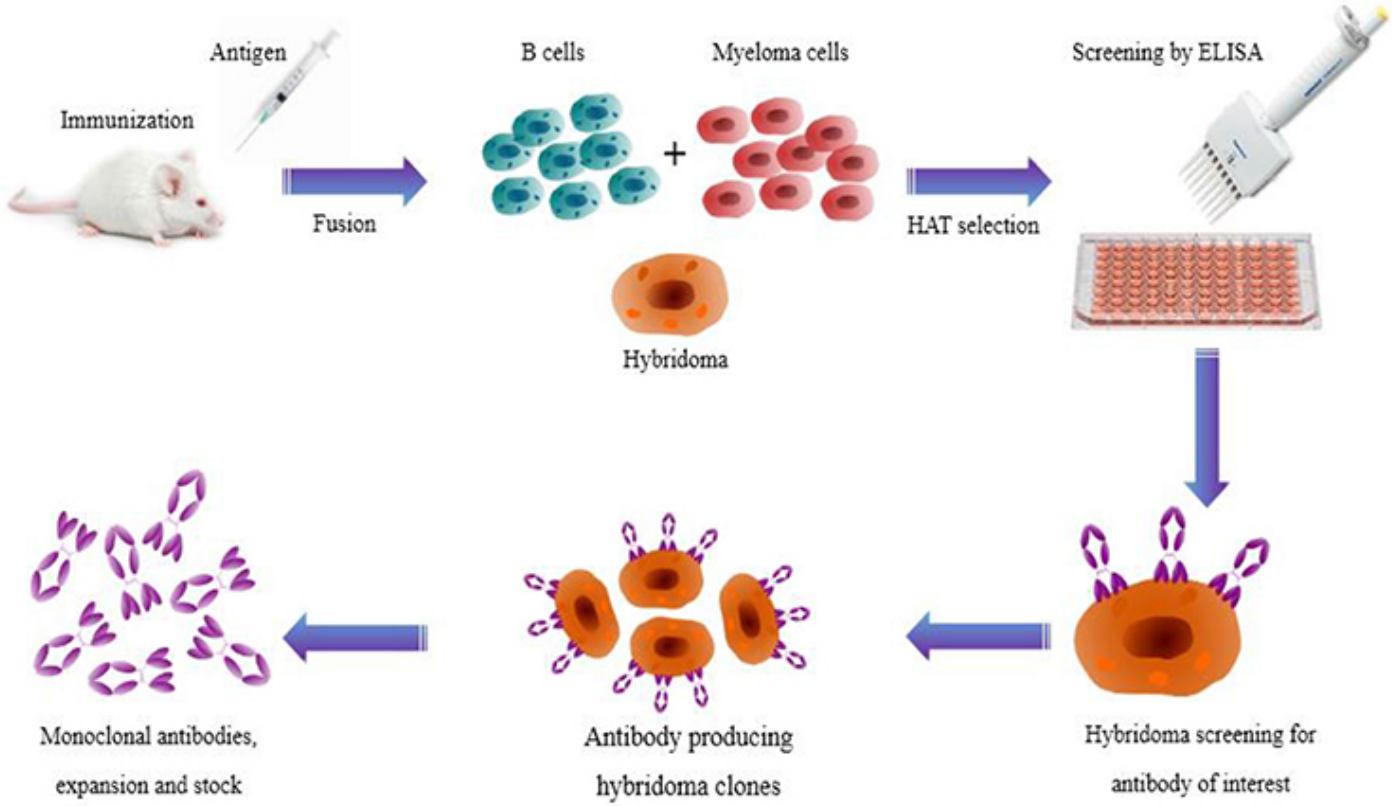
- Conserved N-linked glycan in the Fc domain (CH2)
  - Facing inward
- Critical for effector functions and serum half-life
  - Sialic acid
  - Fucose
  - Galactose
- Aberrant glycosylation can cause complications
  - Aniphylaxis
- Other Ig classes have more glycans
  - IgM, IgA, IgE




