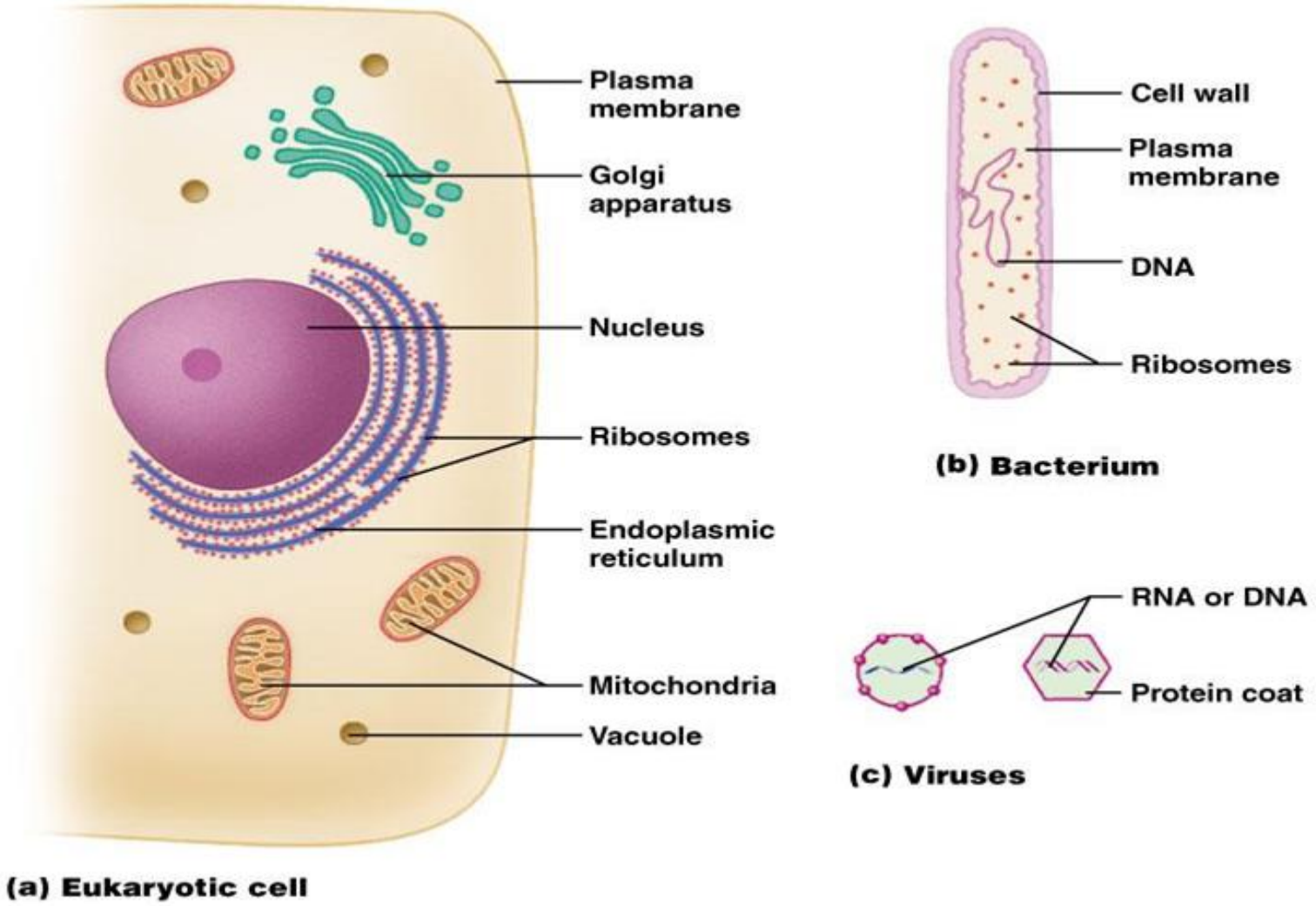
UNIT 5
ImmunoBiology

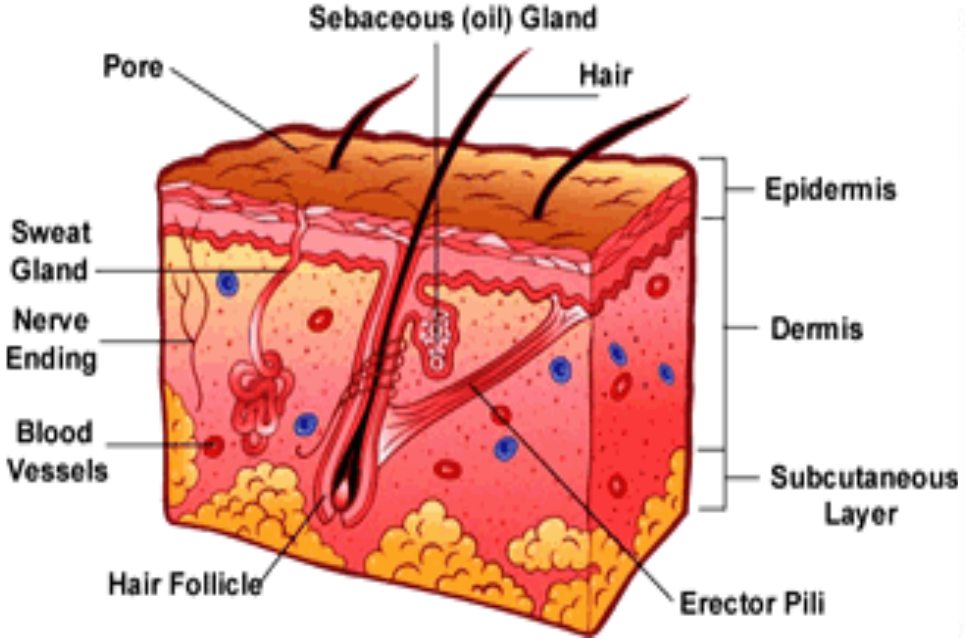
- Elements of the immune system,
- Types of the immune response
- Active and passive immunity
- Immunoinformatics
- epitope prediction tools

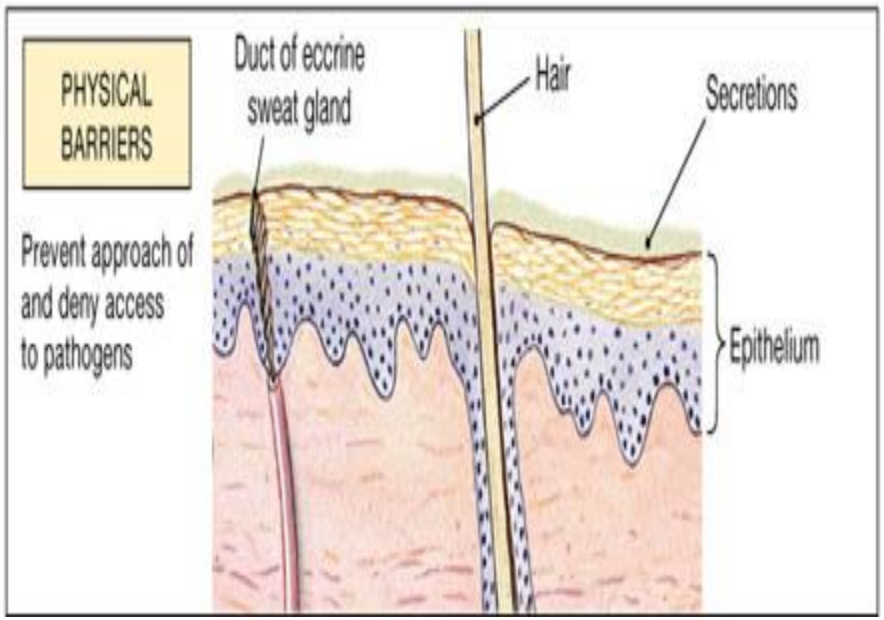
Eukaryotic Cells, Bacteria, and Viruses



Body Defenses: Overview

- **Physical barriers: skin & epithelial linings & cilia**
- **Chemical: acids, mucous & lysozymes**
- **Immune defenses – internal**
  - **Innate, non-specific, immediate response (min/hrs)**
  - **Acquired – attack a specific pathogen (antigen)**
- **Steps in Immune defense**
  - **Detect invader/foreign cells**
  - **Communicate alarm & recruit immune cells**
  - **Suppress or destroy invader**

