My Field for Dummies

Webinar Series

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Immunology and Bats

14 June 2023
9 – 10 AM
Eastern US Time

Live seminar
via Zoom

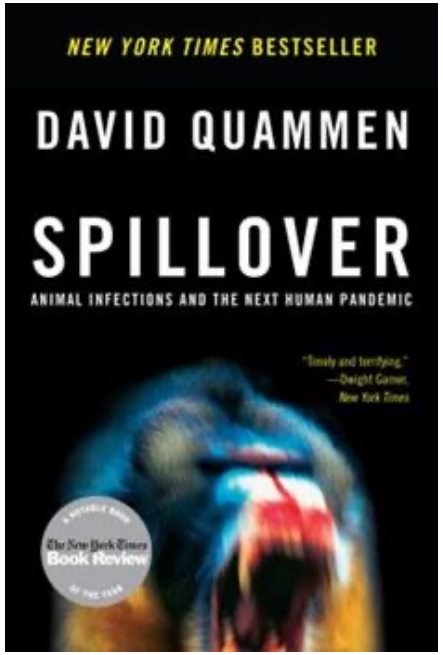
More Info:
www.gbatnet.org/events/my-field-for-dummies-immunology-webinar/

Outline

- **Immune system components:**
  - Innate Immunity
  - Adaptive Immunity
- **Techniques to study Immunology:**
  - ELISA
  - Flow Cytometry
- **Bat Immunology**
  - What do we know?
  - Challenges/ current direction

How did I get into Immunology?

Bachelor's



Master's

-T cell response to influenza

-Innate immune signaling in asthma

-Invertebrate (Shrimp) immune response

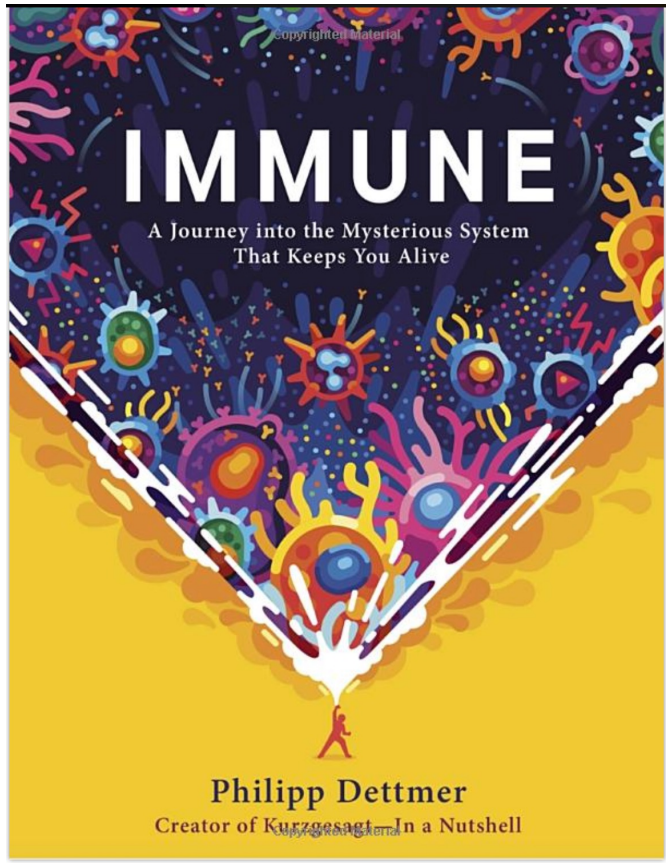
Technician

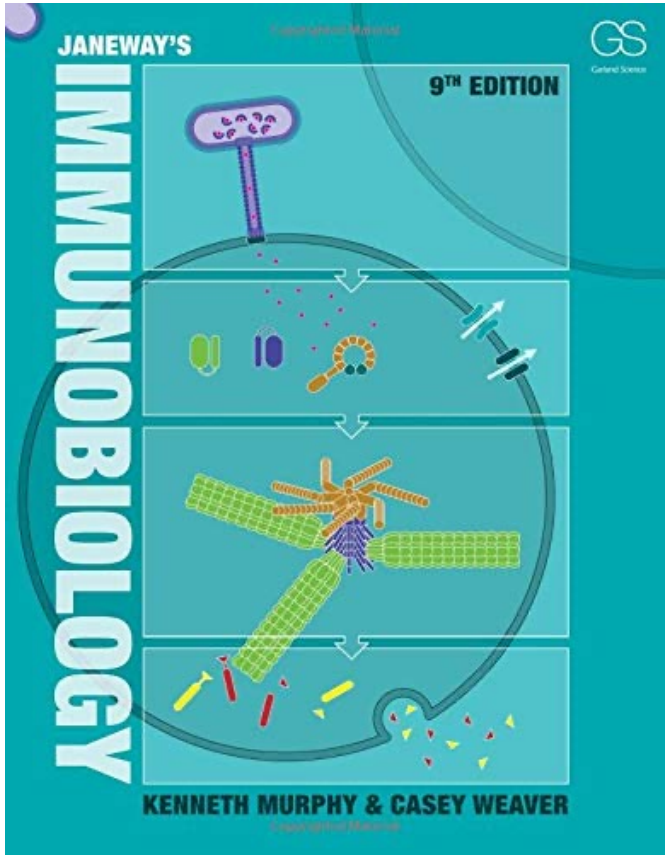
-Oncolytic virus to treat colorectal cancer

PhD (current)

-B cell and immunoglobulin
-Bat Immunoglobulin!

What is Immunology?



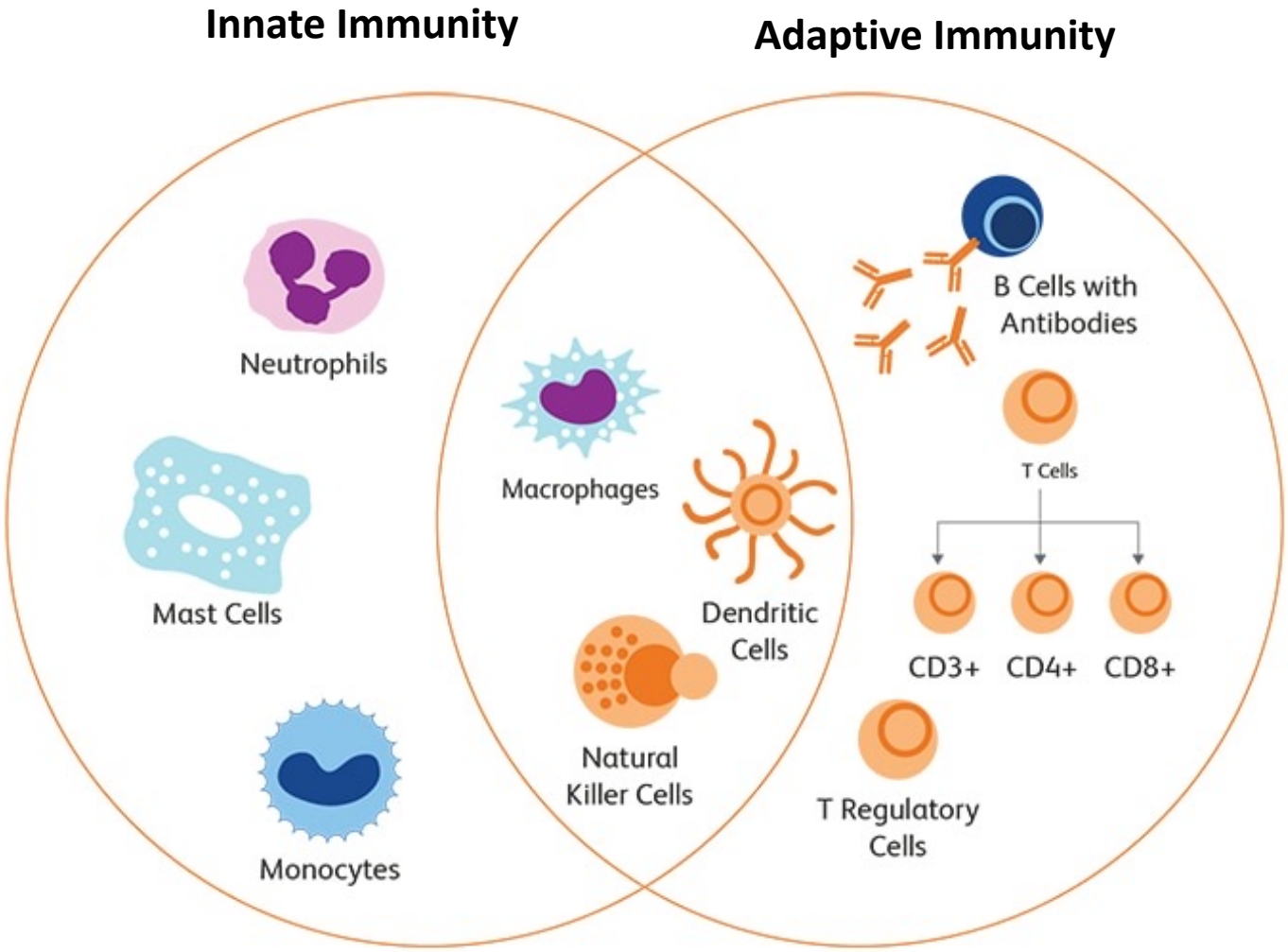


- **Definition**: Study how components of immune system interacts in response against antigens

- **Antigens:** non-self molecules/ substances that elicit immune response e.g. microbial proteins

(Janeway's 9th edition)

Components of Immune system



Innate:

- Fast response
- Broad pathogen recognition

Adaptive:

