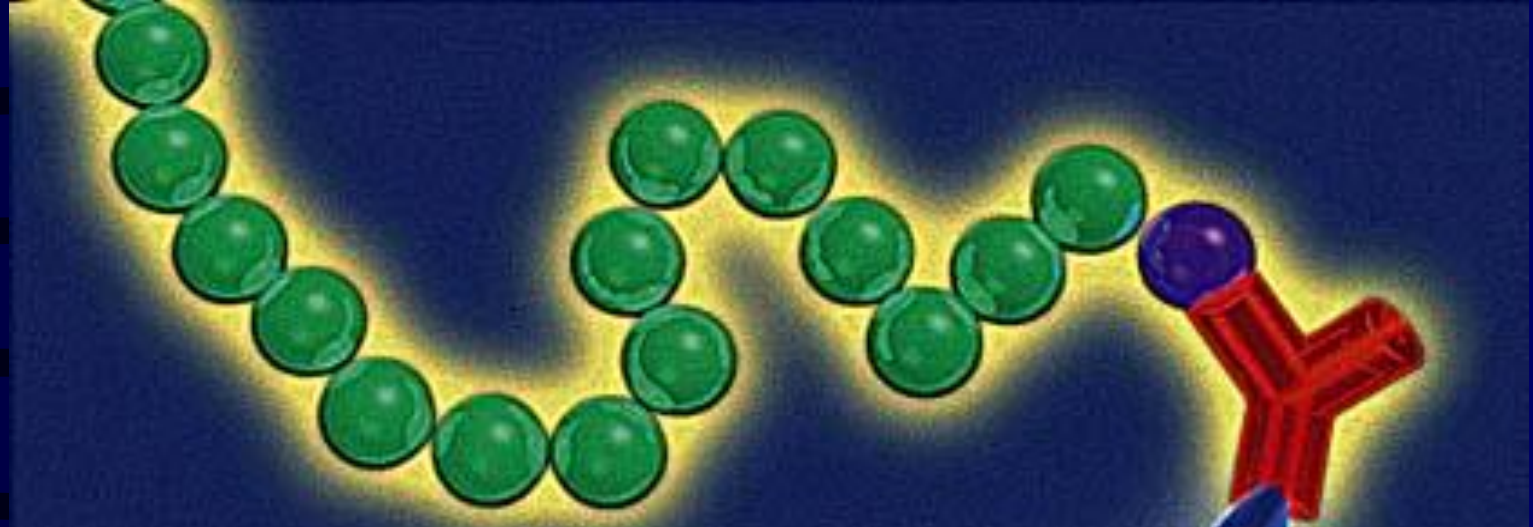


# I. Overview: - The Immune System

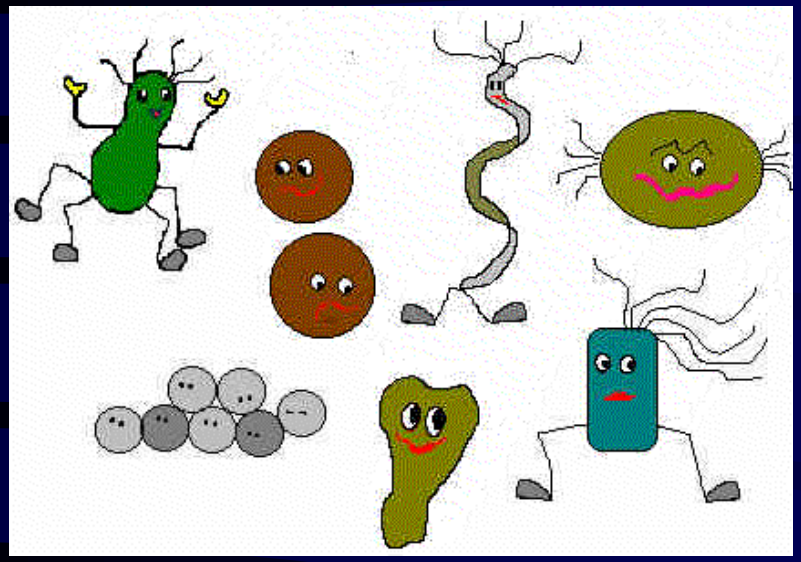
# II. Introduction: - Monoclonal Antibodies

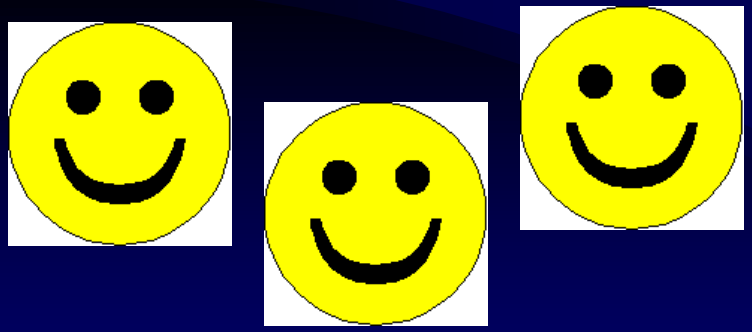
Fatchiyah, JB UB

Source From Medical Information Services
Dr Rucha Ponkshe
Senior Manager - Medical Information
Ms Anahita Gouri
Executive – Medical Information

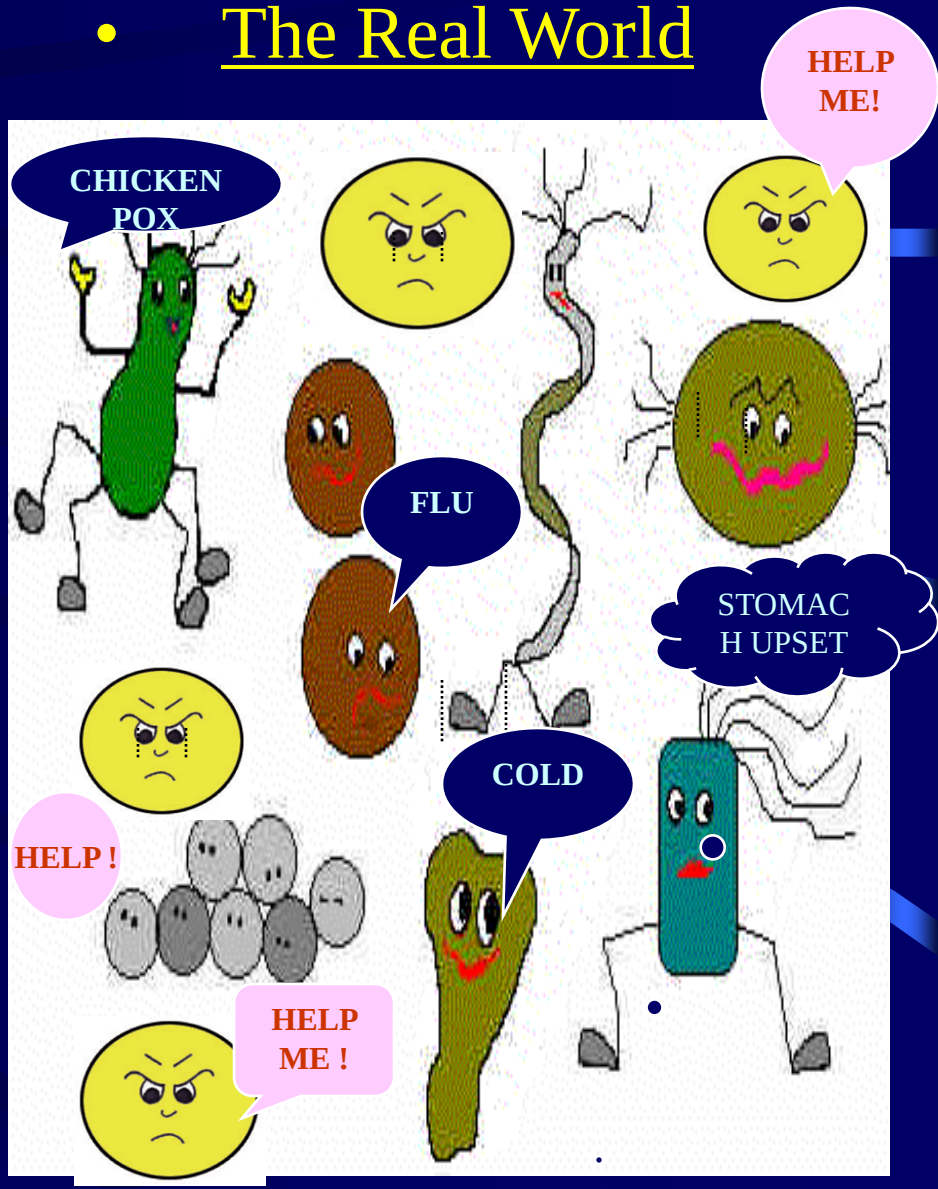
- Goal : - To develop a basic foundation of how the immune defenses operate in order to understand the science behind Monoclonal Antibodies
- Problem: - Too much information to be compressed
- Strategy : - To focus on the big Picture – Not the details
- What we have done to help ?: -
- Monoclonal Antibody Folder on I colon –
  * FDA Documents
  * List of mAB with International PI
  * Exclusive database on Abciximab and Infliximab
  * Presentation with key concepts identified.