Maverakis, E. et al., *J. Autoimmunity* 2015

# Commercial antibodies

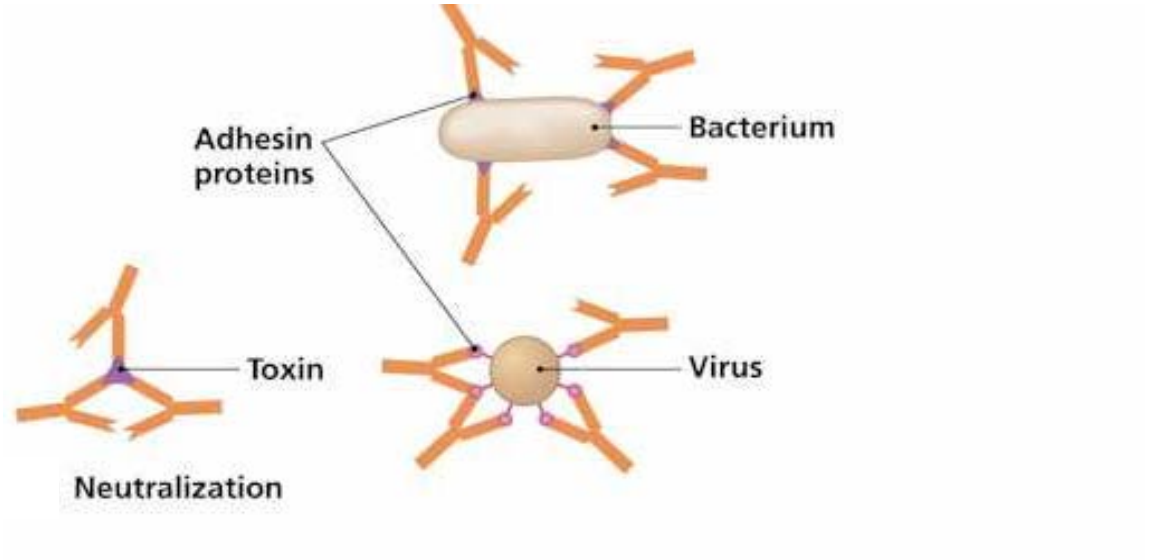
- Polyclonal
  - Isolated from an immunized animal plasma
  - Has 1000's of different antibodies
  - Target many epitopes
- Monoclonal
  - Originate from hybridomas
    - Immortalized plasma cells
  - A single clone
  - A single epitope
- Recombinant antibody expression
  - Chinese hamster ovary (CHO)
  - Human embryonic kidney (HEK)
  - Yeast / E. coli / plants
    - Glycosylation issues