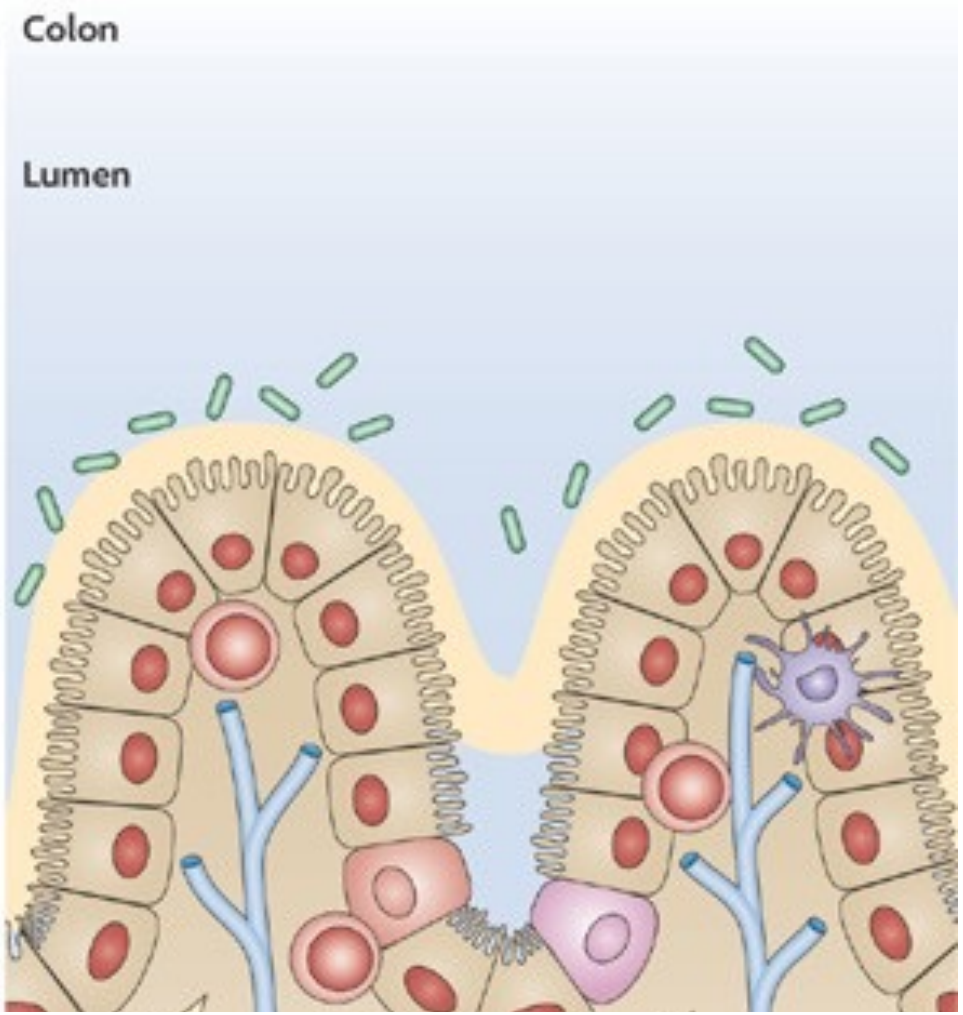
- Delayed response
- Specific-pathogen recognition
- Longer lasting

(Janeways, 9th edition)

Immune Cell Types: Immune System Responses: BD Biosciences, 2023

Innate Immunity-Natural barriers

- **Physical Barriers:**
  - Skin
  - Mucosal layers
- **Chemical Barriers:**
  - Lysozymes
  - Antimicrobial peptides
  - Low pH e.g. stomach acid



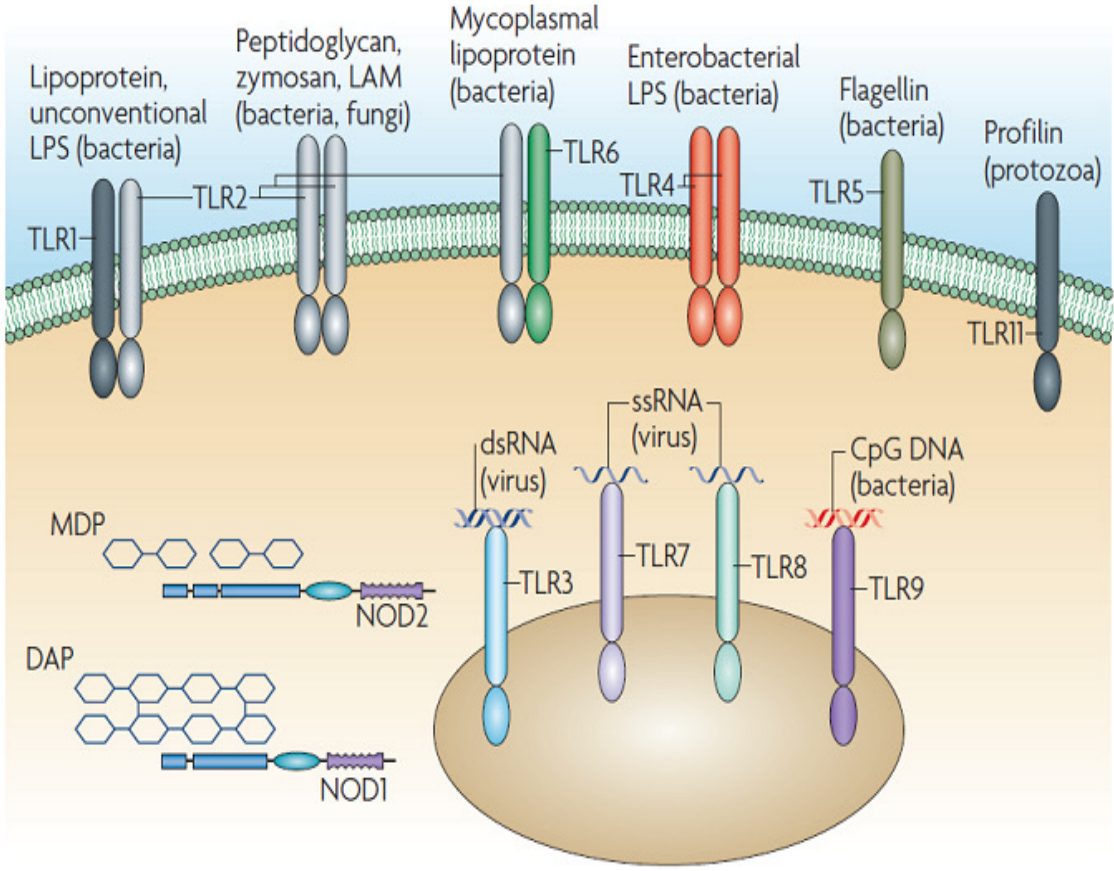
(Abreu, 2010)

Innate Immunity-Pattern Recognition Receptors (PRRs)

- **<u>Recognizes:</u>**
- **PAMPs**
  - Pattern associated molecular patterns
- **DAMPs**
  - Damage-associated molecular patterns

<u>Functions:</u>

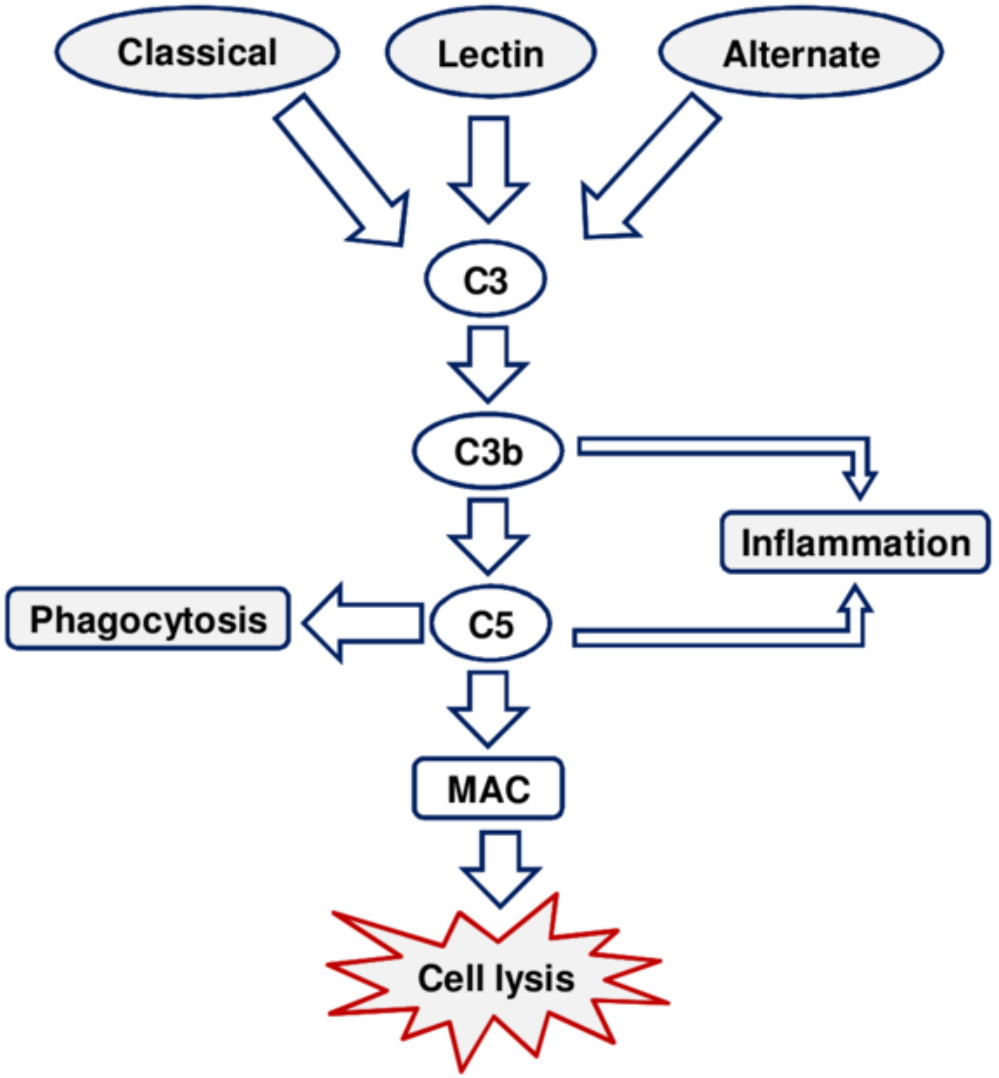
- Recognizes specific molecules:
- E.g. liposaccharides, nucleic acids



(Li and Wu, 2021)

Innate Immunity-Complements

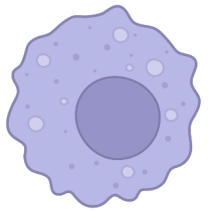
- Highly conserved in many organisms
- Work with antibody and other immune cells



Innate Immune cells

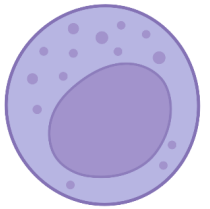
Example of Innate Immune cells:

- Macrophages
- Neutrophils
- Natural Killer (NK) cells
- Dendritic cells

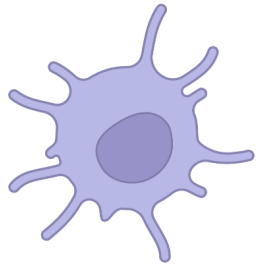


Macrophage

Neutrophil



Natural killer cell

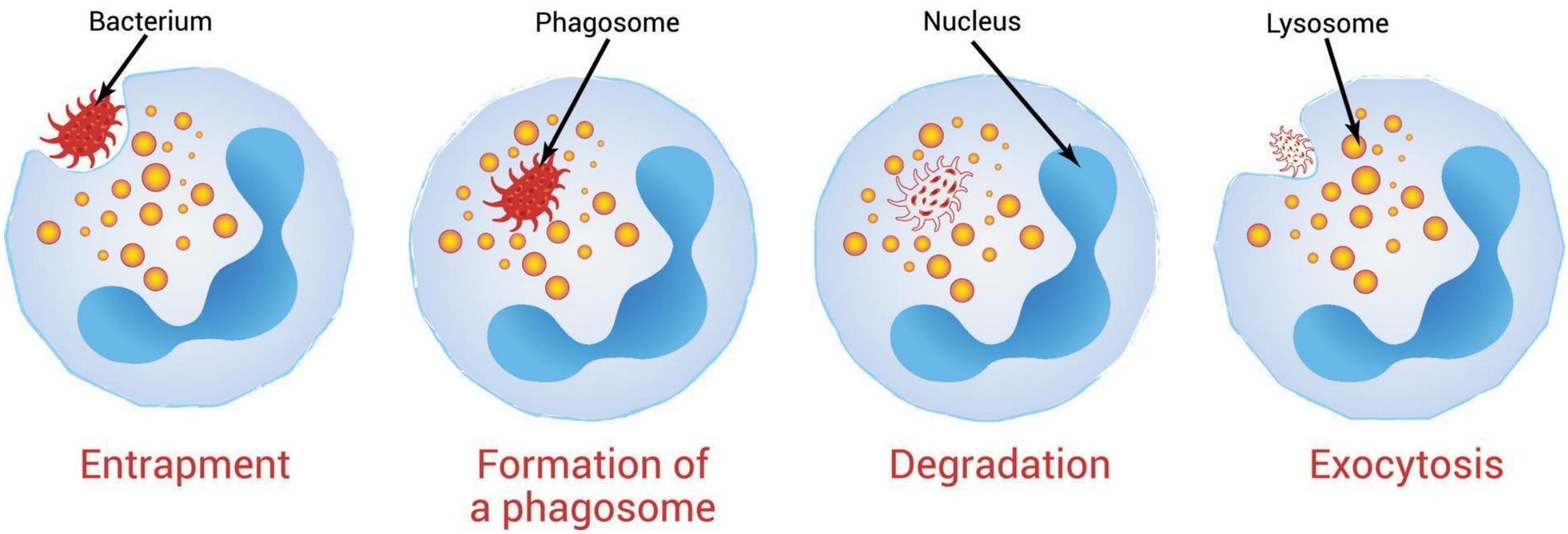


Dendritic cell

Three main Functions:

- **Phagocytosis**-Seek and eat target
- **Chemical assault**- Kill microbes with cytotoxic molecules
- **Present antigen** to Adaptive immune cells

Phagocytosis (Cell-eating)



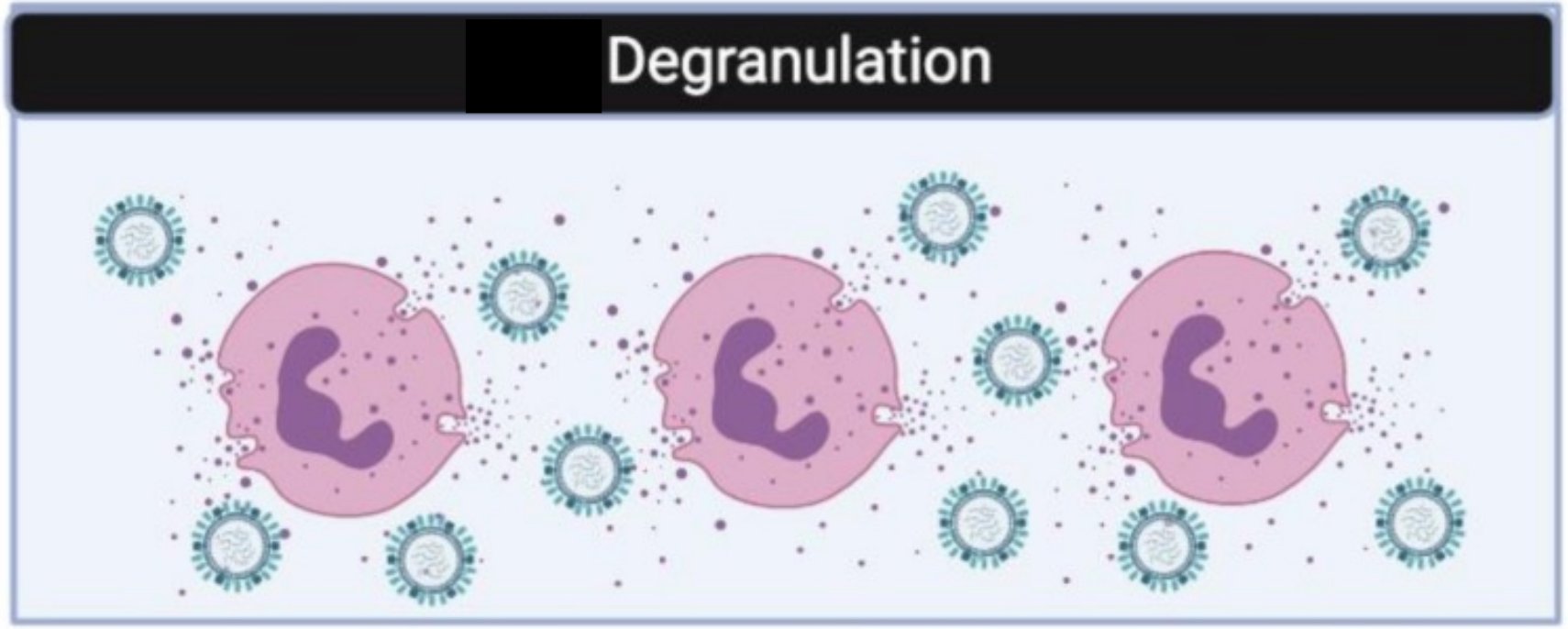
Seek and eat the invading microbe/antigen

Degranulation- Chemical Assault

Granulocytes:

- Neutrophils, Basophils,Eosinophils, mast cells

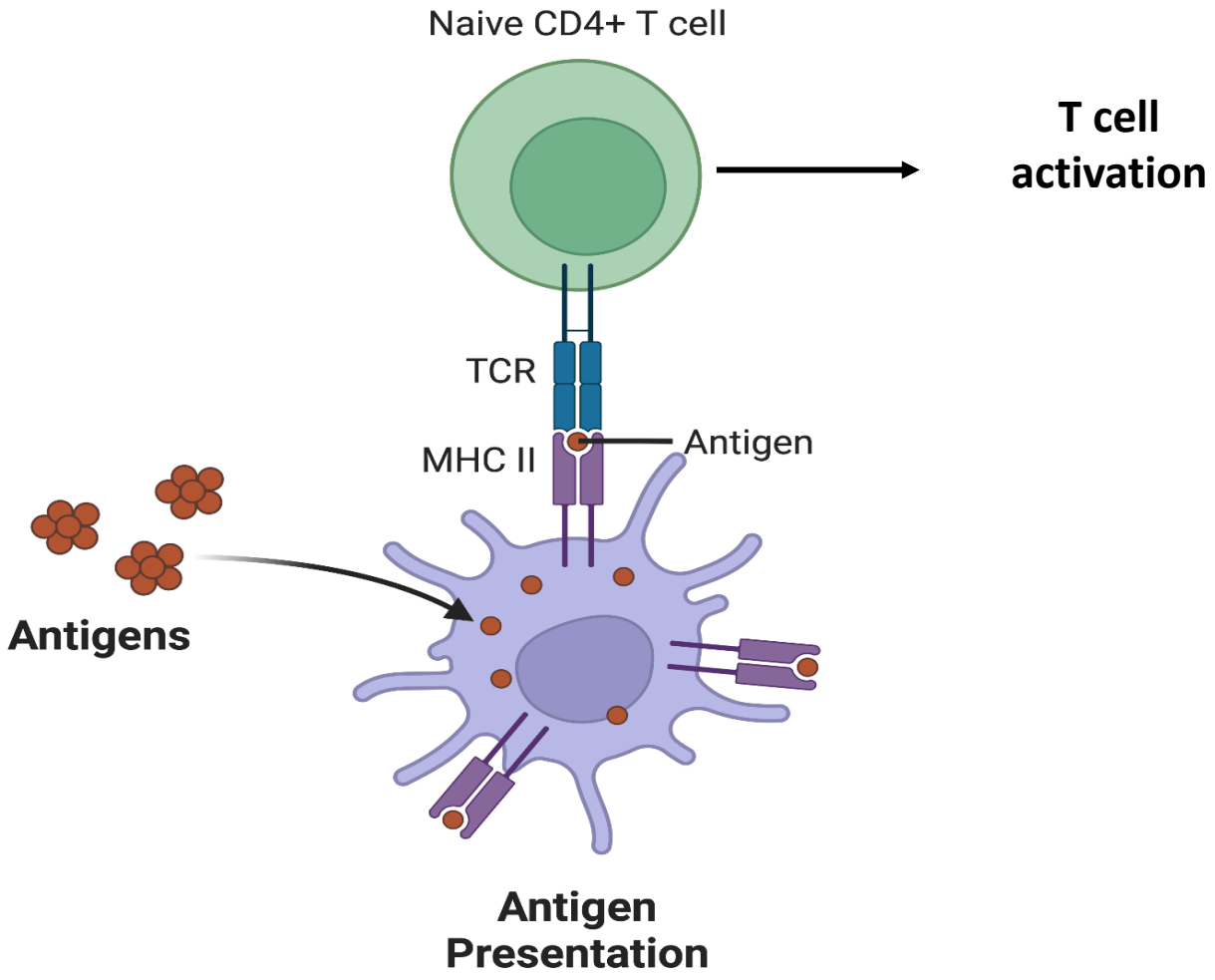
- granules filled with chemicals e.g. ROS, heparin, histamine



(Sneha et al, 2021)

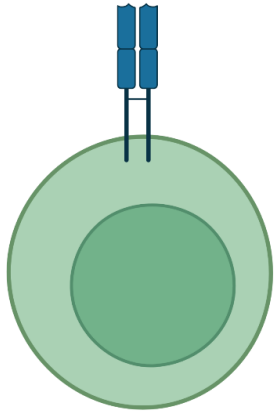
Innate Immune cells-Antigen Presentation