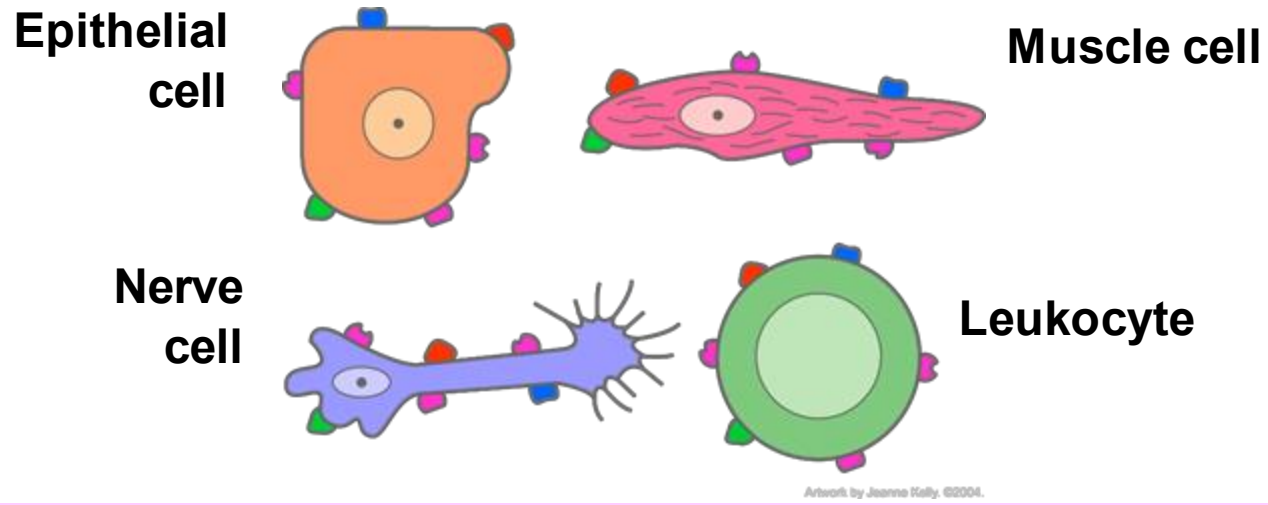




Discrimination of self from non-self

"The success of the immune system depends on its ability to discriminate between foreign (nonself) and host (self) cells. Survival requires both the ability to mount a destructive immune response against nonself and the inability to mount a destructive response against self."

Markers of Self

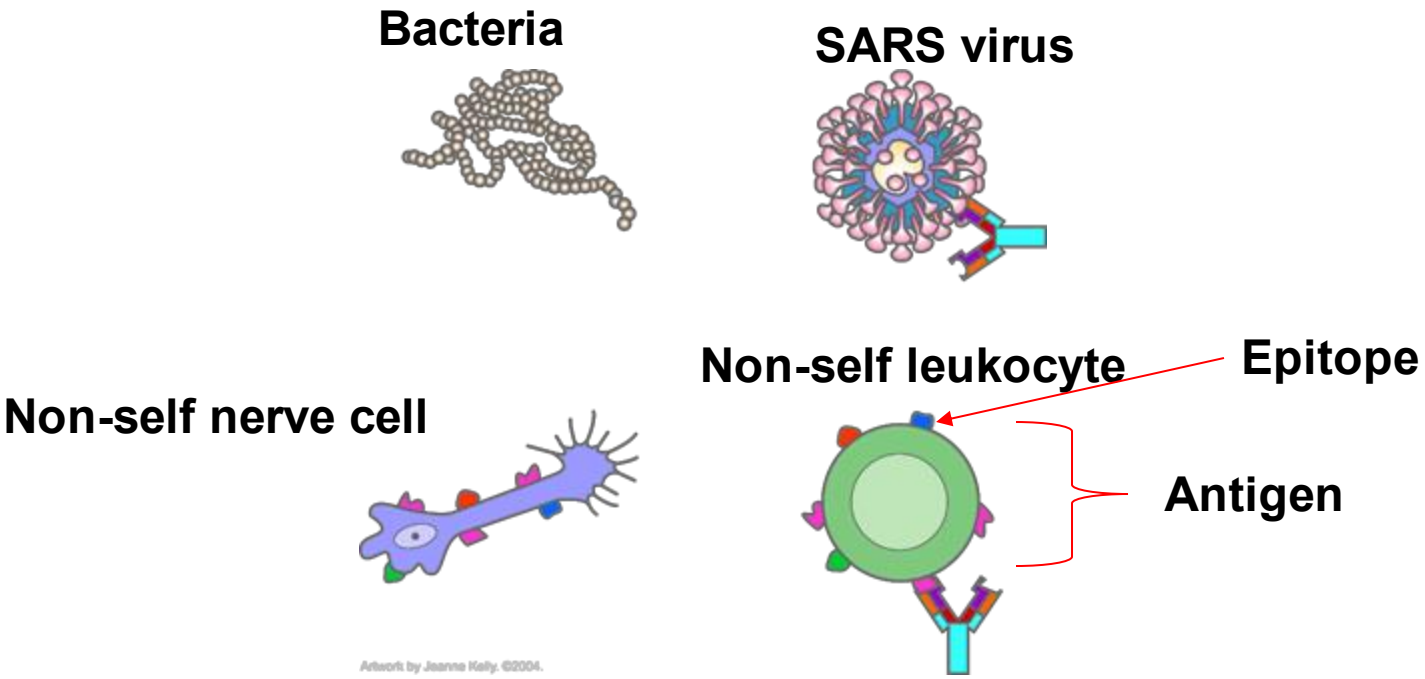


At the heart of the immune response is the ability to distinguish between "self" and "non-self."

- **Every cell in your body carries the same set of distinctive surface proteins that distinguish you as "self."**
- **Normally your immune cells do not attack your own body tissues, which all carry the same pattern of self-markers; rather, your immune system coexists peaceably with your other body cells in a state known as self-tolerance.**

This set of unique markers on human cells is called the major histocompatibility complex (MHC) proteins.

Markers of Non-self



Antigen = any non-self substance

- Virus
- Bacteria
- Non-self cell (foreign cell)

Epitope = The distinctive markers on antigens that trigger an immune response

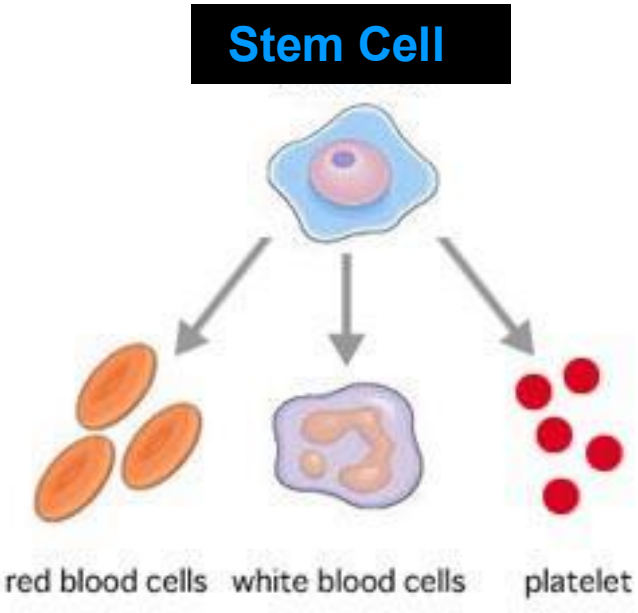
Blood

- Blood is 55% liquid (plasma) and 45% cellular

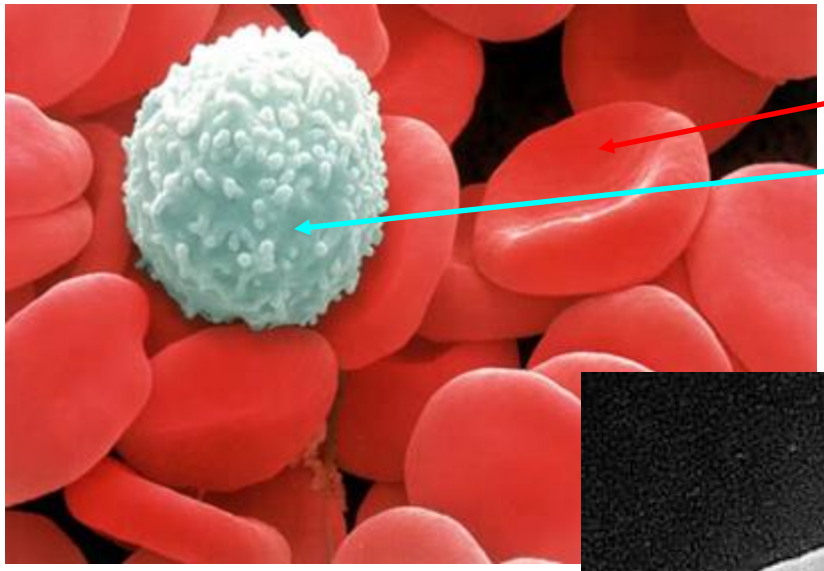
- Cellular component of blood:
  - Red blood cells = carry oxygen
  - White blood cells = immune system
  - Platelets = clot blood