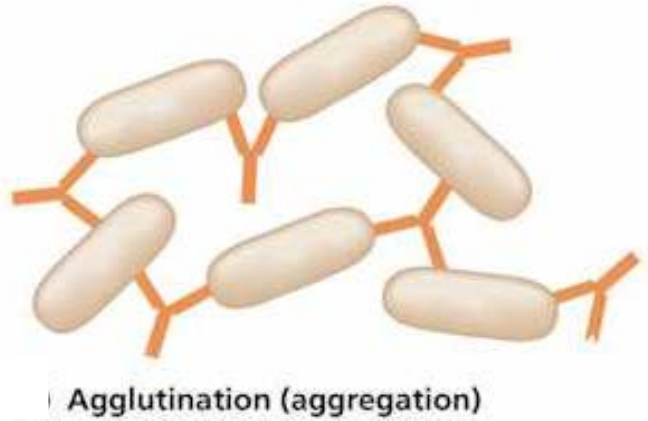



www.frontiersin.org

# Antibodies neutralize antigens

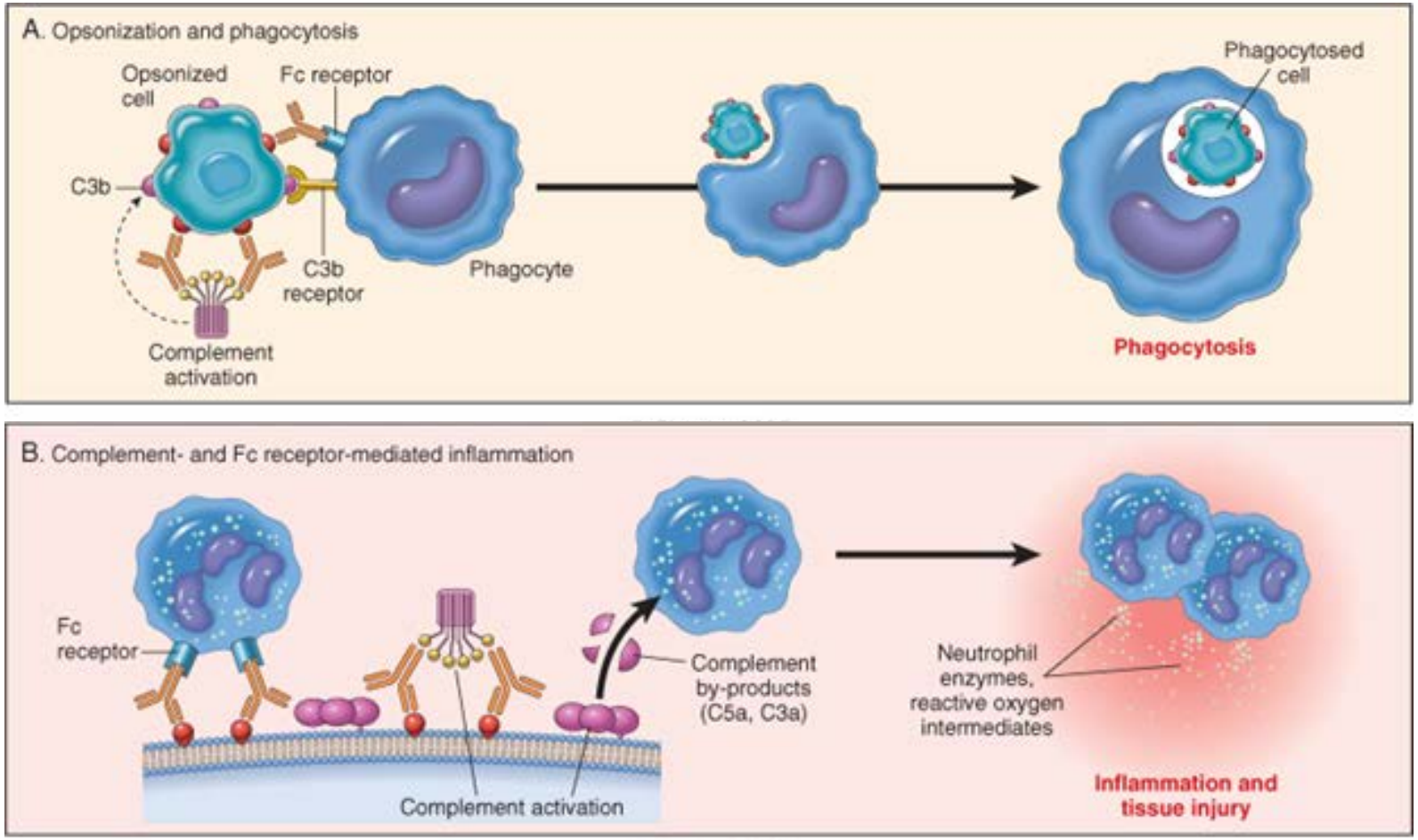
- Neutralization
  - Directly block activity of toxins
  - Block entry of pathogen
- Fab are sometimes still effective
  - Often not as much as the full IgG
- Agglutination/aggregation
  - Tissue or mucosal surface