Antigen presentation links Innate Immunity to Adaptive Immunity

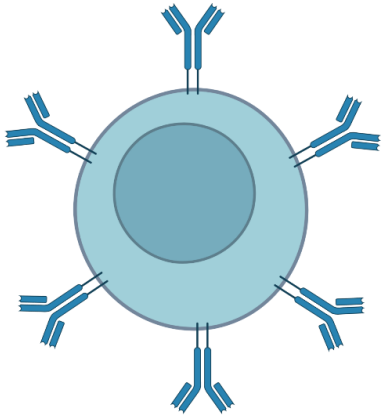


Adaptive Immunity

- **Characteristics:**
  - Specific recognition of antigen
  - Memory functions

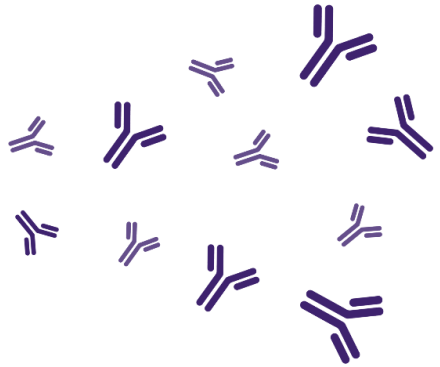


T cell



B cell

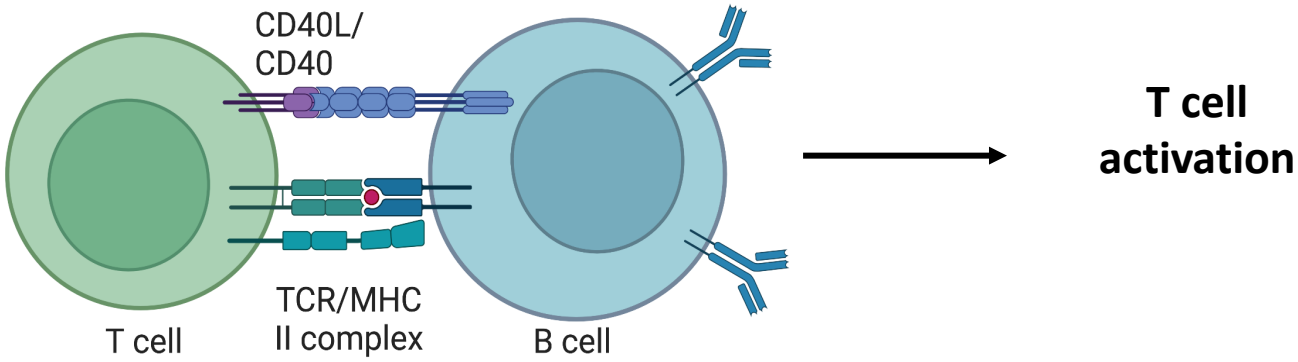
- **Components:**
  - **Cells:**
    - T and B cells
  - **Proteins:**
    - Immunoglobulins (aka antibodies)

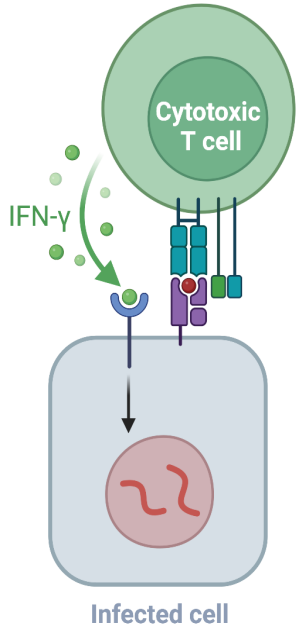


Immunoglobulins

Adaptive Immune Cells

- **T cells:**
  - **CD4+ T cells:**
    - T-helper (Th) cells
    - Regulatory T (T-reg cells)
  - **CD8+ T cells:**
    - Cytotoxic T cells



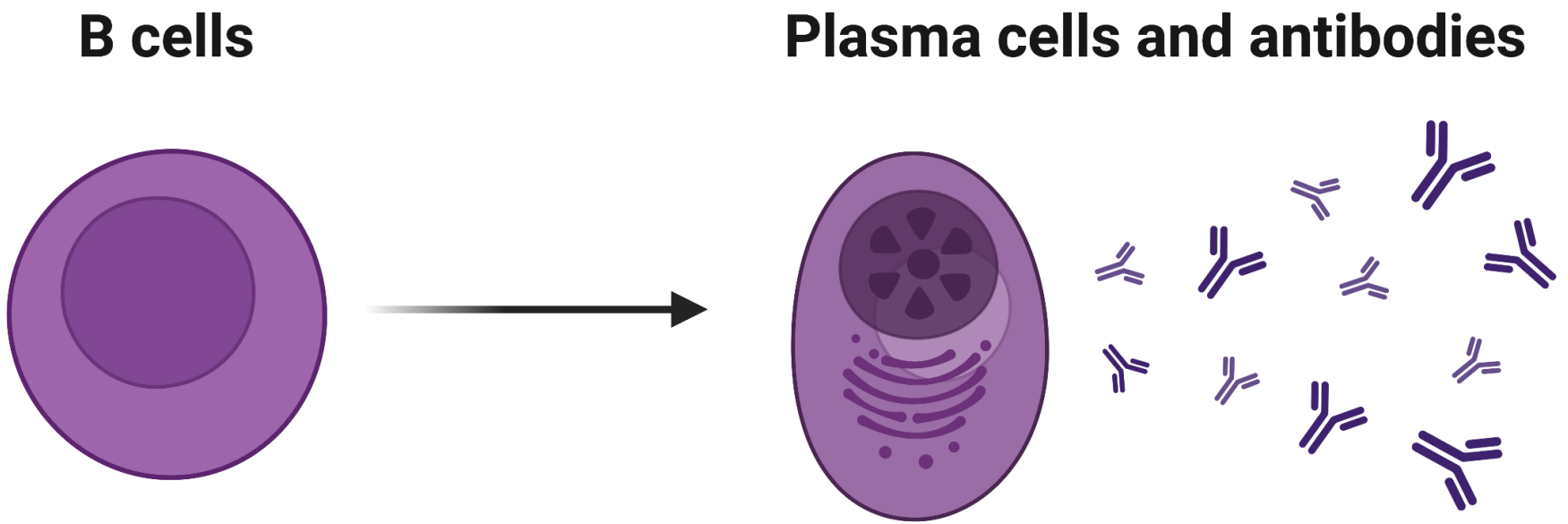


Cytotoxic T cell induce cell-death

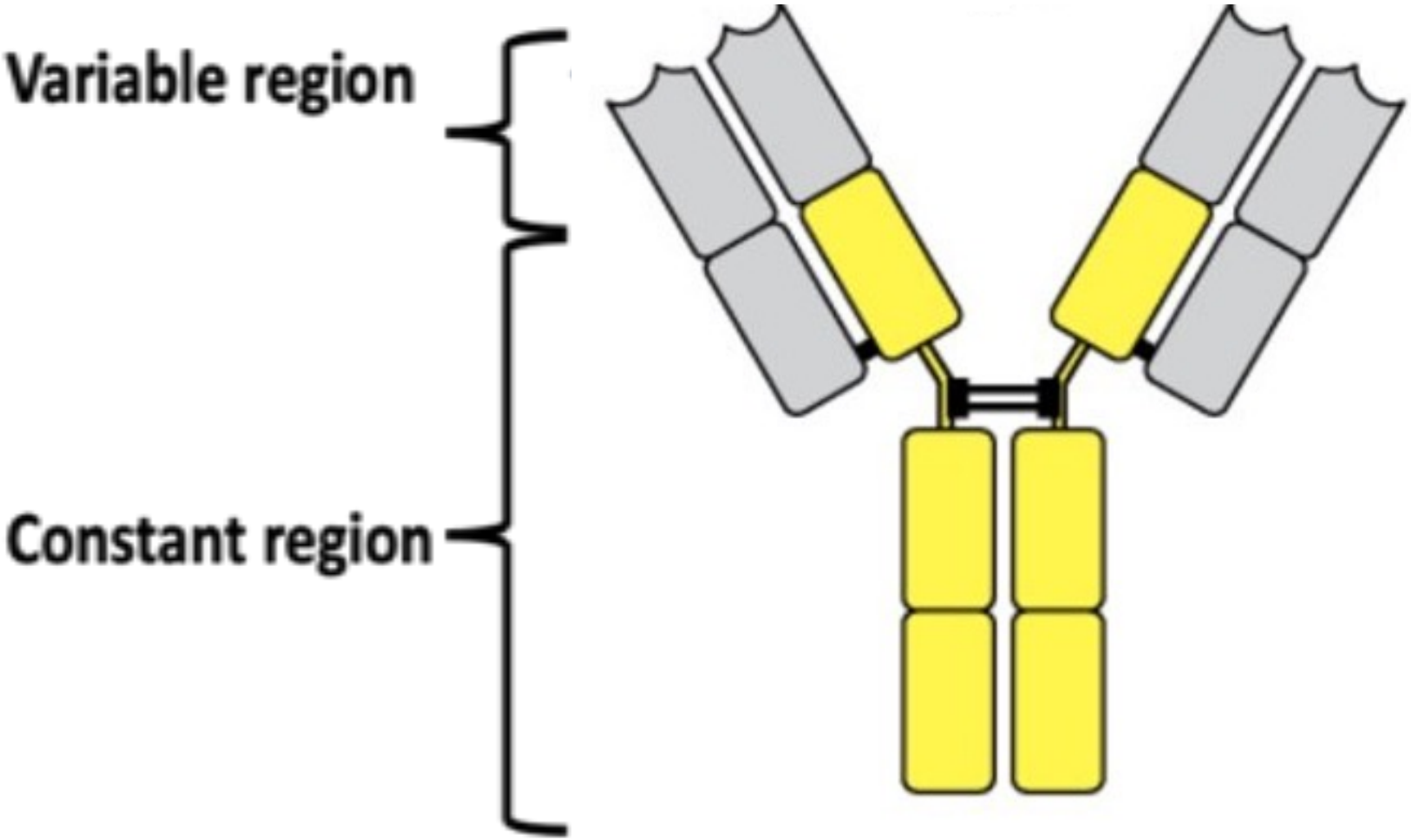
B cells

<u>General Functions</u>:

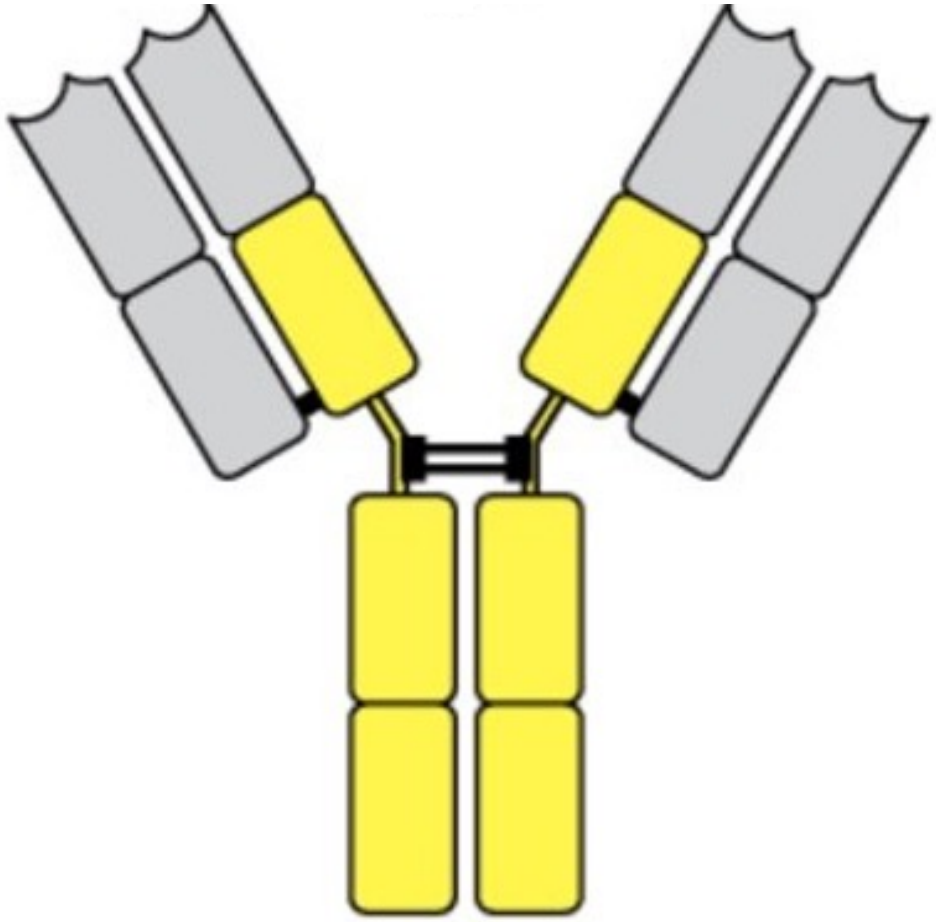
- Produce Immunoglobulins/ antibodies

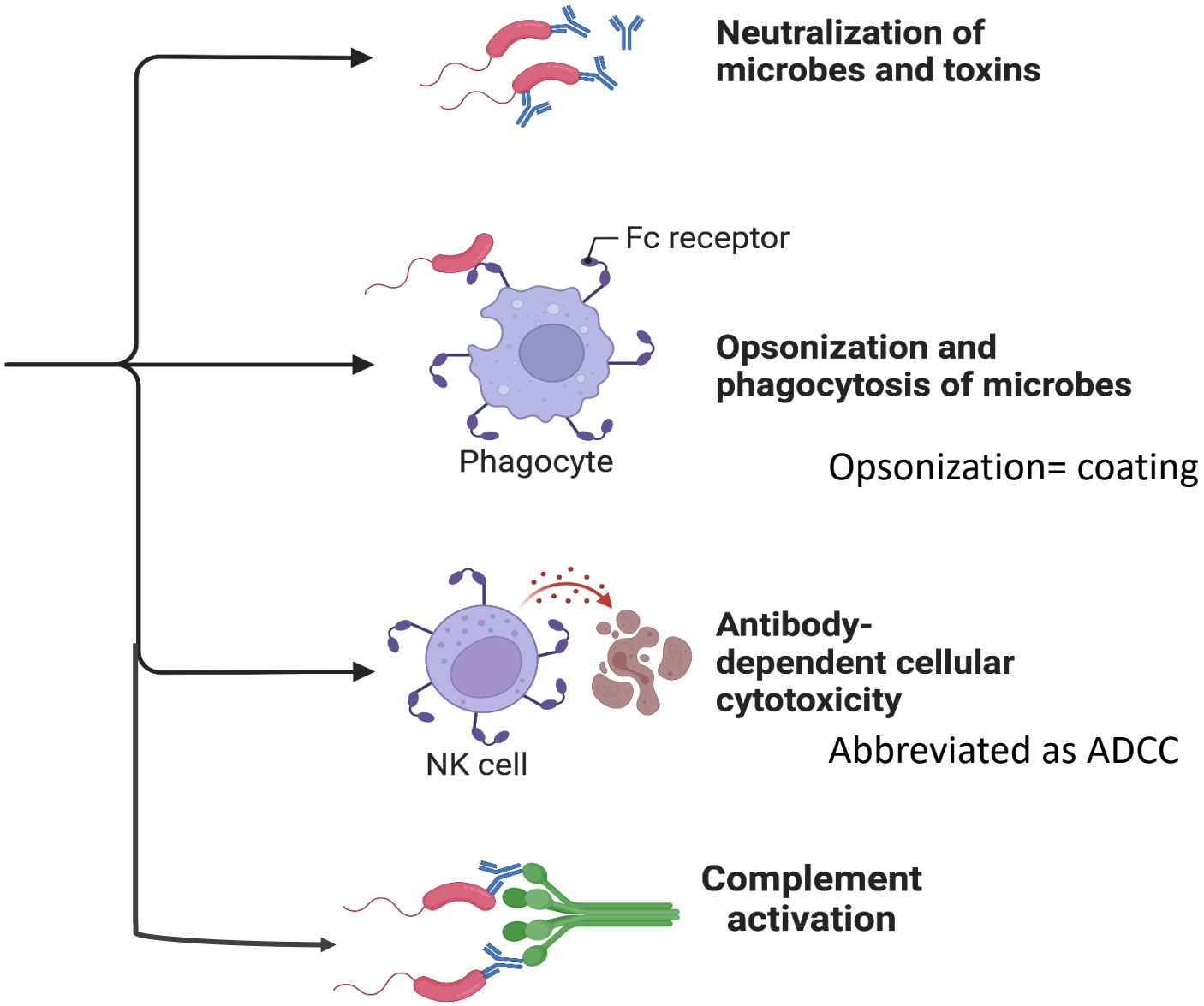


Immunoglobulin Structure



Immunoglobulin Functions





Functional activity	IgD	IgE	IgM	IgA	IgG1
Neutralization	---	---	+	++	++
Opsonization	---	---	+	+	+++
Sensitization for killing by NK cells	---	---	---	---	++
Sensitization of mast cells	---	+++	---	---	+
Activates complement system	---	---	+++	+	++

- Constant region determines isotypes
- Constant region=function

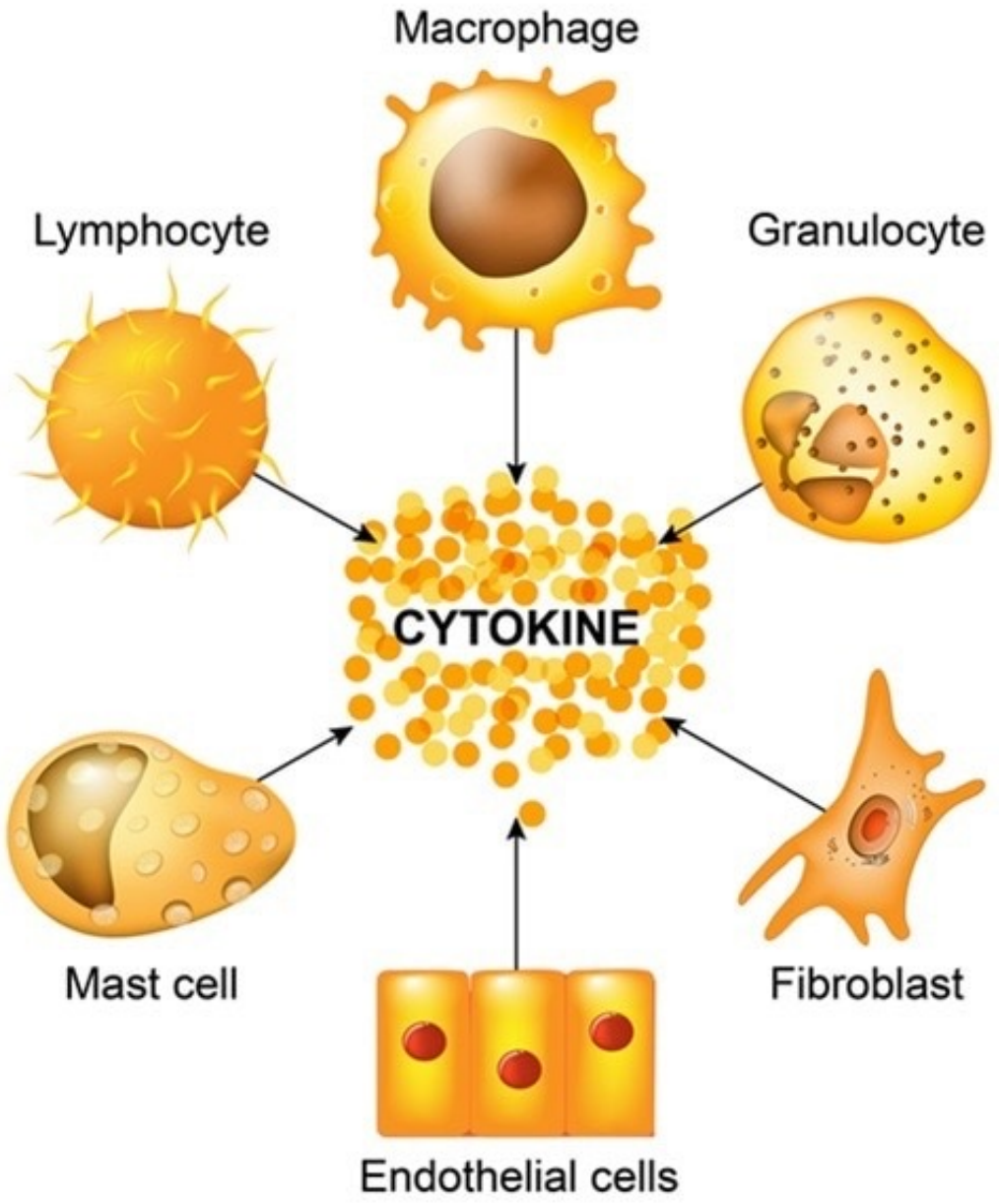
---	Does not apply
+	Applies
++	Strongly applies
+++	Heavily applies