Leukocytes

- All blood cells arise from a pluri-potent stem cell found in bone marrow



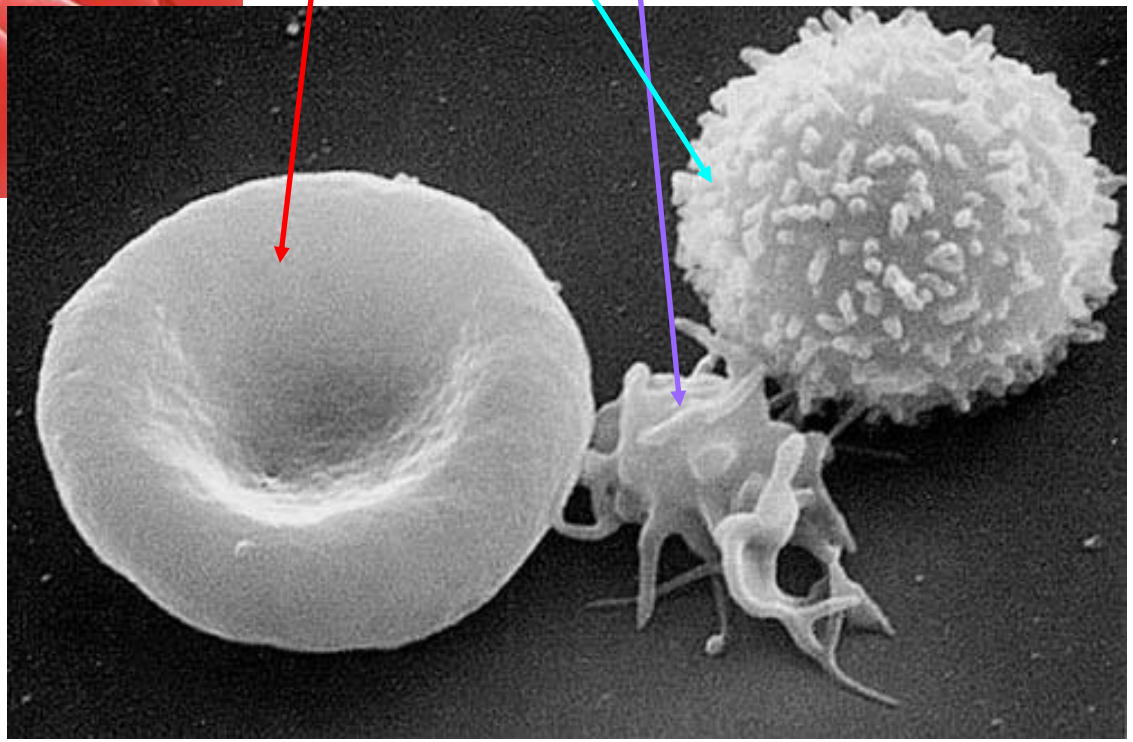
Blood cells



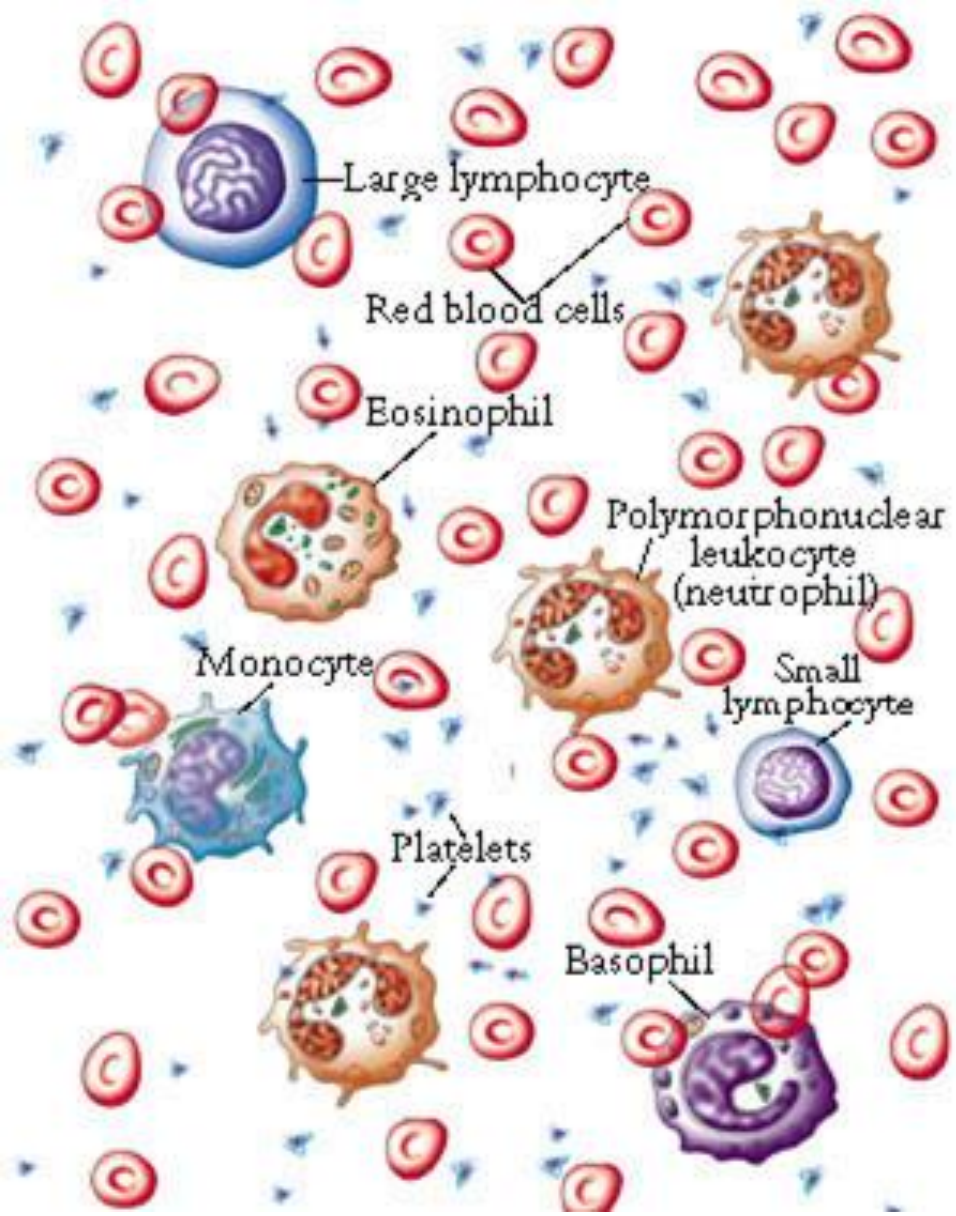
Red Blood cells

White blood cells (immune cells)

Platelets



Leukocytes in the Blood

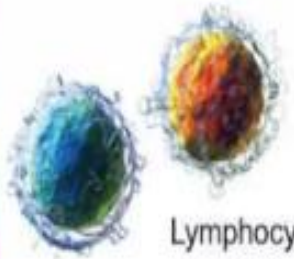


Red Blood Cells		**5.0 X 10^6/mm^3**	
Platelets		**2.5 X 10^5/mm^3**	
Leukocytes (WBC)		**7.3 X 10^3/mm^3**	
1	**Neutrophil**		**50-70%**
2	**Lymphocyte** → agranular		**20-40%**
3	**Monocyte**		**1-6%**
4	**Eosinophil**		**1-3%**
5	**Basophil** → granular		**<1%**

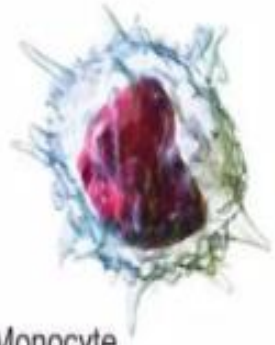
TYPES OF WHITE BLOOD CELLS:

Leukocytes
(white blood cells)