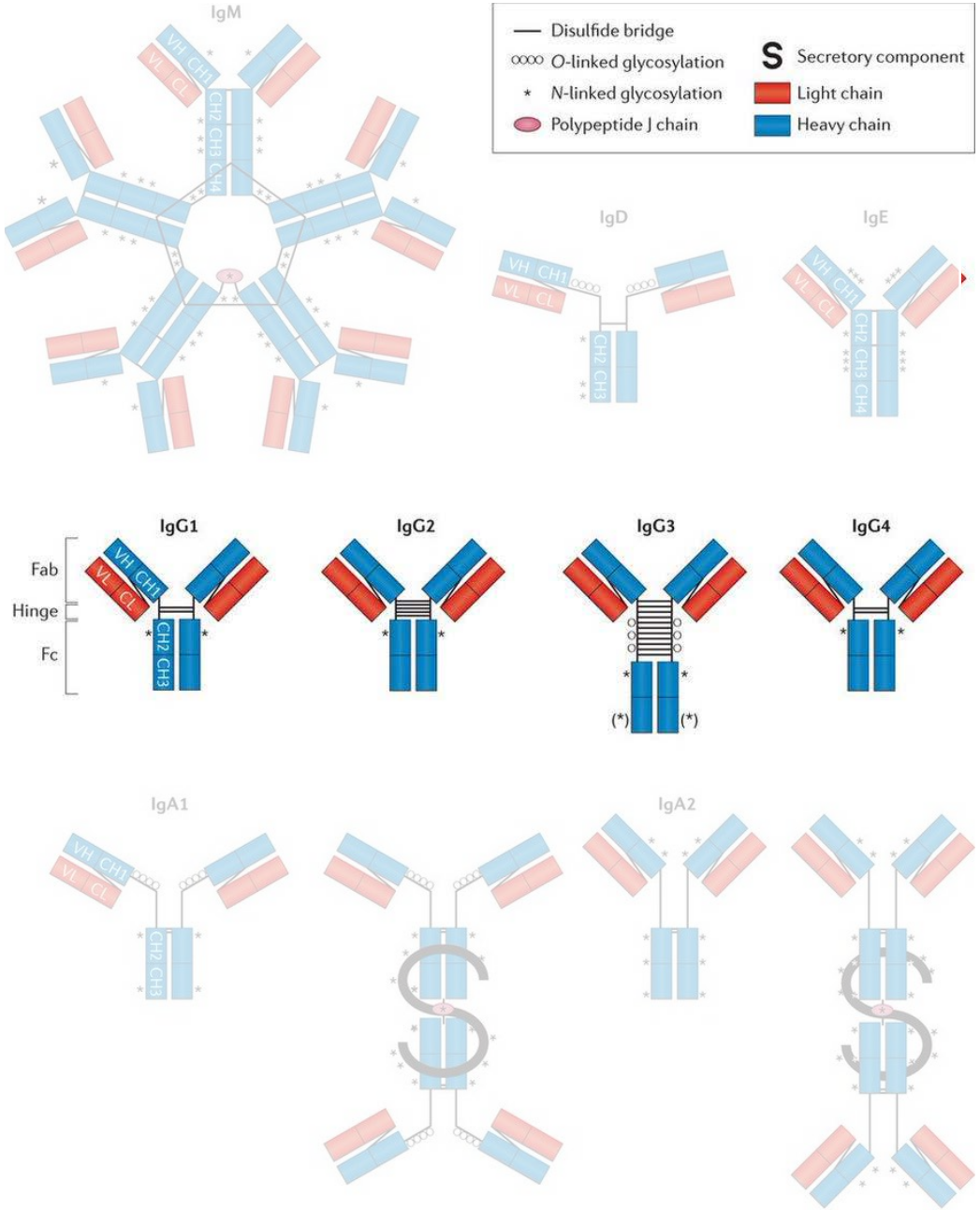
# Antibody mediated effector functions

- Opsonization (labeling)
- Dependent on other immune cells/factors
- Fc-mediated
- Interactions with Fc-receptors (FcR)
  - Phagocytes
  - Natural killer cells
  - Complement factors