- The Perfect World





- The Real World



# THE IMMUNE SYSTEM

**The Latin term *"IMMUNIS"* means EXEMPT, referring to protection against foreign agents.**

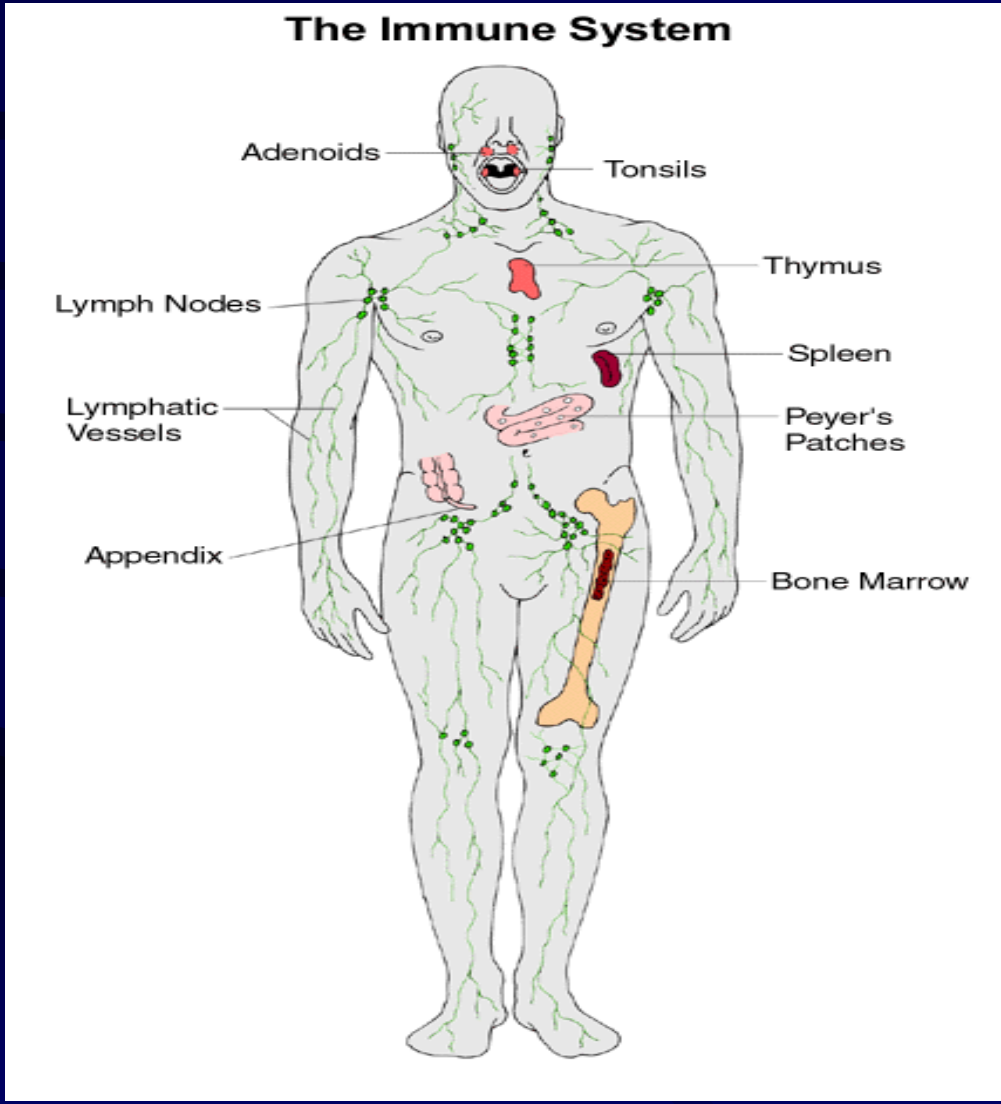
**DEFINITION: - The integrated body system of organs, tissues, cells & cell products that differentiates self from non – self & neutralizes potentially pathogenic organisms.**