agranular



Lymphocytes
20-25%



Monocyte
3-8%

granular

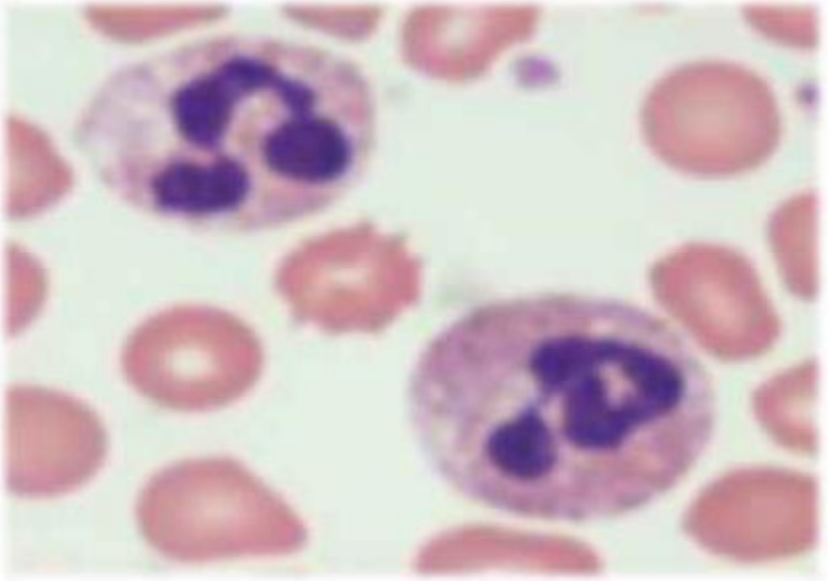
Basophil
0.5-1%

Neutrophil
60-70%

Eosinophil
2-4%

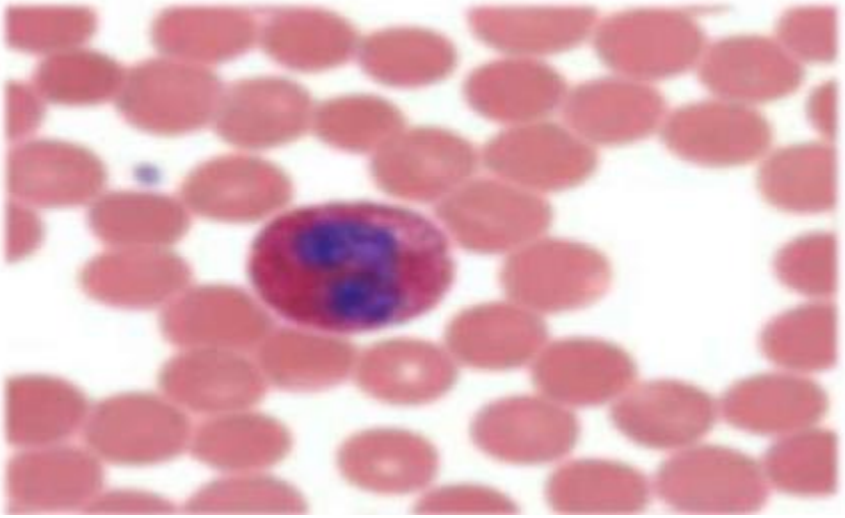
Neutrophil

Neutrophil•60% of WBC•Lifespan is 10 hours in blood•Seek out and destroy ingested bacteria inconnective tissues•100 billion manufactured daily



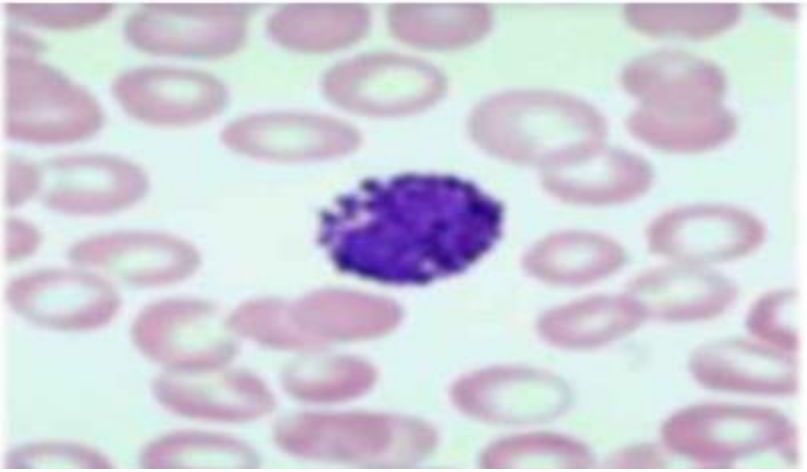
Eosinophil

- 1-3 % of WBC
- Help control allergic reactions
- Release an enzyme histamine – a chemical released during allergic reactions



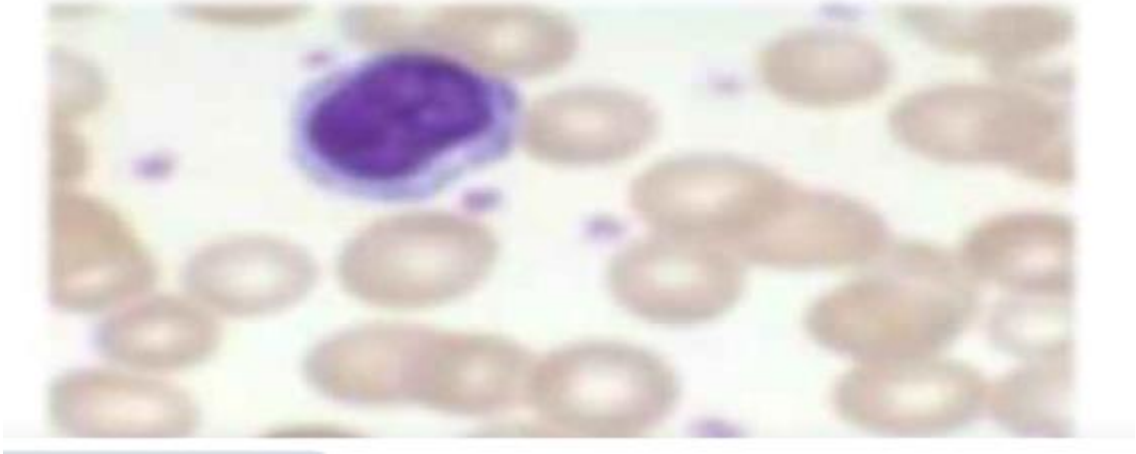
Basophil

- Less than 1% of WBC
- Involved in allergic and inflammatory reactions
- Contains large amounts of histamines which may be released in injured tissue in order to increase inflammation
- Contains heparin an anti-clotting chemical



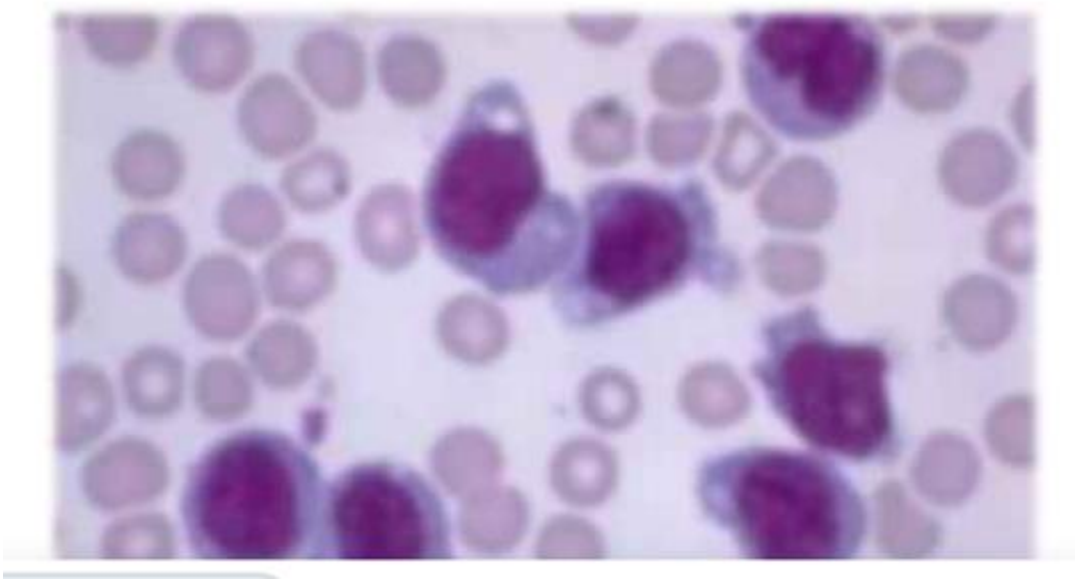
Lymphocytes

- 30% of all leukocytes
- Made from stem cells, but are released from lymph nodes, thymus, spleen and bone marrow
- Produce antibodies and destroy foreign cells found in infectious mononucleosis

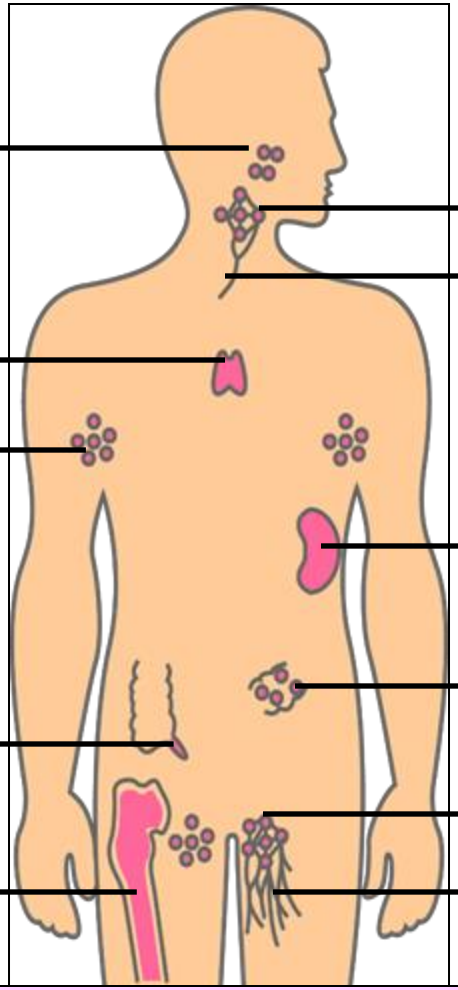


Monocyte

- 6% of WBC's
- Enter connective tissue
- Eat bacteria, dead cells and other littering tissue



Organs of the Immune System



Tonsils and adenoids

Thymus

Lymph nodes

Appendix

Bone marrow

Lymph nodes

Lymphatic vessels

Spleen

Peyer's patches

Lymph nodes

Lymphatic vessels

Bone marrow, the soft tissue in the hollow center of bones, is the ultimate source of all blood cells, including the immune cells.