Cytokines

Functions of cytokines:

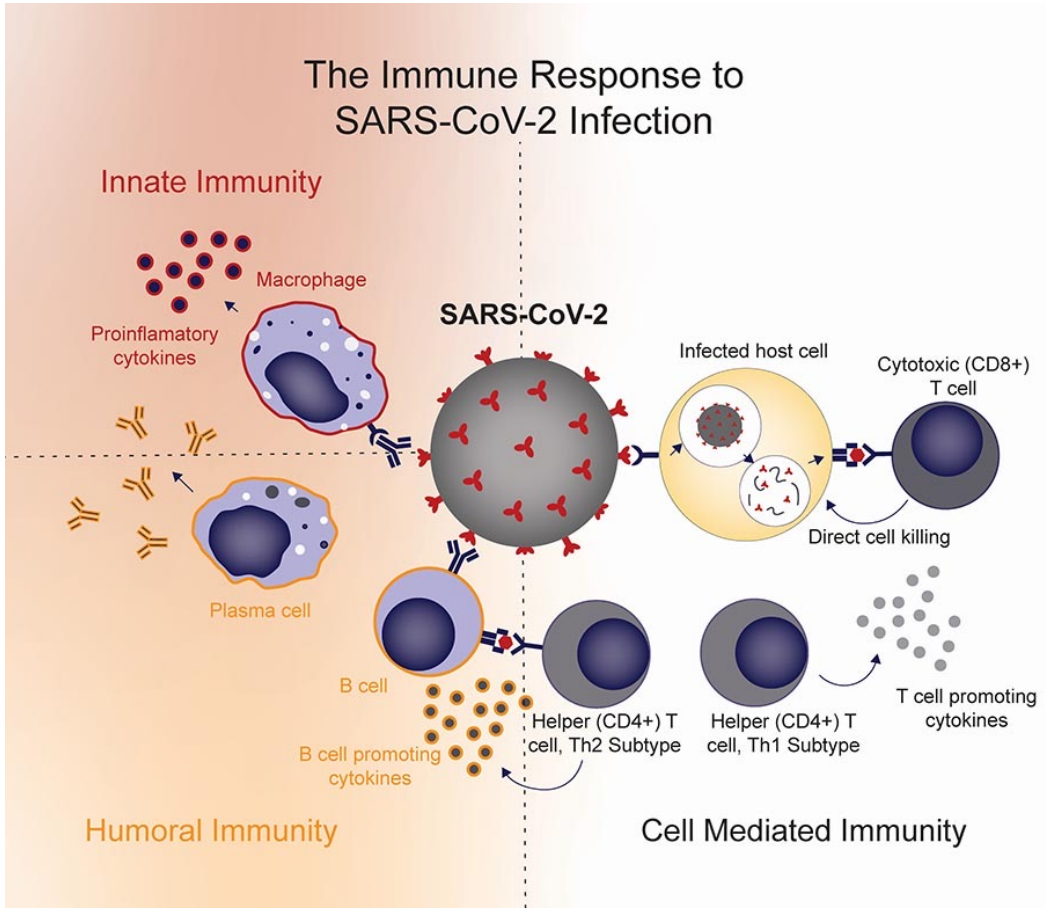
- Mediate cell-cell communication
  - Differentiation
  - Migration

- Example of cytokines:
  - IFNs



The immune system involved coordination of difference components

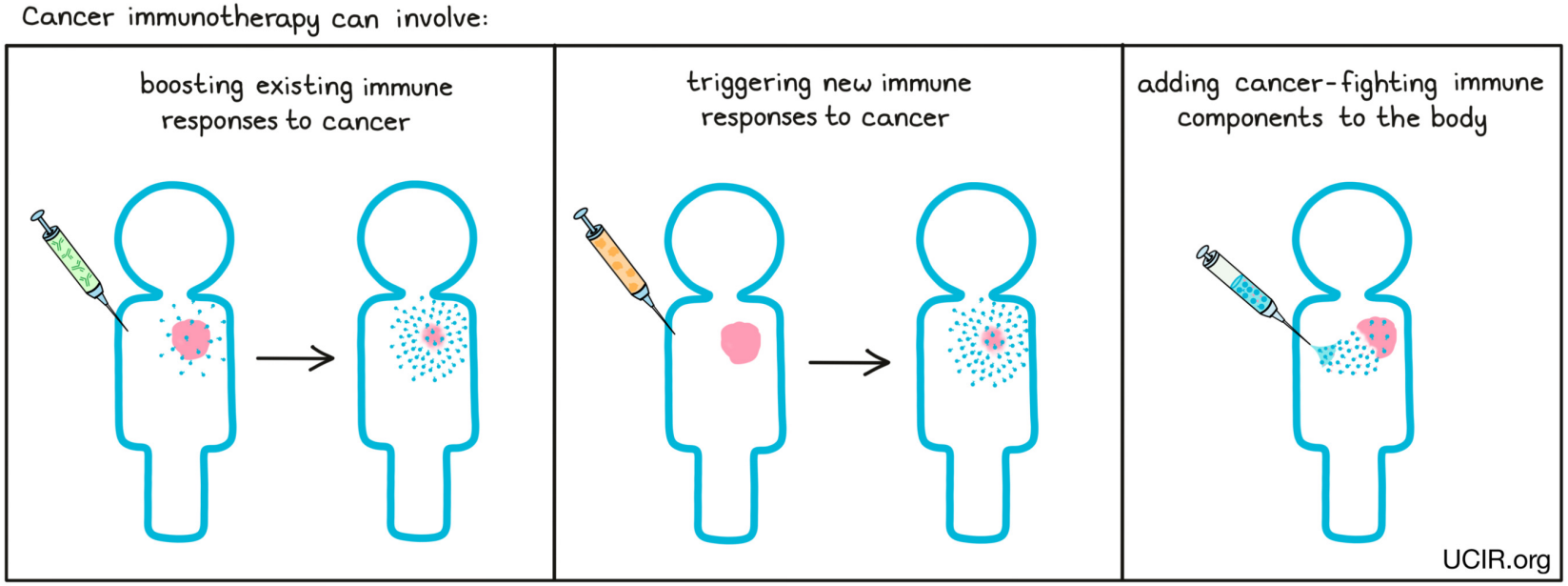
- Basically, immunologists either study how each of these components work alone or together