***(The American Heritage Stedman's Medical Dictionary)***

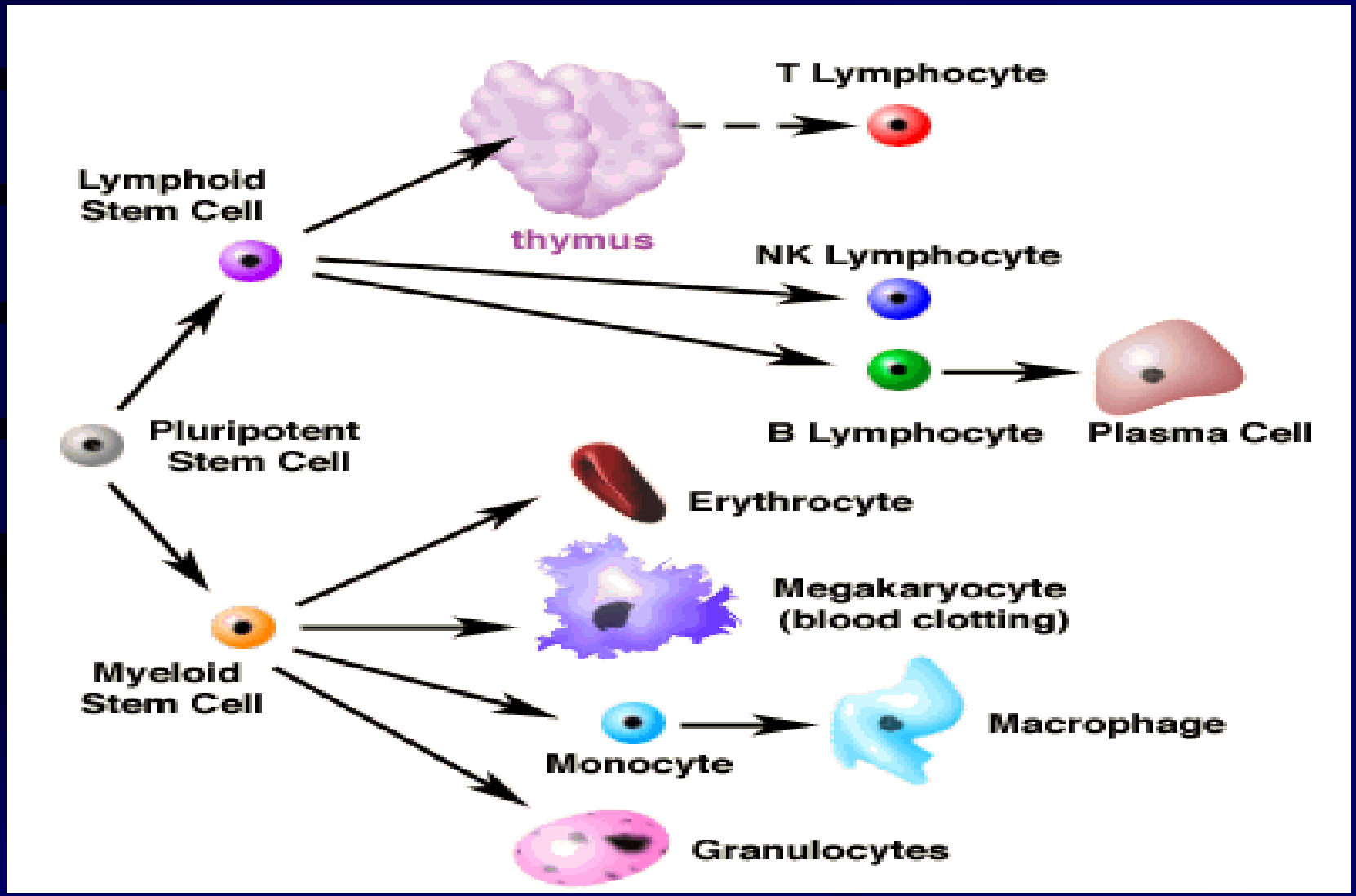
**The Immune System consists of**

1. **Innate Immunity** — **Primary Response**
2. **Acquired Immunity** — **Secondary Response**

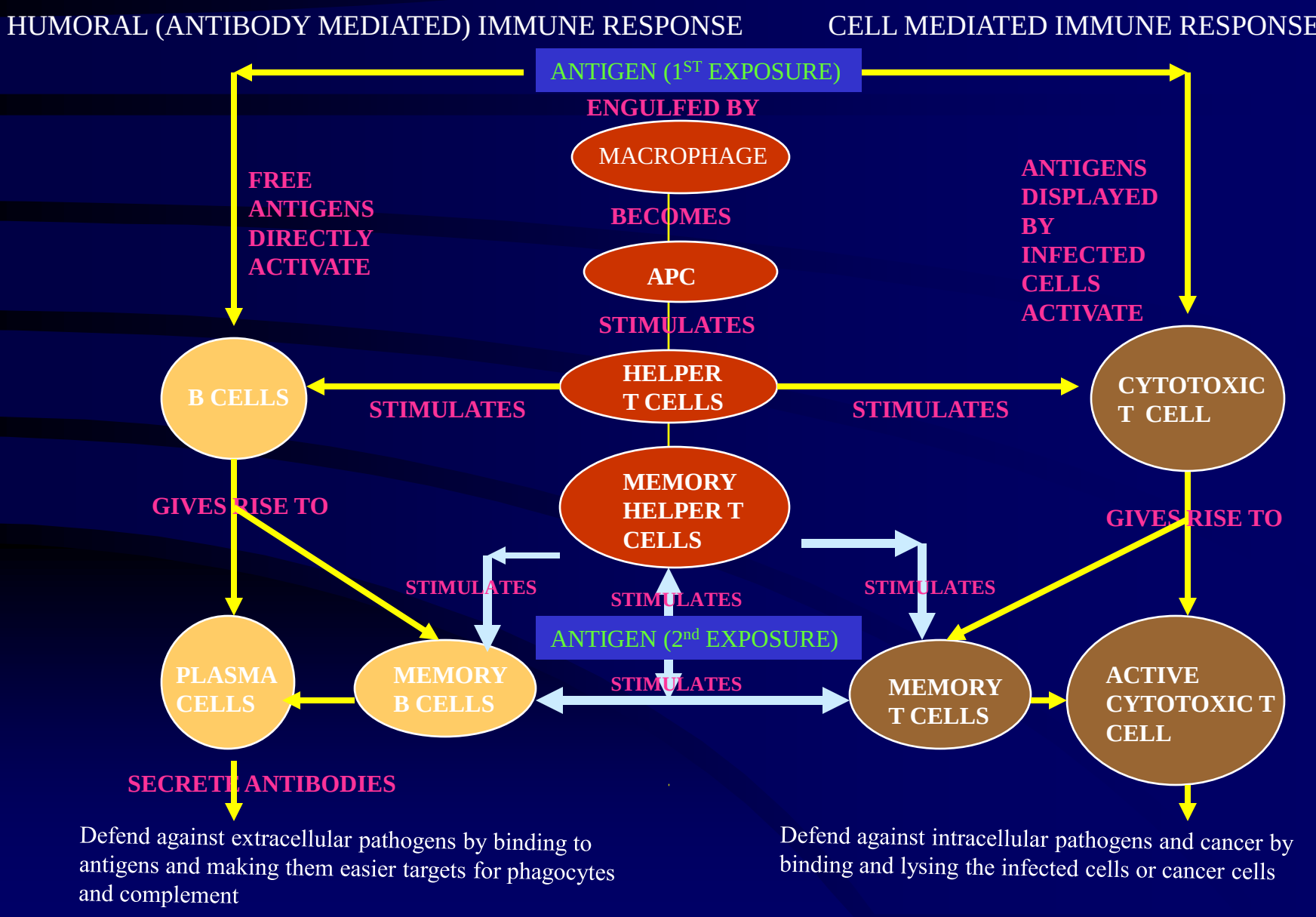
# ANATOMY OF THE IMMUNE SYSTEM



# CELLS OF THE IMMUNE SYSTEM



# FUNCTIONING OF THE IMMUNE SYSTEM



# IMMUNOTHERAPY

**Treatment of the disease by Inducing, Enhancing or Suppressing the Immune System.**

**Active Immunotherapy: -**

**It stimulates the body's own immune system to fight the disease.**

**Passive Immunotherapy: -**

**It does not rely on the body to attack the disease, instead they use the immune system components ( such as antibodies) created outside the body.**