Lymphatic System

The organs of your immune system are connected with one another and with other organs of the body by a network of lymphatic vessels.

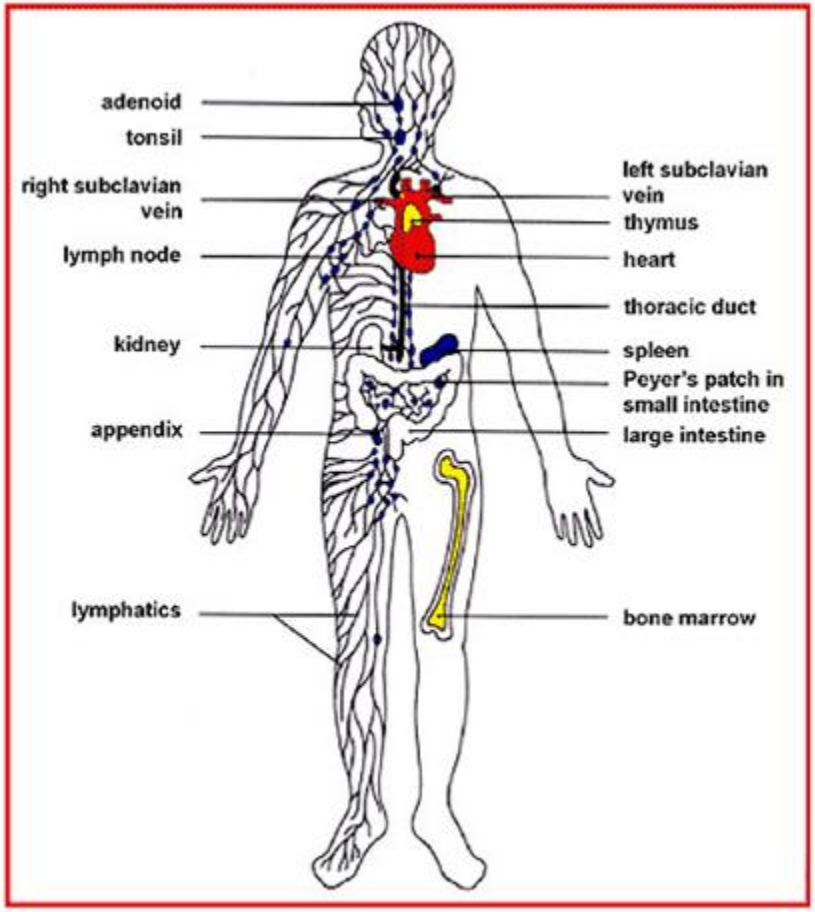
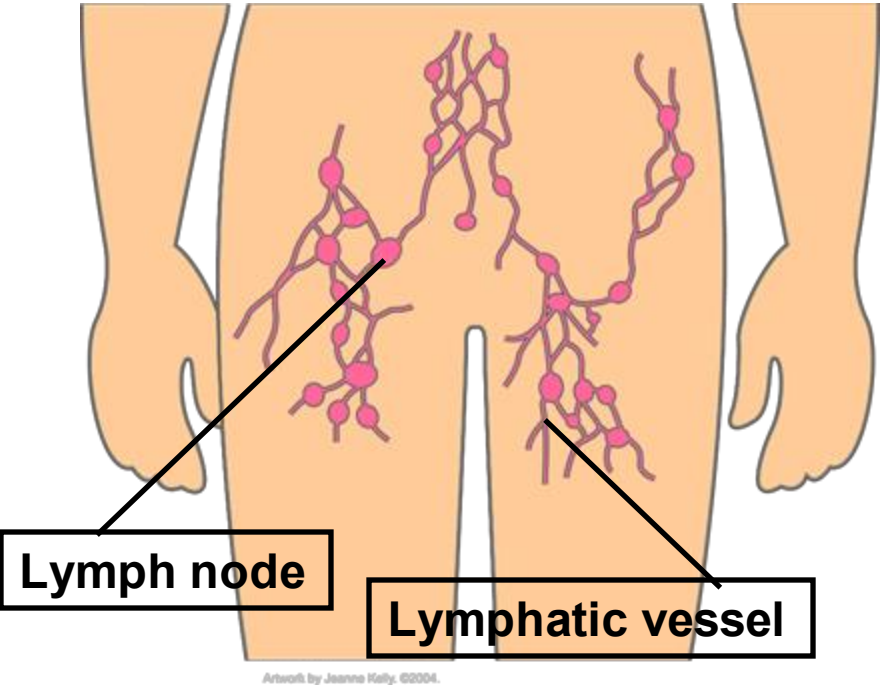


Figure 1. The immune system.

Lymphatic System

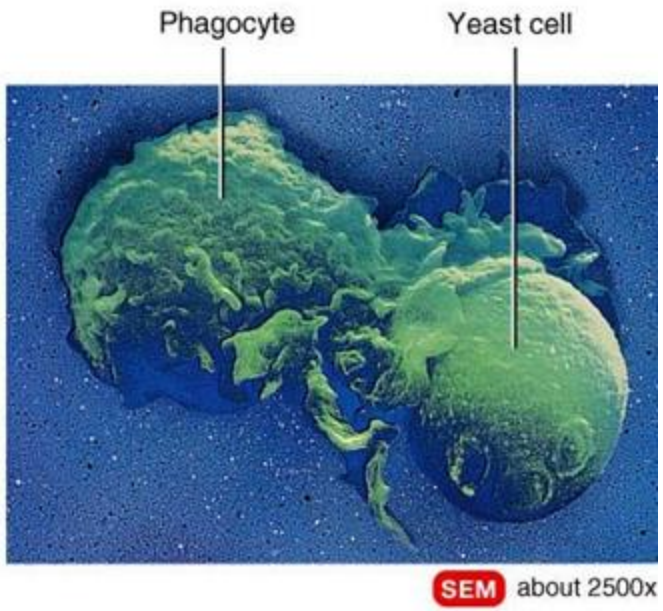
The organs of your immune system are connected with one another and with other organs of the body by a network of lymphatic vessels.



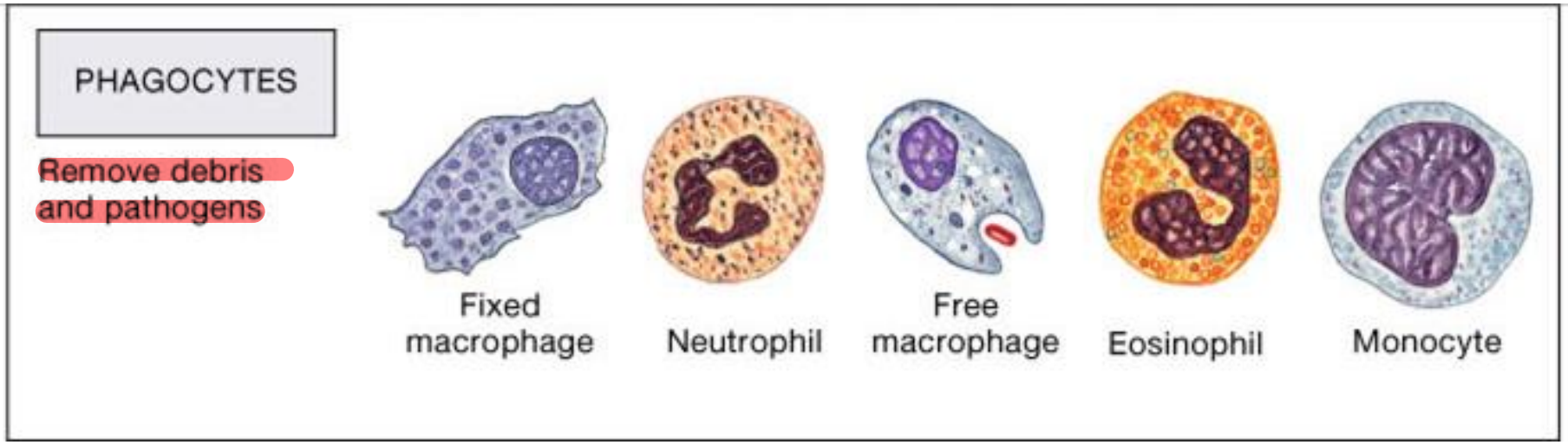
1. **Lymphatic vessels closely parallels the body's veins and arteries**
   - **Lymphatic vessels carry *lymph*, a clear fluid that bathes the body's tissues**
   - **Cells/fluids are exchanged between blood and lymphatic vessels, enabling the lymphatic system to monitor the body for invading microbes.**
2. **Lymph nodes contain high levels of immune cells**

Nonspecific Defenses, Phagocytes

- Remove cellular debris and respond to invasion by foreign pathogens
  - Monocyte-macrophage system - Fixed and free
  - Microphages – Neutrophils and eosinophils
  - Move by diapedesis
  - Exhibit chemotaxis