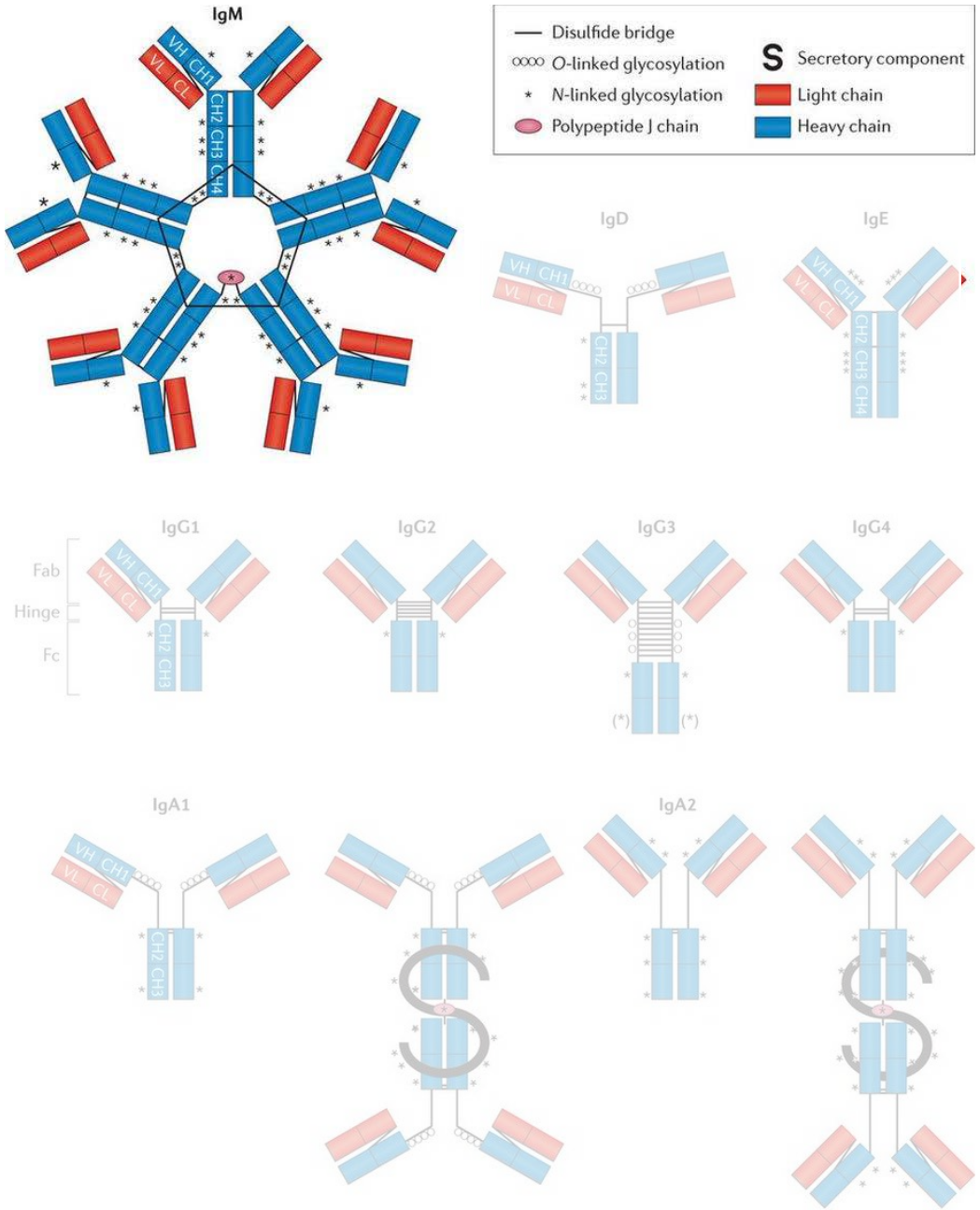
# IgGs

- Most abundant Ig in serum
  - ~90% of all serum antibodies
- Longest serum half-life
  - Around 21 days
- Only ones to cross placental barrier
  - Passive immunity for early infant
- Slight variation in effector function
  - Different affinities for FcRs
- Vast majority of commercial antibodies
  - Cheap and easy to produce