# HISTORY OF IMMUNOLOGY

•**1798 Edward Jenner, Smallpox vaccination**

•1862 Ernst Haeckel, Recognition of phagocytosis

•1877 Paul Erlich, recognition of mast cells

•1879 Louis Pasteur, Attenuated chicken cholera vaccine development

•1883 Elie Metchnikoff Cellular theory of vaccination

•1885 Louis Pasteur, Rabies vaccination development

•1888 Pierre Roux & Alexandre Yersin, Bacterial toxins

•1888 George Nuttall, Bactericidal action of blood

•1891 Robert Koch, Delayed type hypersensitivity

•1894 Richard Pfeiffer, Bacteriolysis

•1895 Jules Bordet, Complement and antibody activity in bacteriolysis

•1900 Paul Erlich, Antibody formation theory

•1901 Karl Landsteiner, A, B and O blood groupings

•1901-8 Carl Jensen & Leo Loeb, Transplantable tumors

•1902 Paul Portier & Charles Richet, Anaphylaxis

•1903 Almroth Wright & Stewart Douglas, Opsonization reactions

•1906 Clemens von Pirquet, coined the word allergy

•1907 Svante Arrhenius, coined the term immunochemistry

•1910 Emil von Dungern, & Ludwik Hirszfeld, Inheritance of ABO blood groups

•1910 Peyton Rous, Viral immunology theory

•1914 Clarence Little, Genetics theory of tumor transplantation

•1915-20 Leonell Strong & Clarence Little, Inbred mouse strains

•1917 Karl Landsteiner, Haptens

•1921 Carl Prausnitz & Heinz Kustner, Cutaneous reactions

•1924 L Aschoff, Reticuloendothelial system

•1926 Lloyd Felton & GH Bailey, Isolation of pure antibody preparation

•1934-8 John Marrack, Antigen-antibody binding hypothesis

•1936 Peter Gorer, Identification of the H-2 antigen in mice

•1940 Karl Lansteiner & Alexander Weiner, Identification of the Rh antigens

•1941 Albert Coons, Immunofluorescence technique

•1942 Jules Freund & Katherine McDermott, Adjuvants

•1942 Karl Landsteiner & Merrill Chase, Cellular transfer of sensitivity in guinea pigs (anaphylaxis)

•1944 Peter Medwar, Immunological hypothesis of allograft rejection

•1948 Astrid Fagraeus, Demonstration of antibody production in plasma B cells

•1948 George Snell, Congenic mouse lines

•1949 Macfarlane Burnet & Frank Fenner, Immunological tolerance hypothesis

•1950 Richard Gershon and K Kondo, Discovery of suppressor T cells

•1952 Ogden Bruton, discovery of agammaglobulinemia (antibody immunodeficiency)

•1953 Morton Simonsen and WJ Dempster, Graft versus-host reaction

•1953 James Riley & Geoffrey West, Discovery of histamine in mast cells

•1953 Rupert Billingham, Leslie Brent, Peter Medwar, & Miriam Hasek, Immunological tolerance hypothesis

•1955 Niels Jerne, David Talmage, Macfarlane Burnet, Clonal selection theory

•1957 Ernest Witebsky et al., Induction of autoimmunity in animals

•1957 Alick Isaacs & Jean Lindemann, Discovery of interferon (cytokine)

•1958-62 Jean Dausset et al., Human leukocyte antigens

•1959-62 Rodney Porter et al., Discovery of antibody structure

•1959 James Gowans, Lymphocyte circulation

•1961-62 Jaques Miller et al., Discovery of thymus involvement in cellular immunity

•1961-62 Noel Warner et al., Distinction of cellular and humoral immune responses

•1963 Jaques Oudin et al., antibody idiotypes

•1964-8 Anthony Davis et al., T and B cell cooperation in immune response

•1965 Thomas Tomasi et al., Secretory immunoglobulin antibodies

•1967 Kimishige Ishizaka et al., Identification of IgE as the reaginic antibody

•1971 Donald Bailey, Recombinent inbred mouse strains

•1974 Rolf Zinkernagel & Peter Doherty, MHC restriction

•**1975 Kohler and Milstein, Monoclonal antibodies used in genetic analysis**

•1984 Robert Good, Failed treatment of severe combined immunodeficiency (SCID, David the bubble boy) by bone marrow grafting. 1985 Tonegawa, Hood et al., Identification of immunoglobulin gene

•1985- Leroy Hood et al., Identification of genes for the T cell receptor

•1990 Yamamoto et al., Molecular differences between the genes for blood groups O and A and between those for A and B

•1990 NIH team, Gene therapy for SCID using cultured T cells.

•1993 NIH team, Treatment of SCID using genetically altered umbilical cord cells.

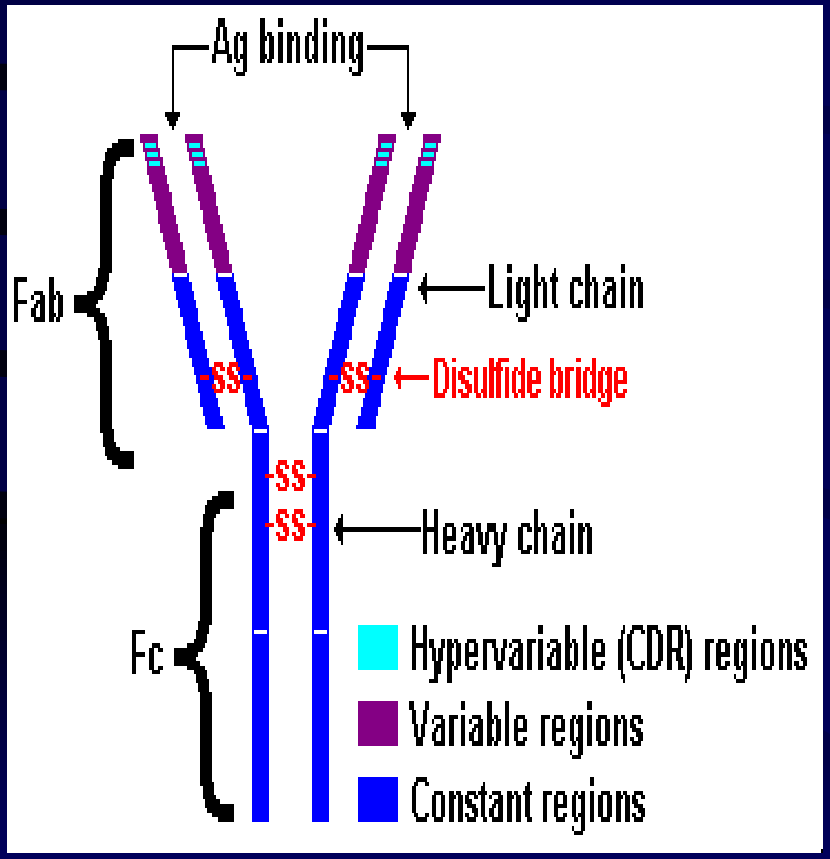
•1985-onwards Rapid identification of genes for immune cells, antibodies, cytokines and other immunological structures.