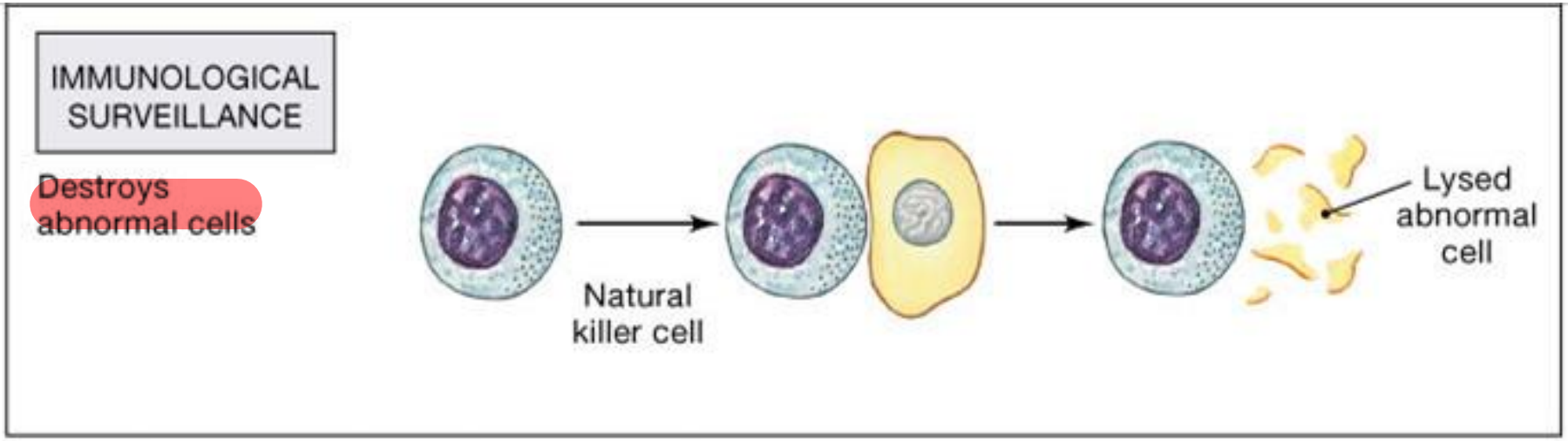


(b) Phagocyte engulfing a yeast cell

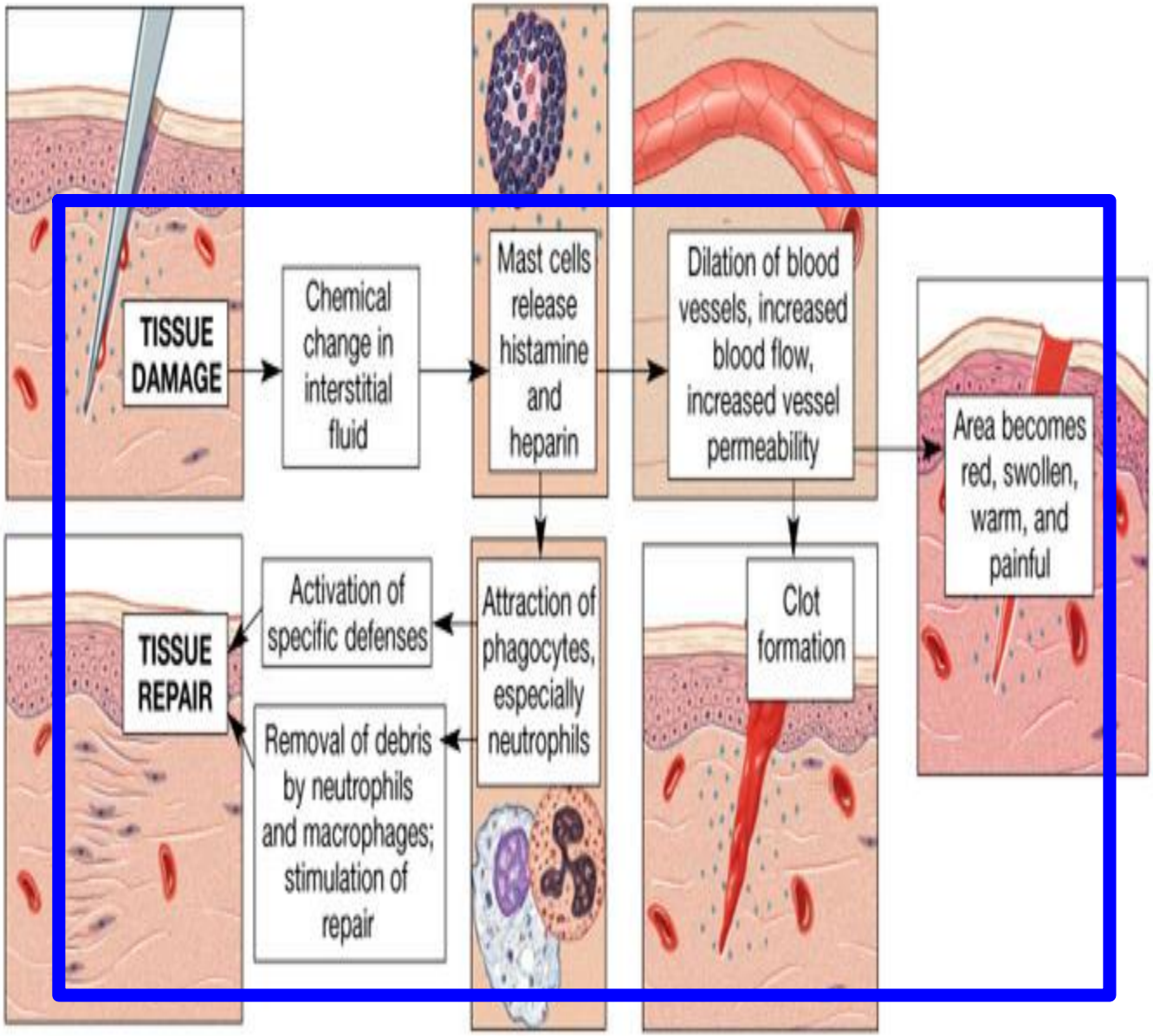


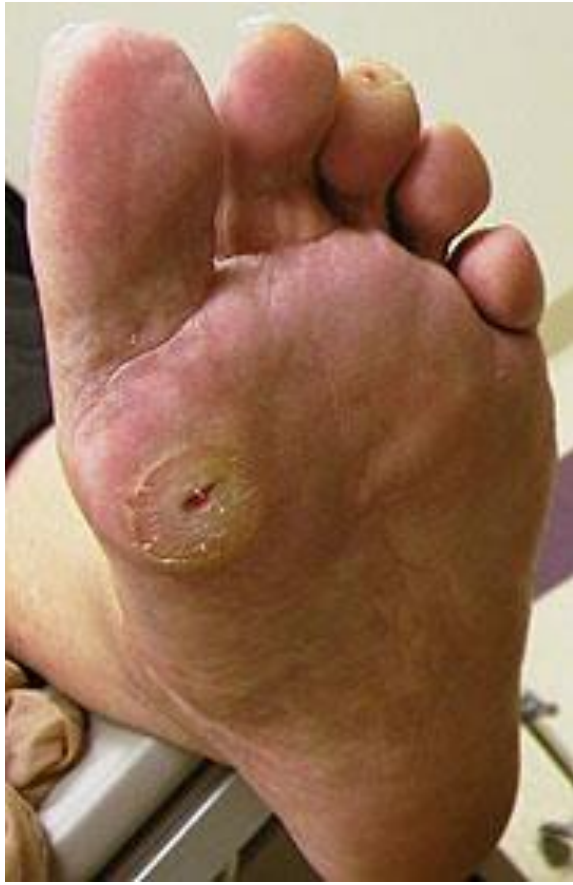
Nonspecific Defenses, Immunological surveillance