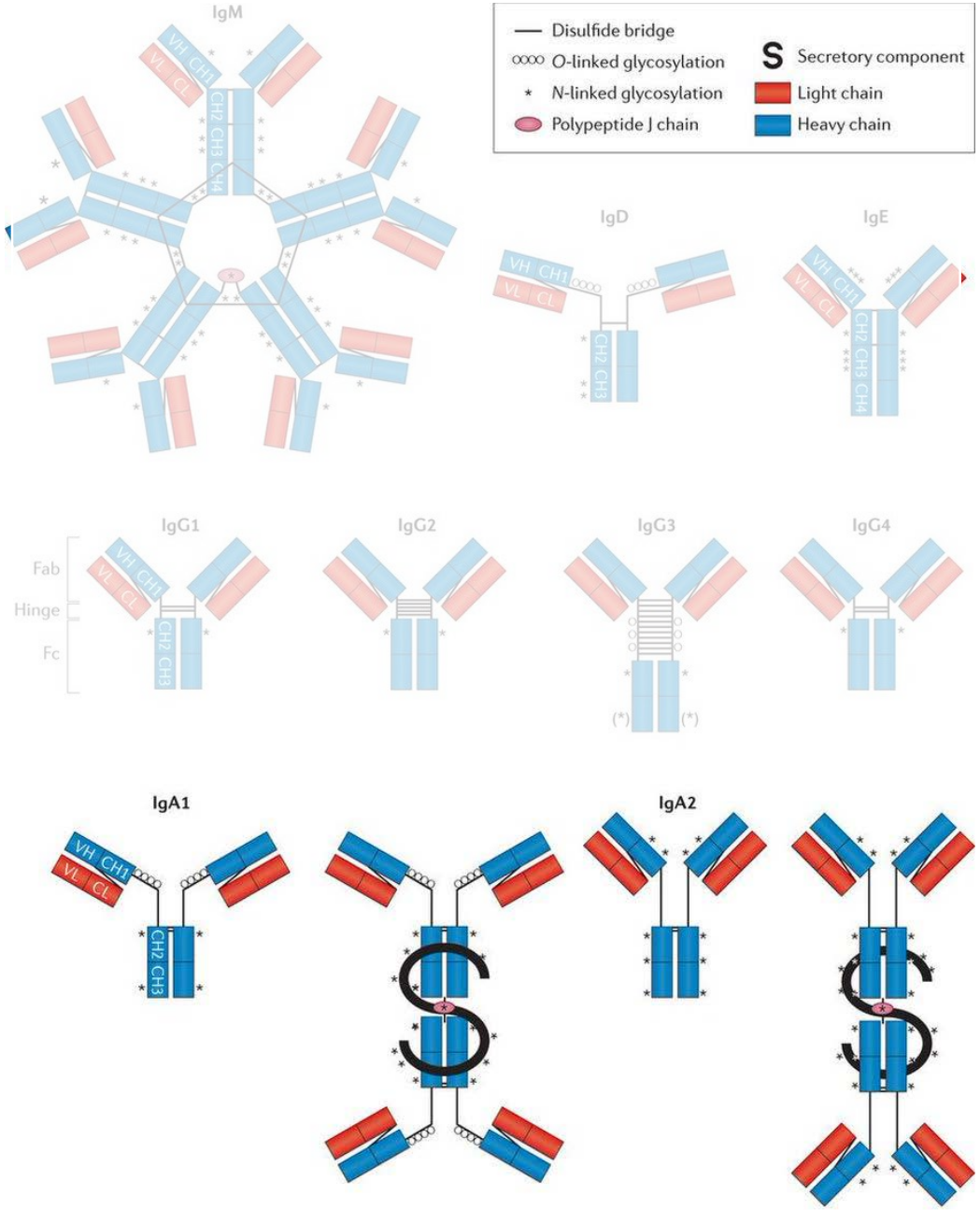
# IgM

- 2nd most abundant Ig in serum
  - ~5-10% of serum antibodies
- Hexamers or pentamers (1 MDa)
  - Include a joining (J) chain
  - Can be secreted
- First produced in response to infection
- Generally have weaker antigen affinity
  - But have high avidity
- Natural cancer defense
- Blood compatibility (ABO)
- Xenograf rejection (pig organs)