•1798 Edward Jenner, Smallpox vaccination

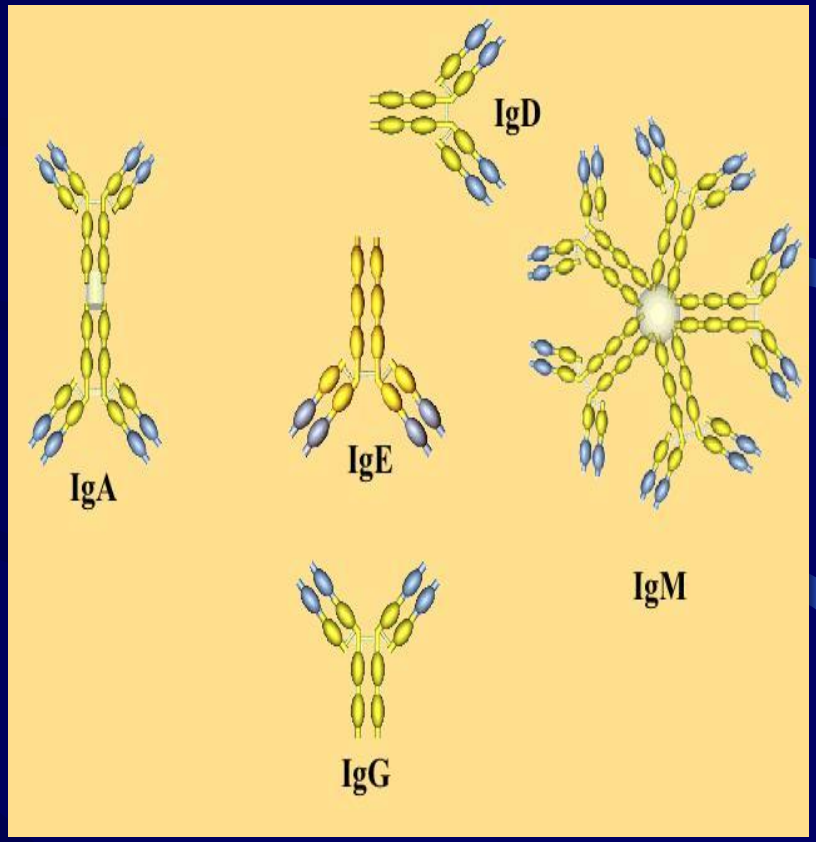
•1975 Kohler and Milstein, Monoclonal antibodies used in genetic analysis

# ANTIBODIES

## STRUCTURE




## CLASS

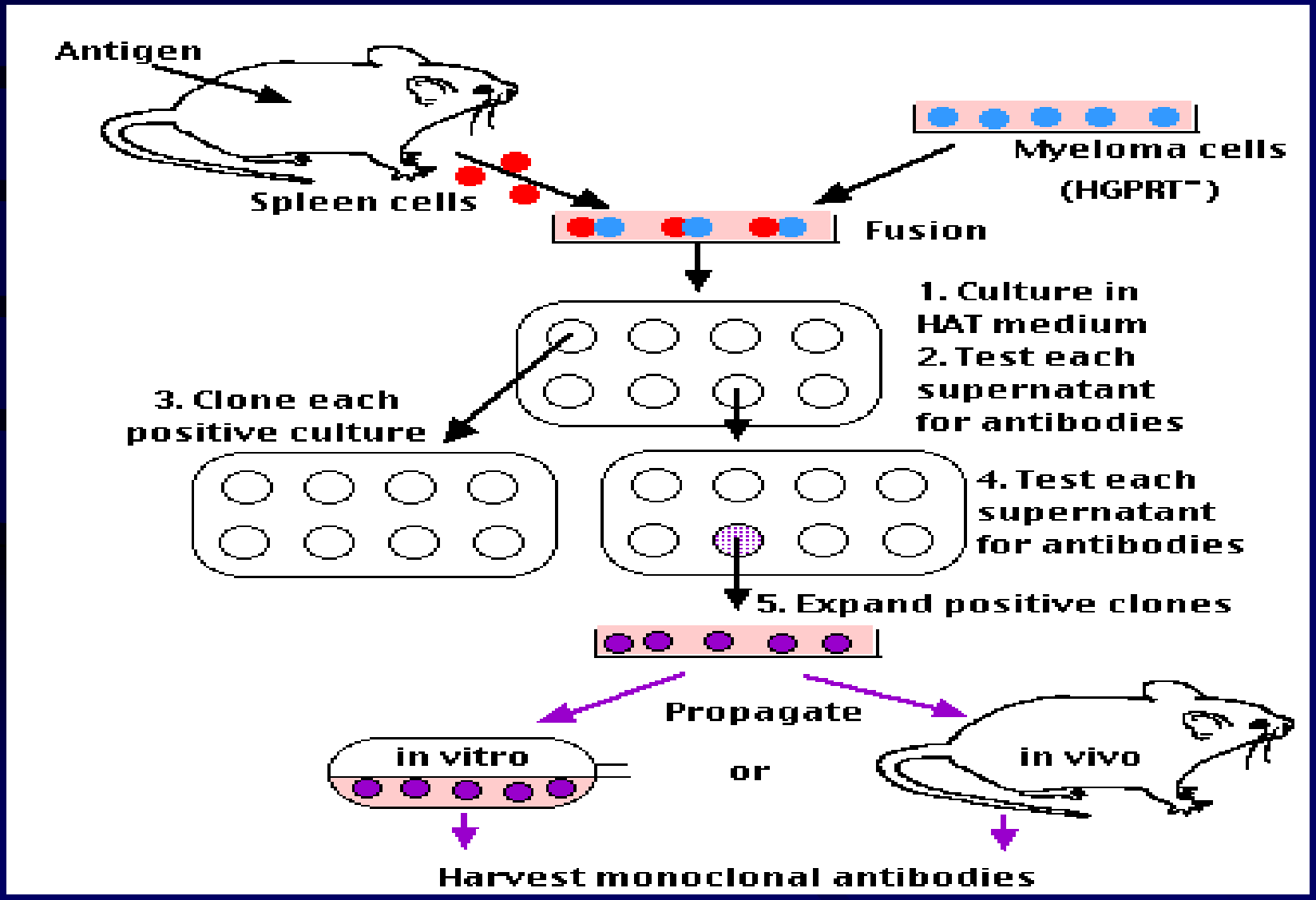


# ANTIBODIES

| POLYCLONAL. | MONOCLONAL. |
| --- | --- |
| Derived from different B Lymphocytes cell lines | Derived from a single B cell clone |
| Batch to Batch variation affecting Ab reactivity & titre | mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity |
| NOT Powerful tools for clinical diagnostic tests | Enable the development of secure immunoassay systems. |