Applications of Immunology

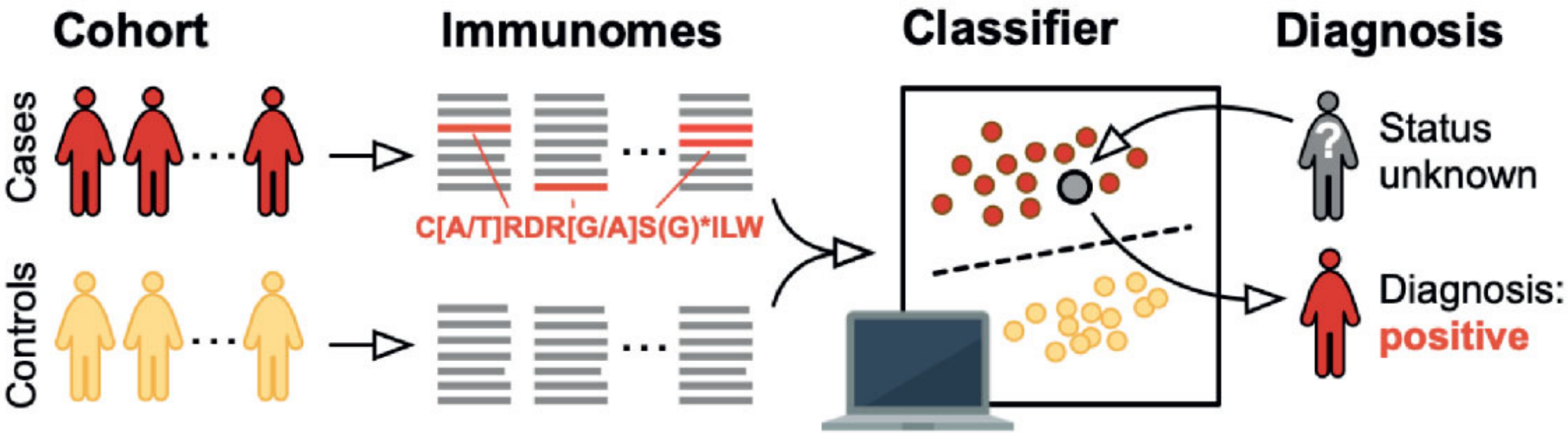
Vaccine

Therapeutics



Applications of Immunology

Diagnosis

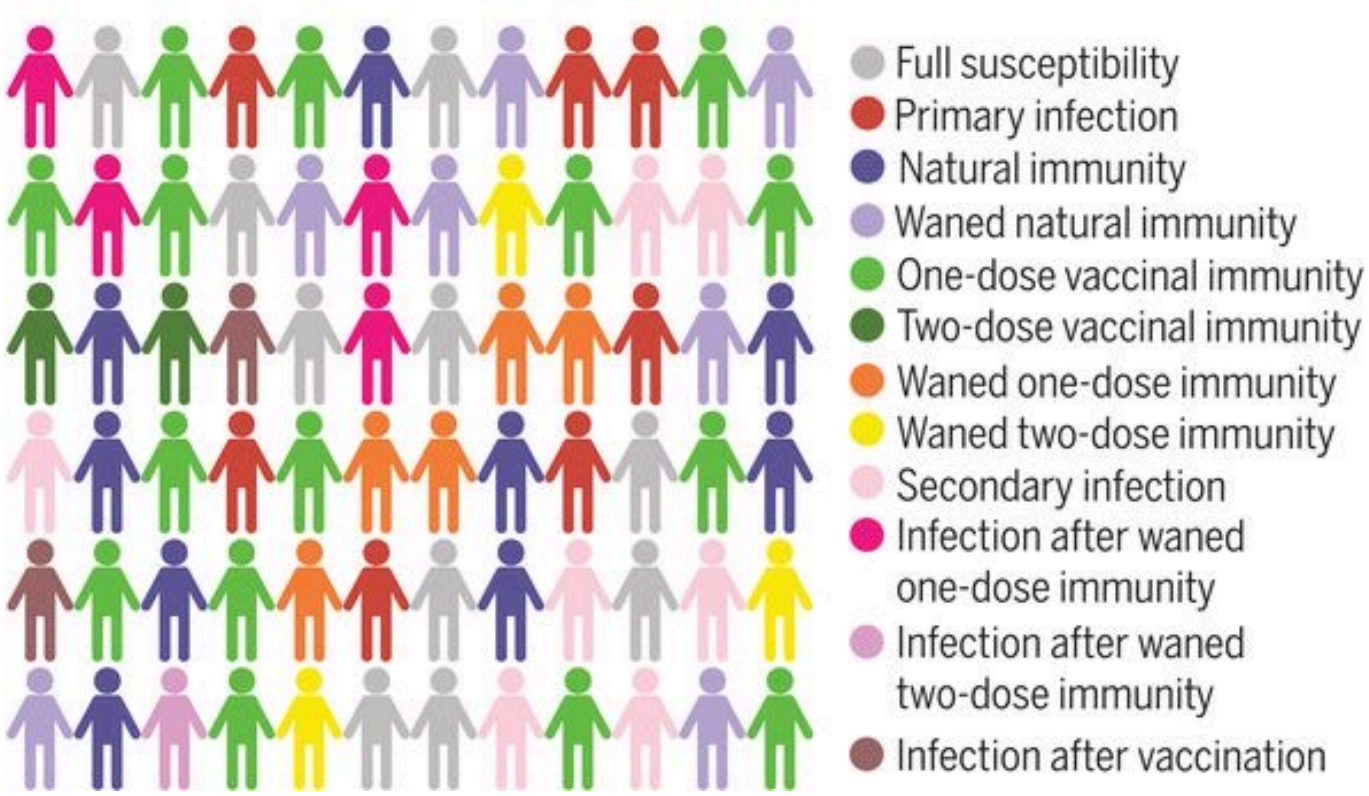


Identification of specific immune markers relevant for particular disease

Applications of Immunology

Epidemiology& Population health

Compartmental immuno-epidemiological model with natural and vaccinal immune waning

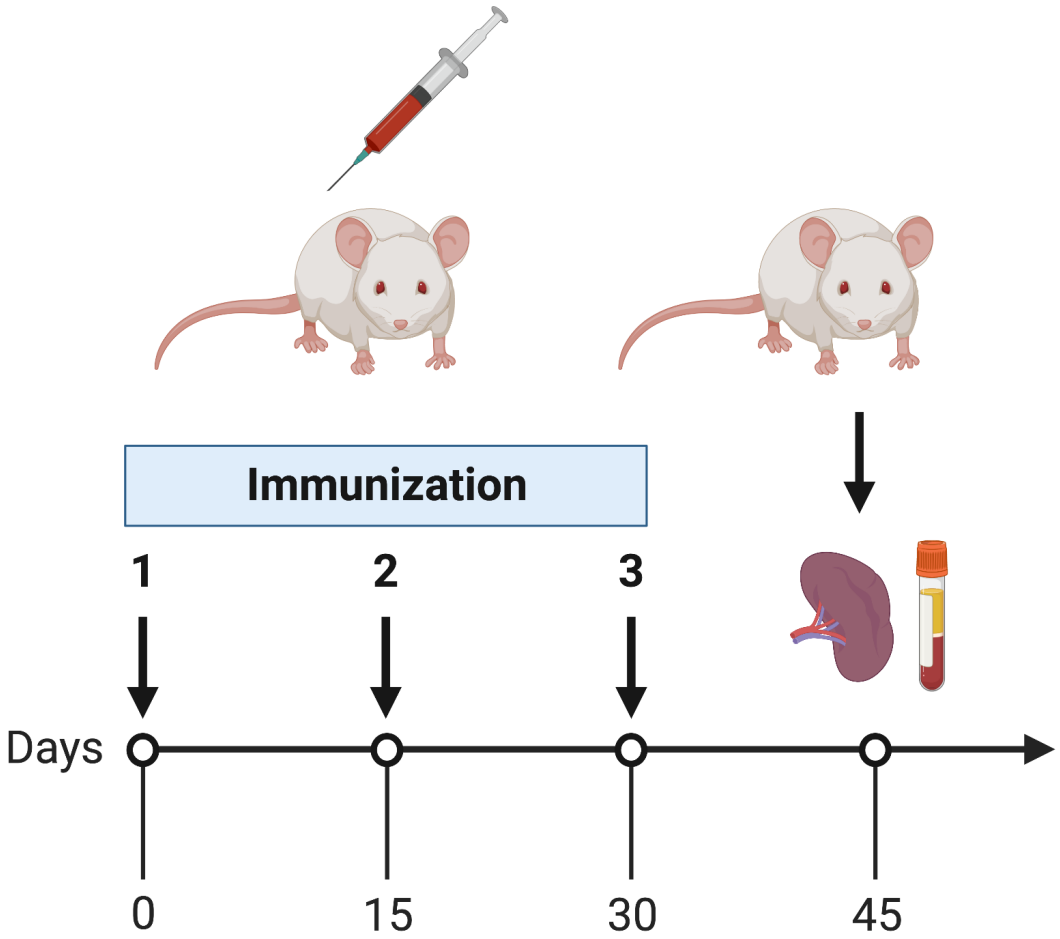


(Arnaout et al, 2021)

Common Laboratory Techniques Utilized in Immunological Research

- Induction of Immune response
- Enzyme- linked immunosorbent Assay (ELISA)
- Flow Cytometry

Induce Immune response in mice



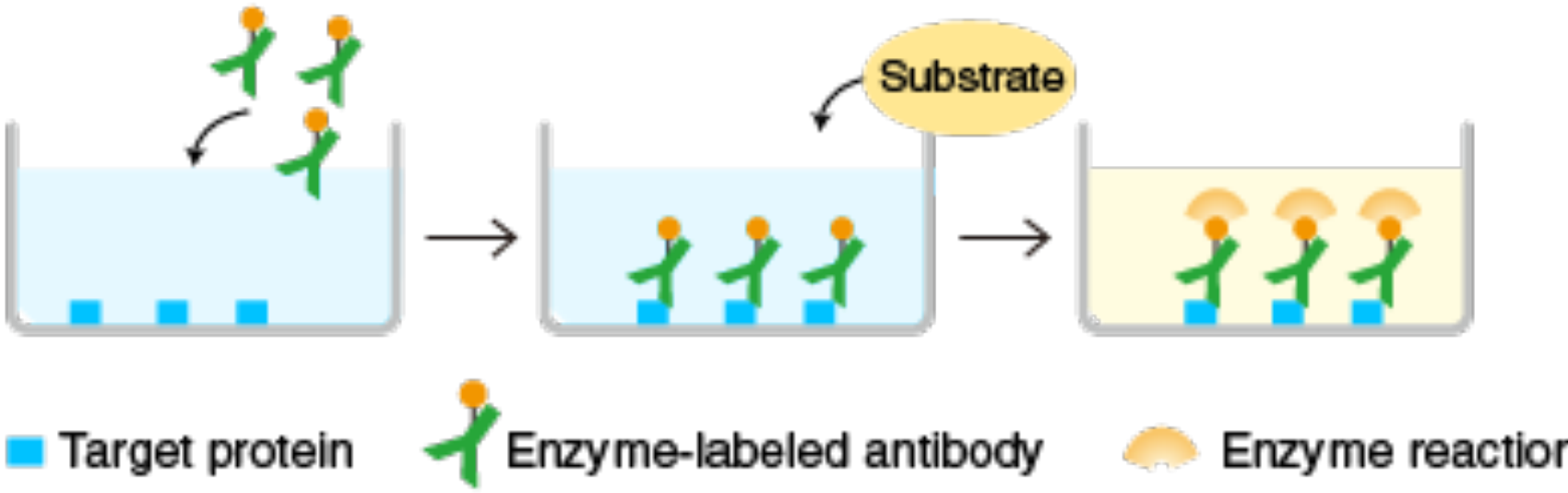
- **Immunization:** with specific antigen e.g. LPS or Chicken gamma globulin
- **Challenge:** with pathogen e.g. Influenza A
- **In the field:** samples from animal with sign of disease vs healthy

ELISA

ELISA= **E**nzyme-**L**inked **I**mmunosorbent-**A**ssay

Applications:

- Usually use blood as a sample
- Measure quantity of specific proteins
- Snapshot of specific point in time