# IgA

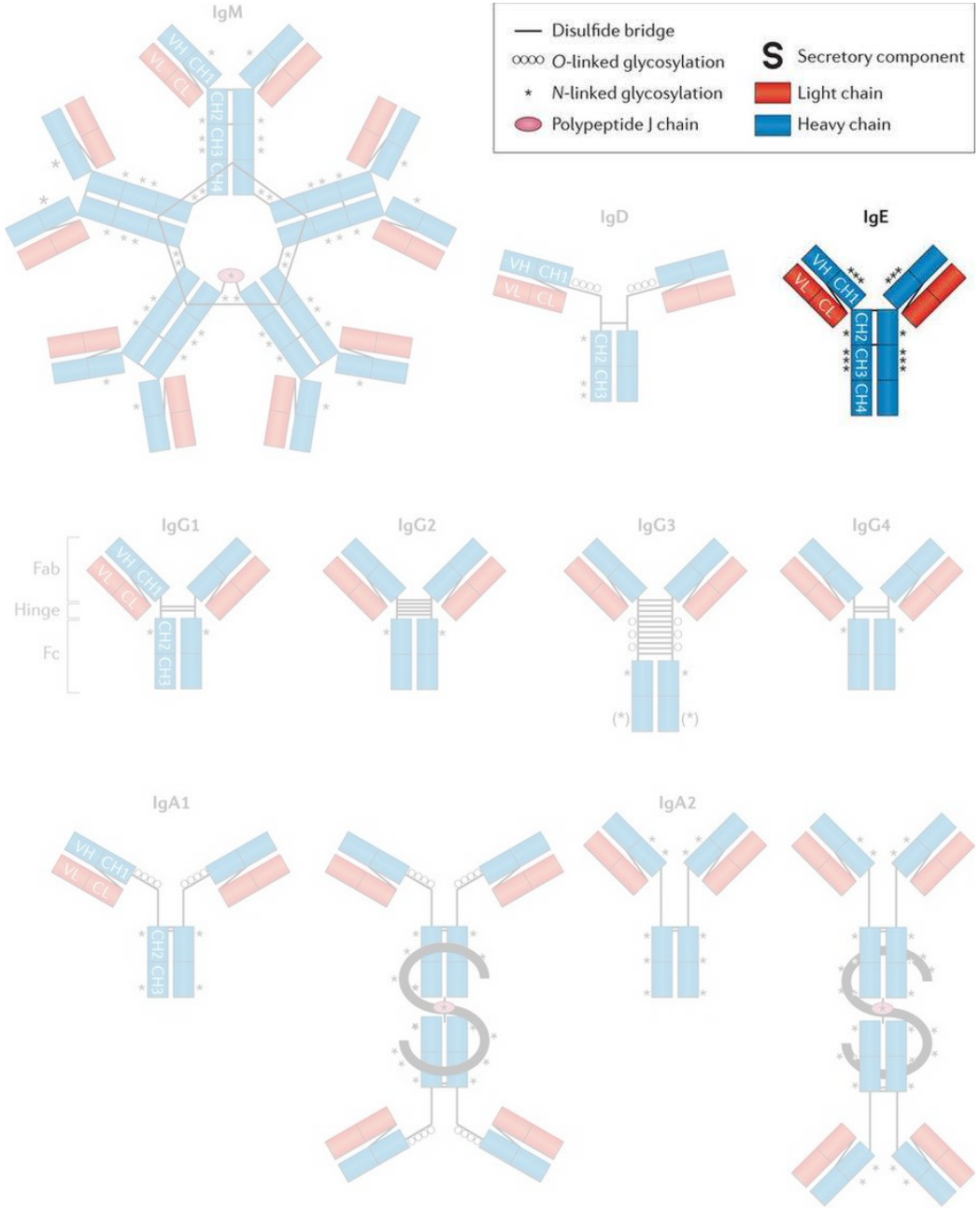
- Disulfide bonded dimers
  - Include a joining (J) chain
  - Mostly secreted
  - Very little in serum
- Protect mucosal surfaces
  - Gut, nasal etc.
  - Breastmilk
- Neutralizing toxins in digestive tract




Nature Reviews | Immunology

# IgE

- Low serum concentration
  - All cell-surface bound
  - Ultra high affinity for FcRε
- "Arms" immune cells
  - Activation induces cytokines
  - Inflammation
  - Allergy symptoms
- Protects from multicellular pathogens
  - Helminths (hookworms)
- Evolutionary baggage
  - Major mediator of allergies
  - Helmith therapy



# FcRs and immune regulation

- FcRs present on many types of immune cells
  - Regulate the type of response to antigen/antibody class
- Affinity for Fc is well tuned to keep effector functions regulated
- Other FcRs used for sorting/recycling Igs
  - FcRn
  - Longer half-life