# PRODUCTION OF MONOCLONAL ANTIBODY

## HYBRIDOMA TECHNOLOGY



# PRODUCTION OF MONOCLONAL ANTIBODY

## HYBRIDOMA TECHNOLOGY

### Step 1: - Immunization Of Mice & Selection Of Mouse Donor For Generation Of Hybridoma cells

**ANTIGEN ( Intact cell/ Whole cell membrane/ micro-organisms ) + ADJUVANT (emulsification)**


**Ab titre reached in Serum**

**Spleen removed**

**(source of cells)**

# PRODUCTION OF MONOCLONAL ANTIBODY

## HYBRIDOMA TECHNOLOGY

### Step 2: - Screening Of Mice For Antibody Production

**After several weeks of immunization**


**Serum Antibody Titre Determined**

**(Technique: - ELISA / Flow cytometery)**

**Titre too low**

**BOOST**
**(Pure antigen)**

**Titre High**

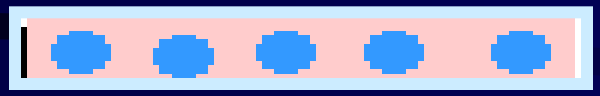
2 weeks

**BOOST**
**(Pure antigen)**

# PRODUCTION OF MONOCLONAL ANTIBODY

## HYBRIDOMA TECHNOLOGY

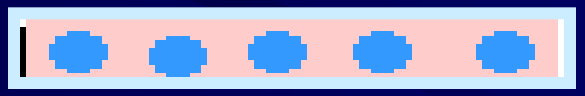
### Step 3: - Preparation of Myeloma Cells