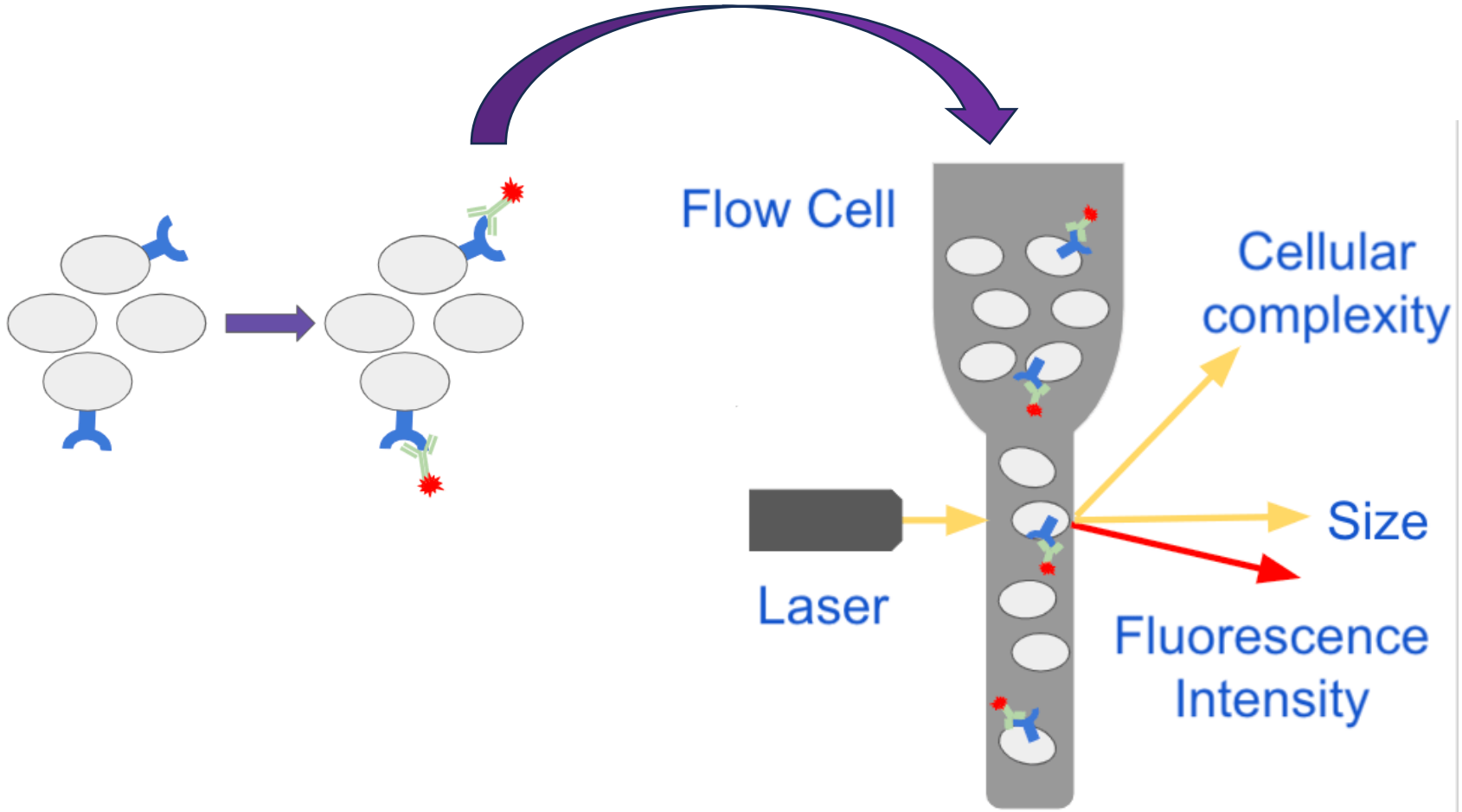
Mblbio.com

Flow cytometry

Flow cytometry-
Fluid + cells

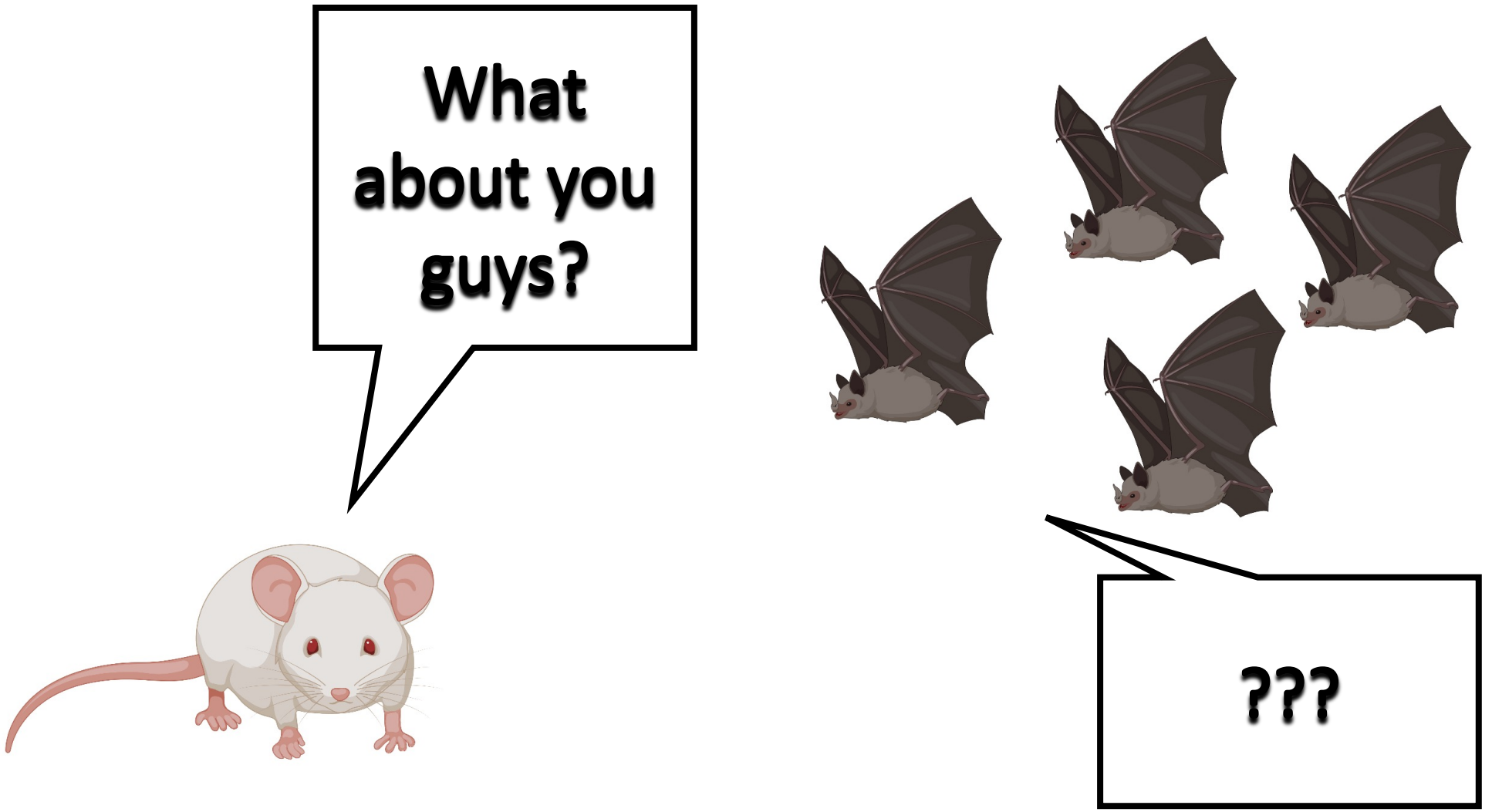
Applications:

- Detect and quantify specific cell population based on surface marker
- Also allow for separation of specific cell populations



Blog.addgene.org

Yeah, but what about bats?



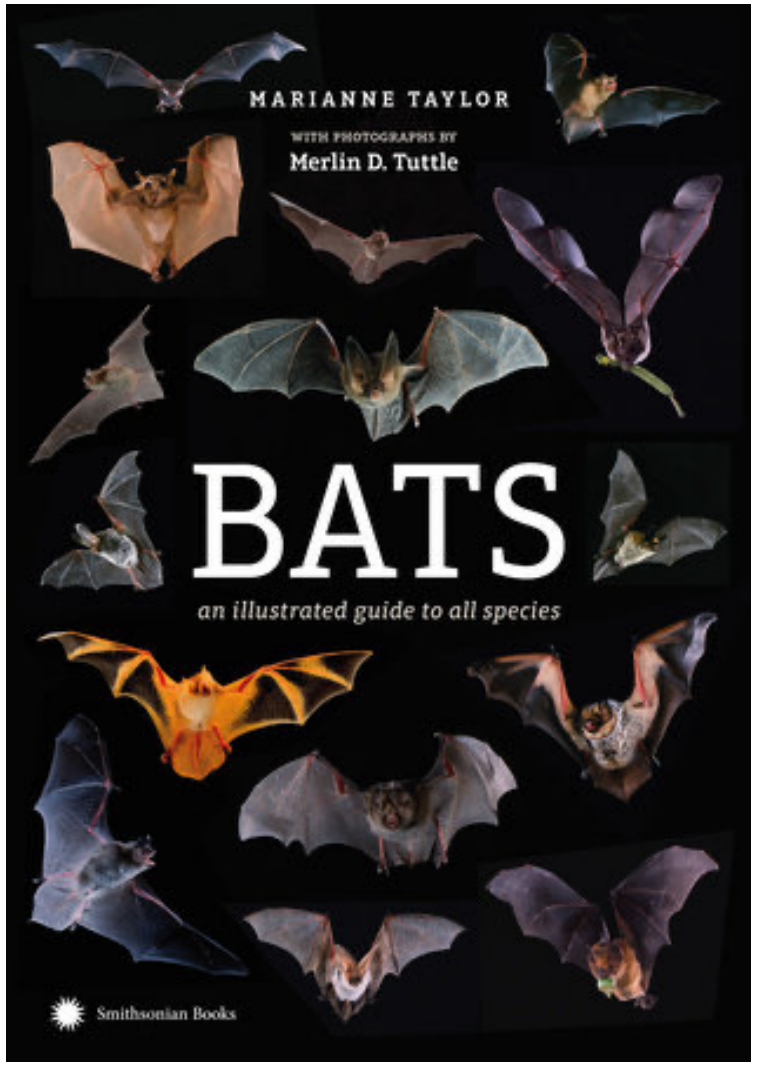
Bat Immunology

- Why do we want to know about bat immune system?
- What do we know about bat immunology?
- Challenges in studying bats

Why bat immune system?

Bats as unique taxa:

- High diversity and distribution
- Unique physiology (flight)
- Some bats are associated with zoonosis



Bat Immunity

- **Innate Immunity:**
  - Positive selection for antiviral PRRs e.g. TLR8, RIG-I
- **Adaptive Immunity:**
  - They have T and B cells- but we don’t know if they function the same as mice
  - Lower Immunoglobulins in response to virus challenge - but we still not sure how they function in bat
- **Other Immune mediators:**
  - Type I IFNs in response to poly I:C stimulation- a type of cytokine (protein) that coordinate with other immune cells to mount an antiviral response

(Banerjee et al ,2020)

Challenges in studying bat immunology

- Technical Feasibility in the field
- Lack of Baseline information
- Lack of other resources

Technical Feasibility in the field

- Certain species are endangered
- Sample collection/ preservation
- Access to proper facility to study immunology

Lack of baseline information

- Not a model organism
- What is a healthy bat?
- Accurate taxonomy?
- Genome sequences and annotation

BAT 1K

Lack of tools for Immunological Studies

- Not a model organism
- Antibody (preferably monoclonal antibodies) required for technique such as ELISA or Flow cytometry
- Difficult/ expensive to keep bats captive- low reproductive rate/ long generation time relative to mice

Bat Immunoglobulin Mouse anti-Bat, Alexa Fluor™ 750, Clone: BT1-4F10, Novus Biologicals™

Antigen	Bat Immunoglobulin
Classification	Monoclonal
Conjugate	Alexa Fluor 750
Formulation	50 mM sodium borate with 0.05% sodium azide
Immunogen	Microchiropteran bat (Eptesicus fuscus) spleen cells.

How GBatNet helps?

Immunology working group:

- How has the bat immune system evolved and influenced bat diversification?

Measuring stress in bat working group:

- What is healthy bat? What is stressed bat?

BOHRN

BAT 1K

<u>Objective in common:</u> Share information, generate standardized methods/ practice to study bat health

Thank you so much for your attention!
I am now ready for questions

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