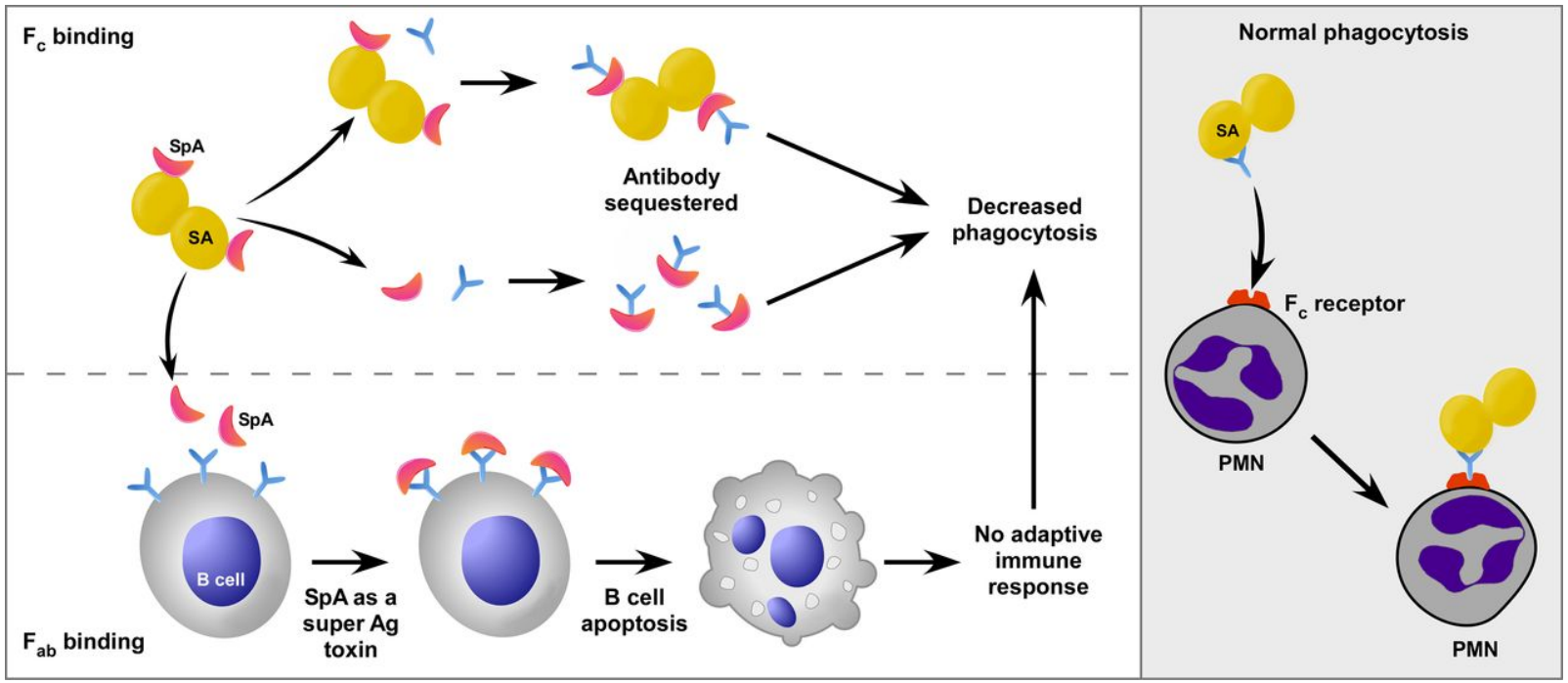
| | | FcγRI | FcγRIIA | FcγRIIB | FcγRIIIA | FcγRIIIB | FcεRI | FcαRI |
|---|---|---|---|---|---|---|---|---|
| Antibody affinity | IgG1 | ++++ | ++ | + | + | +/– | – | – |
| | IgG2 | – | +/– | +/– | +/– | +/– | – | – |
| | IgG3 | ++++ | + | + | ++ | + | – | – |
| | IgG4 | +++ | +/– | + | + | – | – | – |
| | IgA | – | – | – | – | – | – | ++++ |
| | IgE | – | – | – | – | – | ++++ | – |
| Cellular expression | Monocytes | + | +++ | + | + | – | – | ++ |
| | DCs | + | +++ | + | – | – | – | |
| | NK cells | – | – | +/–* | +++ | – | – | – |
| | Neutrophils | + (ind) | + | +/– | – | +++ | – | +++ |
| | Eosinophils | – | ++ | – | – | – | +++ | + |
| | Basophils | – | ++ | ++ | – | +/– | +++ | – |
| | Mast cells | + (ind) | ++ | +/– | – | – | +++ | – |
| | B cells | – | – | +++ | – | – | – | – |

++++: high affinity/expression; +/–: low affinity/expression; ind: inducible expression;
–: no affinity/expression; *: in FcγRIIC polymorphic individuals.

Trends in Molecular Medicine

# Considerations for antibody therapeutics

- Production/purification
  - Protein A & G
- High affinity for IgG Fc region
- Orients antibody outward to disrupts opsonization




Kobayashi and DeLeo, mBio 2013

# Considerations for antibody therapeutics

- Production/purification (protein A/G)
- Potency (antigen binding affinity/kinetics) determines dose.
- Stability
  - In vitro: degradation/formulation
  - Serum half-life: stability, interactions with recycling receptors (FcRn)
- Effector functions
  - Interactions with FcRs/complement factors
  - Fc domain modifications, glycosylation
- Immunogenicity (induced anti-antibodies)
- Off-target effects / self-reactivity