**Myeloma Cells**

**Immortal Tumor Of Lymphocytes**

**+ 8 - Azaguanine**



**Myeloma Cells**

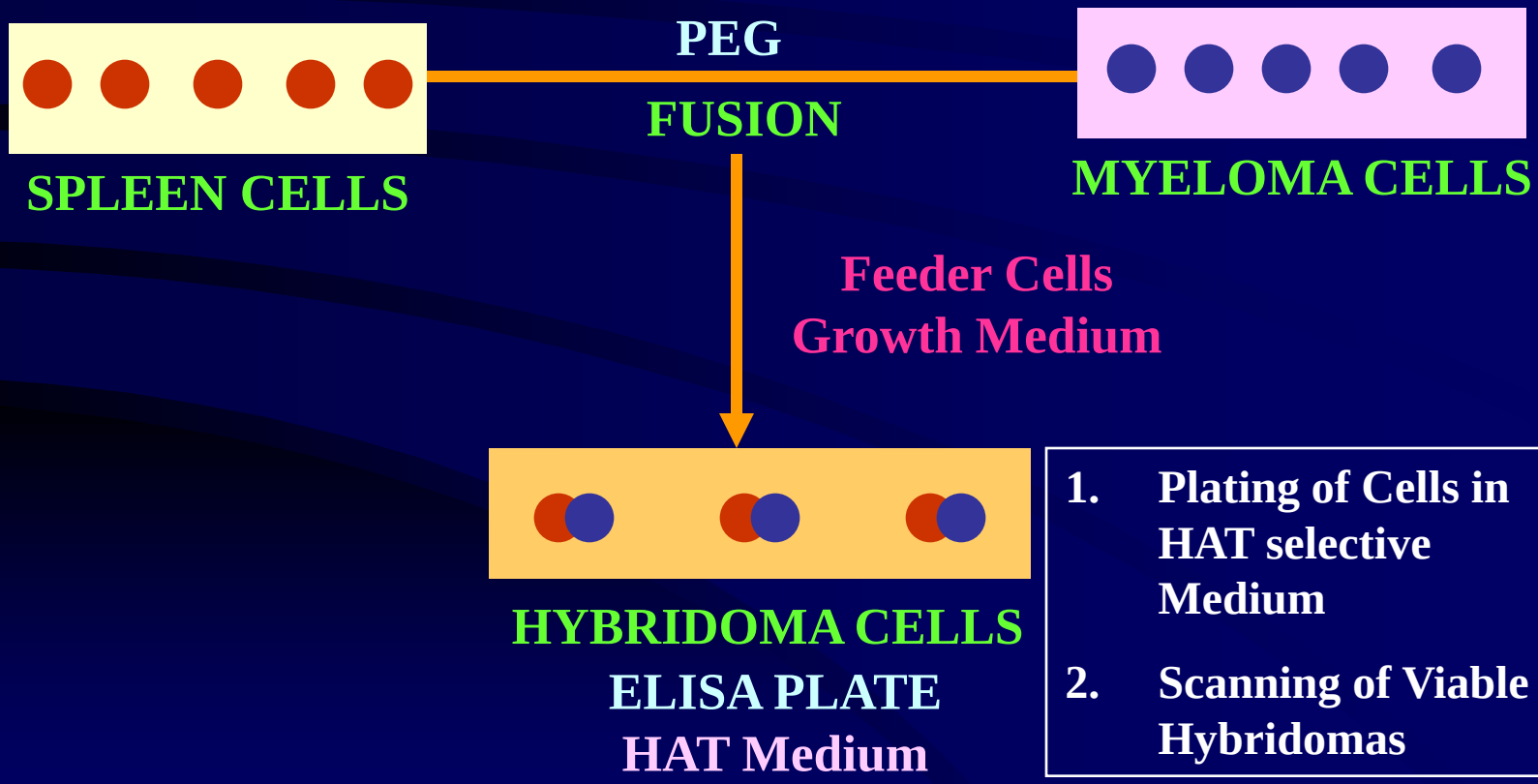
**$HGPRT^-$**

**High Viability & Rapid Growth**

# PRODUCTION OF MONOCLONAL ANTIBODY

## HYBRIDOMA TECHNOLOGY

### Step 4: - Fusion of Myeloma Cells with Immune Spleen Cells & Selection of Hybridoma Cells

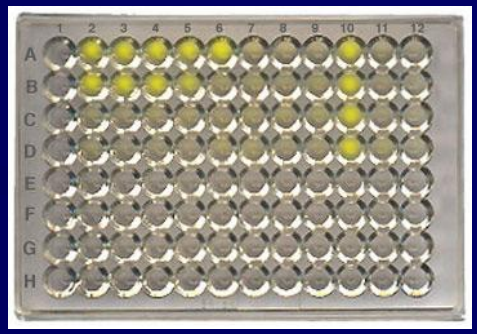



1. Plating of Cells in HAT selective Medium
2. Scanning of Viable Hybridomas

# PRODUCTION OF MONOCLONAL ANTIBODY

## HYBRIDOMA TECHNOLOGY

**Step 4: - Cloning of Hybridoma Cell Lines by " Limiting Dilution" or Expansion**




**A. Clone Each +ve Culture**

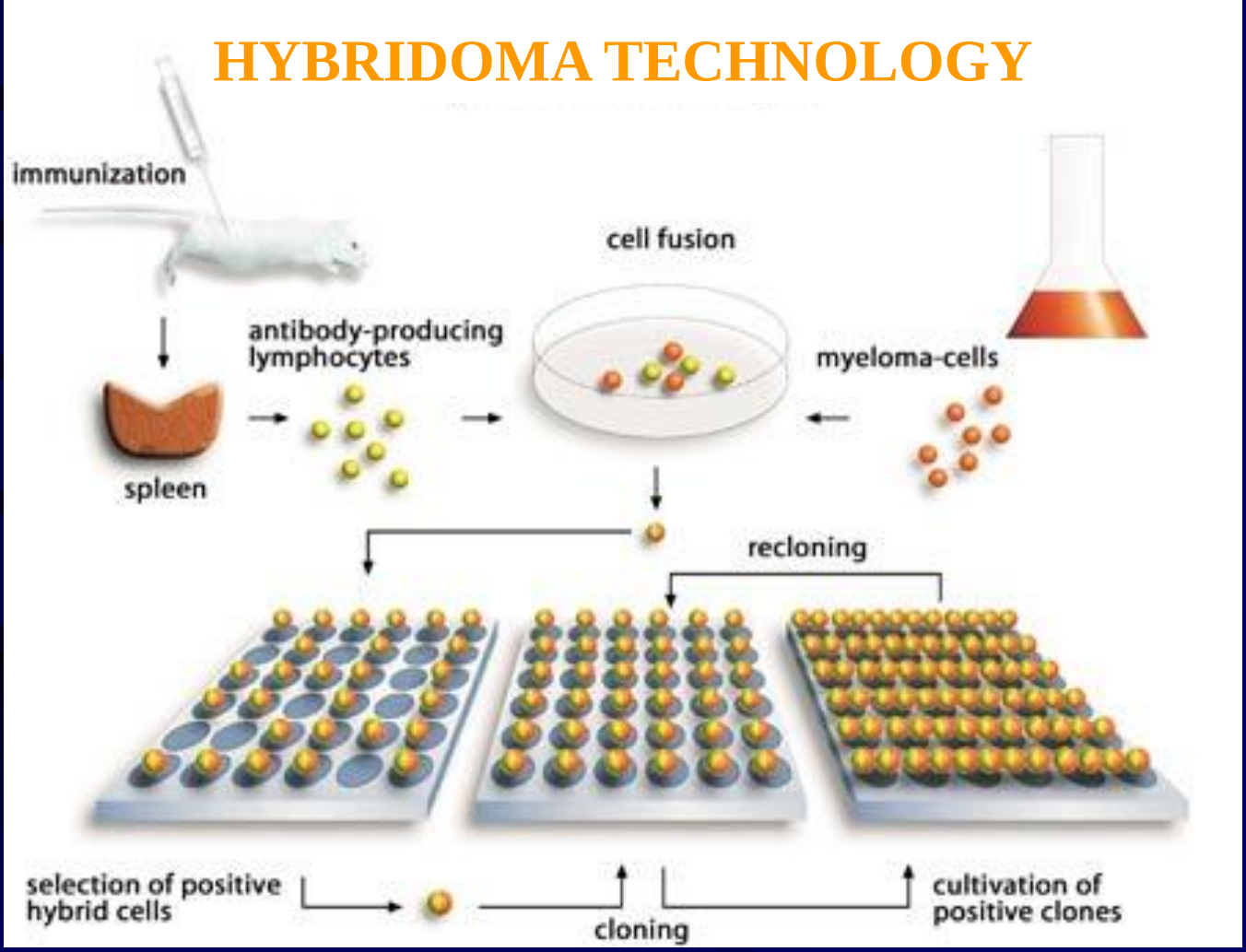
**B. Test Each Supernatant for Antibodies**