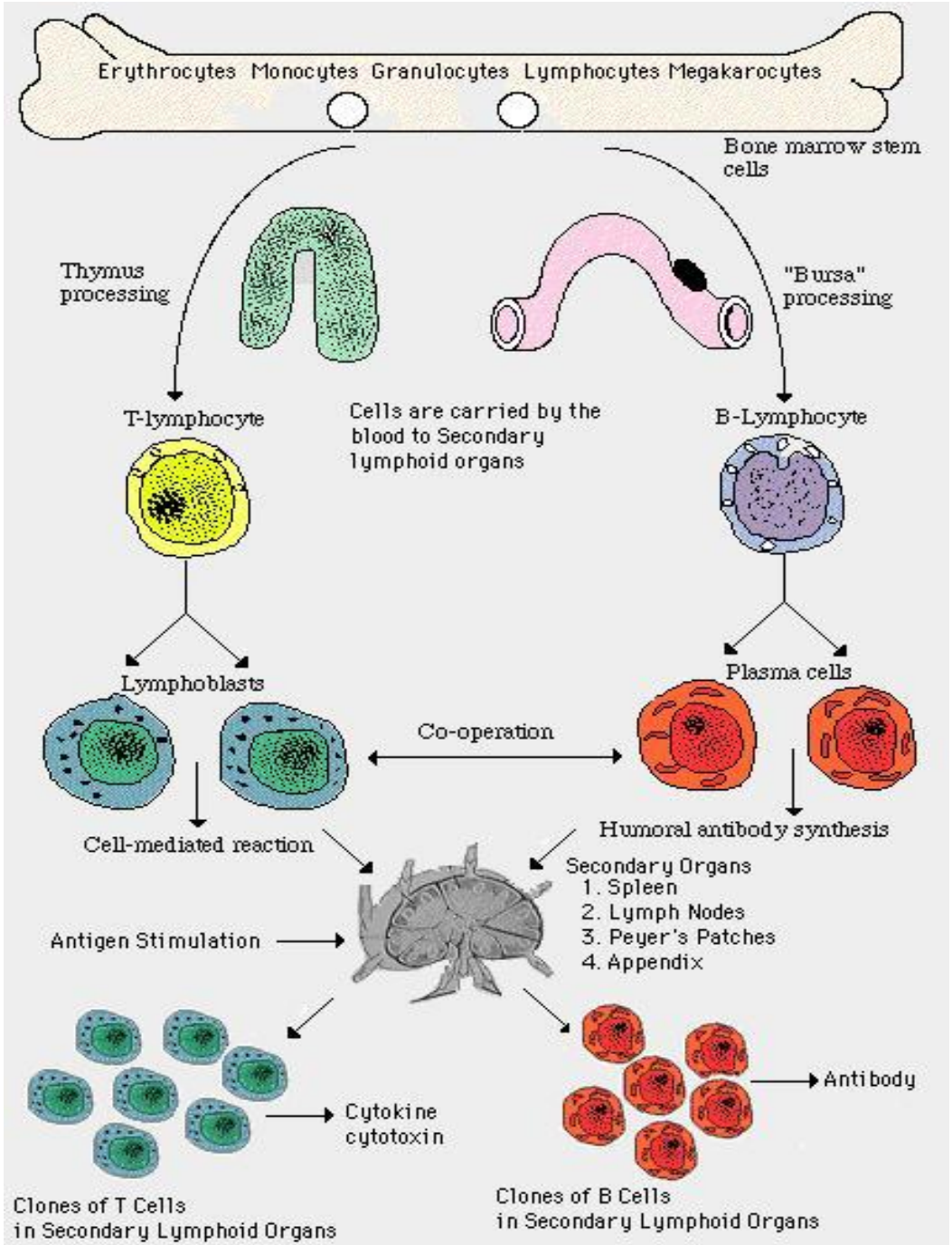
- Constant monitoring of normal tissue by NK cells
- NK cells
  - Recognize cell surface markers on foreign cells
  - Destroy cells with foreign antigens



Inflammation







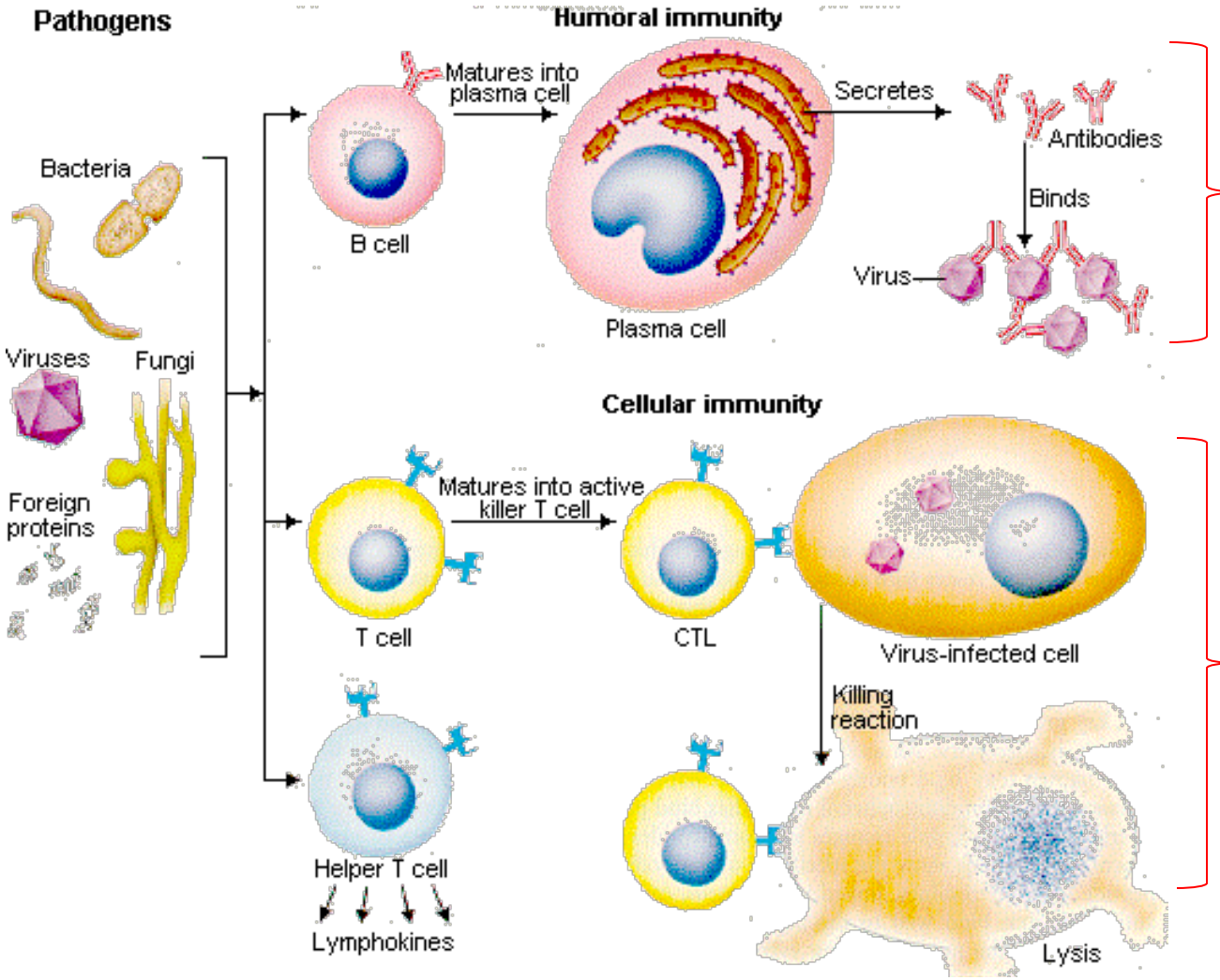
Adaptive or Acquired Immunity

- Acquired after birth
- Seen only in vertebrates
- Characteristic features are:
  - Diversity
  - Specificity
  - Self vs non-self
  - Memory

Immune Response System

Made up of two cellular systems (lymphocytes)