**C. Expand +ve Clones**

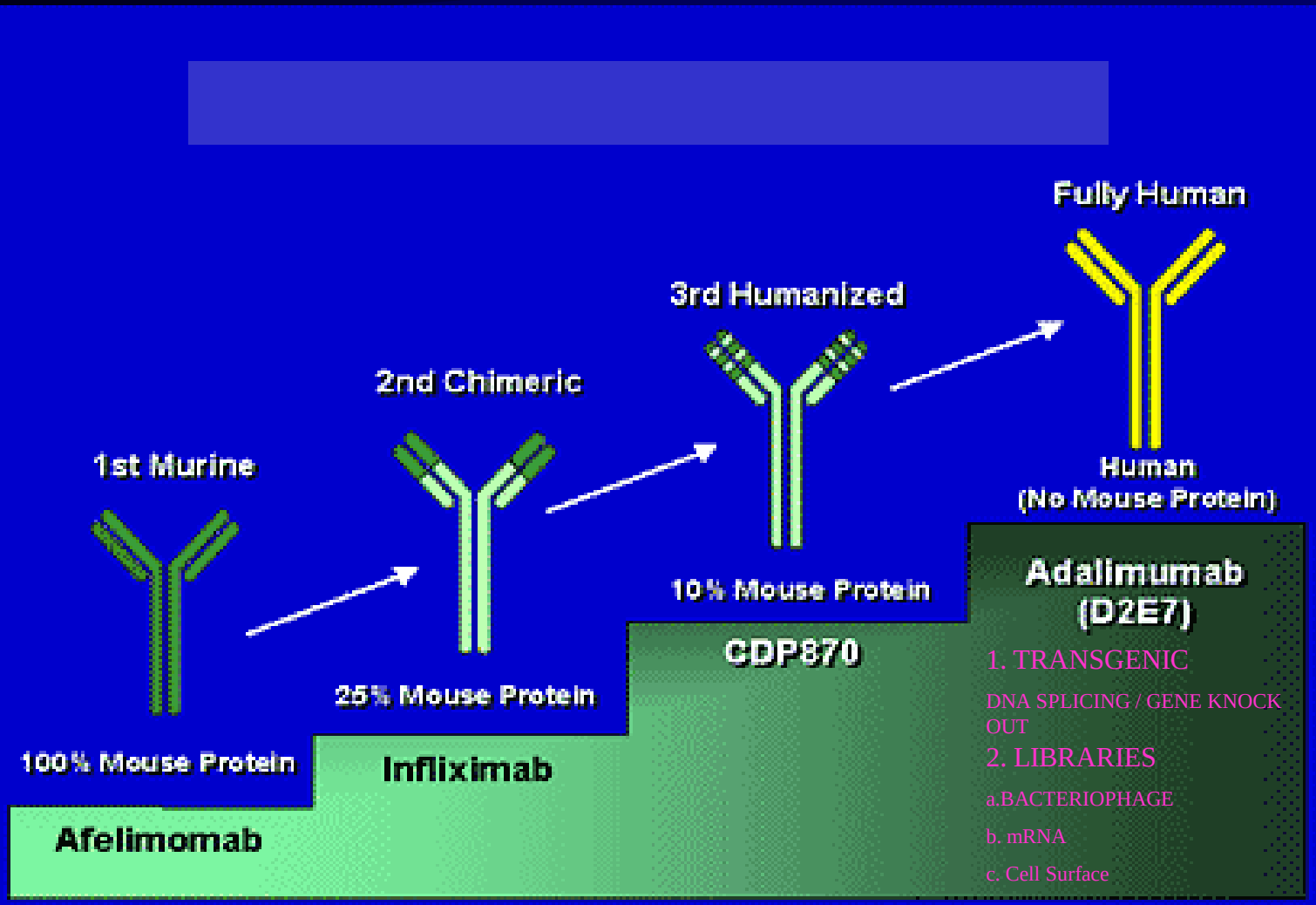
Tissue Culture Method


Mouse Ascites Method

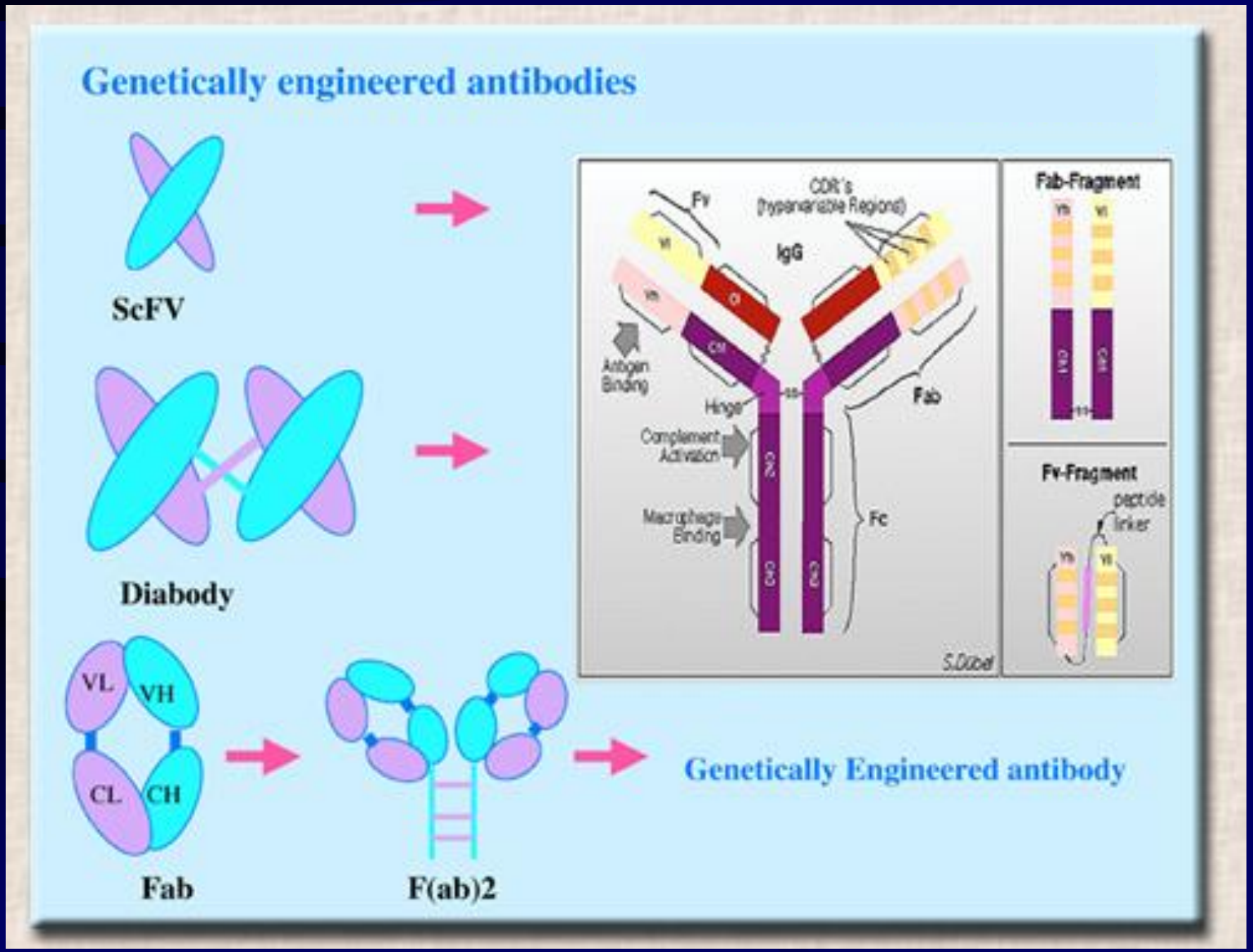
# PRODUCTION OF MONOCLONAL ANTIBODY



# EVOLUTION OF MONOCLONAL ANTIBODY



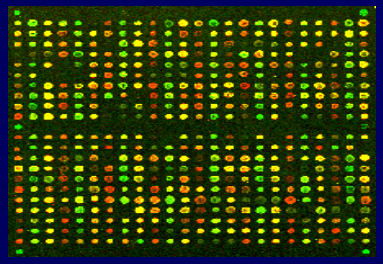
# ENGINNERED ANTIBODIES



| Company Name | Name of Product[1] | Indications | Date of FDA Approval | Antibody Type [2] |
|---|---|---|---|---|
| Ortho Biotech | **Orthoclone-OKT®** | Organ Transplant Rejection | 1986 | M |
| J&J/Eli Lilly | **ReoPro®** | Acute Cardiac Conditions | 1994 | C |
| BiogenIdec/Genentech/Roche | **Rituxan®** | Non-Hodgkin's Lymphoma | 1997 | C |
| BiogenIdec | **Zevalin™** | Non-Hodgkin's Lymphoma | 2002 | M |
| PDLI | **Zenapax®** | Acute Transplant Rejection | 1997 | H |
| MedImmune/Abbott | **Synagis®** | Viral Respiratory Disease | 1998 | H |
| Genentech/Roche | **Herceptin®**<br>**Avastin ®** | Breast Cancer<br>Colorectal Cancer | 1998<br>2004 | H<br>H |
| J & J | **Remicade®** | Crohn's, Rheumatoid Arthritis | 1998 | C |
| Novartis | **Simulect®** | Acute Transplant Rejection | 1998 | C |
| Wyeth | **Mylotarg™** | Acute Myleoid Leukemia | 2000 | H |
| Schering /ILEX Oncology | **Campath®** | Chronic Lymphocytic Leukemia | 2001 | H |
| Abbott/CAT | **Humira™** | Rheumatoid Arthritis | 2002 | PD |
| Novartis/Genentech/Tanox | **Xolair®** | Asthma | 2003 | H |
| Genentech/Xoma | **Raptiva™** | Psoriasis | 2003 | H |
| Corixa/GlaxoSmithKline | **Bexxar®** | Non-Hodgkin's Lymphoma | 2003 | M |
| BMS/ImClone Systems | **Erbitux ™** | Colorectal Cancer | 2004 | C |

# Applications of Monoclonal Antibodies

- Diagnostic Applications
  **Biosensors & Microarrays**
- Therapeutic Applications
  **Transplant rejection Muronomab-CD3**
  **Cardiovascular disease Abciximab**
  **Cancer Rituximab**
  **Infectious Diseases Palivizumab**
  **Inflammatory disease Infliximab**
- Clinical Applications
  **Purification of drugs, Imaging the target**
- Future Applications
  **Fight against Bioterrorism**




# Market Analysis & Forecast

- **Industry participants forecast of the market vary widely, however, a consensus is emerging that the market should reach US$26 billion by the end of the decade. This is a conservative estimate implying an average annual growth rate of 18%.**
- **By 2008, mAbs should account for 32 percent of all revenue in the biotech market**
- **100 mAb Expected By 2010**
- **mAb contributing to the in vitro diagnostics market expected to be worth $34 Billion this year**

# Why should we be interested ?

- **mAbs drive the development of multibillion dollar biotechnology industry.**
- **Many of the leading pharmaceutical companies have entered the mAb sector, attracted by quicker and less costly development, higher success rates, premium pricing, and a potentially reduced threat from generics**
- **The outlook for monoclonal antibody therapeutics is healthy. The ongoing success of existing products, combined with a bulging pipeline of new products awaiting approval and limited generic erosion, point towards robust growth in this segment**

**PAUL EHRLICH**

**“…. I TRUST, THAT WE NO LONGER FIND OURSELVES LOST ON A BOUNDLESS SEA, BUT THAT WE HAVE ALREADY CAUGHT A DISTINCT GLIMPSE OF THE LAND WHERE WE HOPE, NAY, WHICH WE EXPECT, WILL TEILD RICH TREASURES FOR BIOLOGY AND THERAPEUTICS”**