1. Humoral immunity - **B cells**
2. Cell-mediaed immunity - **T cells**



B cells make antibodies

T cells mount direct attack on foreign/infected cell

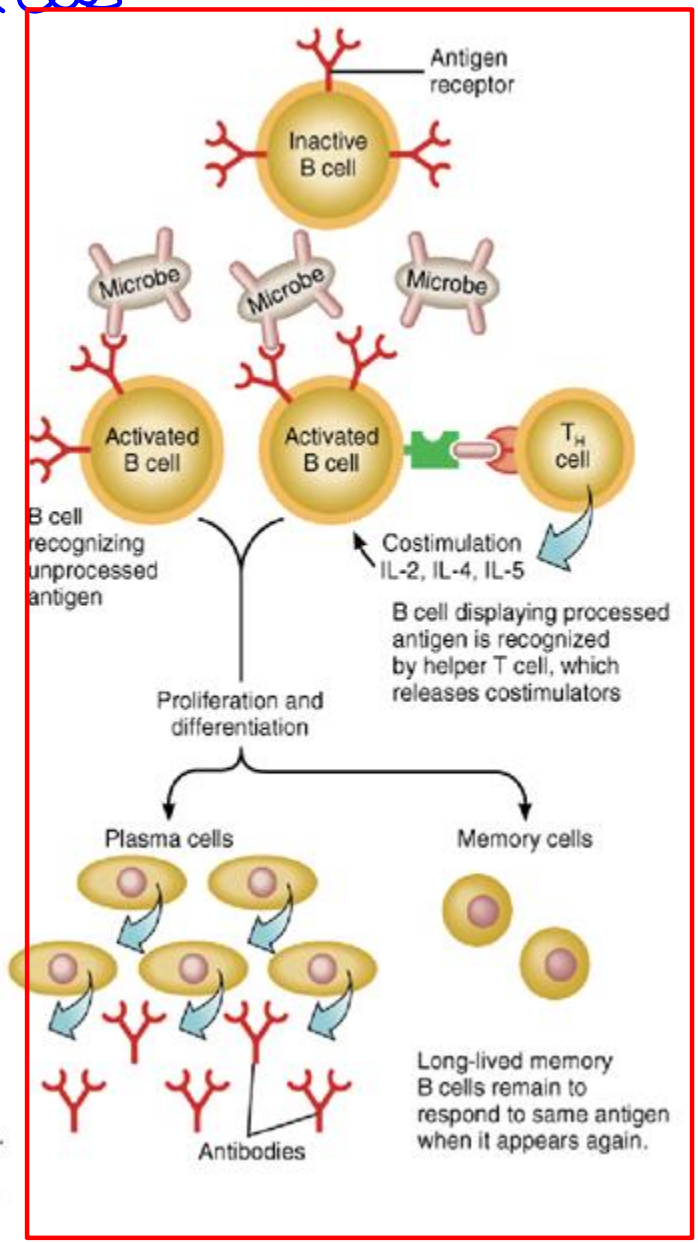
Cell mediated immunity

L T cells

- T cells must be activated
- Must have both surface antigen recognition and costimulation to activate
- T cell receptors recognise and bind to specific antigen presented with MHC complexes
  - T cell only activated if binds to antigen and receives costimulation
    - Co-stimulation provided by cytokines or membrane proteins
  - Need for co-stimulation prevents immune responses occurring accidentally
    - Recognition (binding to receptor) without costimulation results in anergy (prolonged state of inactivity) in both B and T cells
  - Once T cell co-stimulated it is activated
    - Proliferates
    - Differentiates (forms more highly specialised cells)
  - Activation, proliferation and differentiation occurs in secondary lymphatic organs and tissues

Humoral (Antibody-mediated) immunity

- Mediated by B cells
- Antigen can activate B cell in two ways:
  - direct binding
    - provokes less vigorous response
  - B cells process antigen (act as APC) and display processed antigen with MHC proteins
    - T_H cells recognise processed antigen
      - T_H cells provide co-stimulation for B cell
- Activated B cell
  - proliferates and differentiates
    - plasma cells
      - secrete antibodies with same antigen binding properties as receptors
    - memory B cells



Innate/Acquired-Adaptive Immunity

- Innate immunity rely on a global distinction of self/non-self recognition
  - Instantaneous
  - Non specific
  - Non adaptative
  - Memory less
- Adaptative immunity involve the cell-mediated and hummoral response.
  - Time limited to the eradication of the antigen
  - Specific to the given antigen
  - Adaptativ
  - Memory full

Immunologic Memory

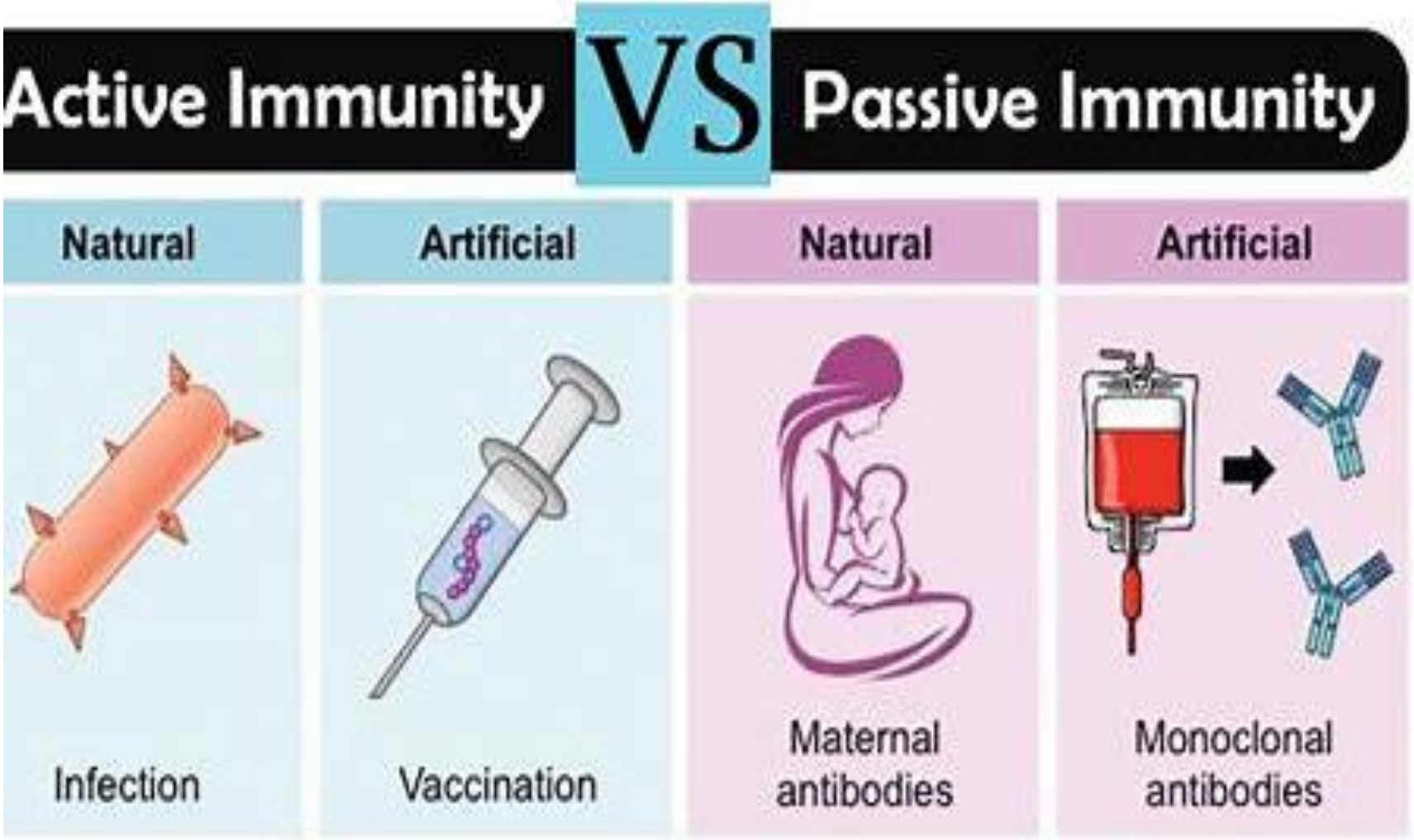
- First Response (4-5 days)
  - First adaptive response against a given antigen
- Secondary Response (1 day)
  - Shorter lag time
  - More rapid buildup
  - Higher overall level of response
  - Better fit to the invading antigen
  - Utilizes IgG instead of IgM

Active and Passive Immunity

- Active immunity and passive immunity are two types of acquired immunity.

Active Immunity	Passive Immunity
Active immunity is usually long-lasting, sometimes life-long. It is produced by the antibodies of the host in response to direct contact with an antigen	Passive immunity lasts only for a few weeks or months. It is produced by the introduction of antibodies from outside into the host
It produces an immunological memory	It does not produce immunological memory
When the antigens enter the body, antibodies and other specialised lymphocytes are produced	Antibodies are introduced from an external source. For instance, a mother introduces antibodies to a fetus through the placenta and to an infant via mother's milk.
There are no side-effects	It may cause reactions
Immunity does not occur immediately	Immunity develops immediately

Immunity: Active and Passive



VACCINES

- Vaccination is the administration of antigenic agents applied to stimulate the immune system of an individual and to develop adaptive immunity to a disease.
- Vaccines can ameliorate, or often even prevent, the effects of infection. Vaccination is generally considered to be the most effective method of preventing infectious diseases
- A multi-stage tuberculosis vaccine and Covid has recently been developed to confer protection after the exposure to the pathogen . There are numerous vaccine examples, including experimental ones against AIDS, cancer and Alzheimer's disease.
- The core mechanism behind all the vaccinations is the ability of the vaccine to initiate an immune response in a quicker fashion than the pathogen itself.

- The purpose of every vaccination is to present a particular antigen or set of antigens to the immune system in order to evoke a relevant immune response.

- The main active component of a vaccine may be inactive, but still intact (attenuated bacteria or viruses), or purified components of the pathogen that are known to induce immune reaction.

Types of Vaccines

1. Inactivated vaccines

- This type of vaccine consists of virus particles grown in cell culture and inactivated by applying high temperature or chemicals such as formaldehyde. Booster shots required Eg., Hepatitis A, Polio, Flu

2. Live attenuated vaccines

- The attenuated vaccines contain live virus particles with low levels of virulence. They have retained their ability to slowly reproduce, and thus they remain a continuous source of antigen for a certain period after the first vaccination, reducing the need of booster shots. Eg., MMR combined vaccine, Small pox, Rotavirus

3. Subunit vaccines

- Subunit vaccines use only the antigenic components that best stimulate the immune system, instead of dealing with the entire micro-organism.Eg., Hepatitis B,HPV (Human papillomavirus), Pertusis

4. Toxoid vaccines

- The toxoid vaccines are typical solution for bacteria that secrete harmful metabolites or toxins. It is common to use them when the main reason for discomfort or sickness is a bacterial toxin. Eg., diphtheria and tetanus.

5. DNA vaccines

- DNA vaccination is a very new approach for induction of humoral and cellular immune responses to protein antigens by administering genetically engineered DNA. DNA vaccines are still in the experimental stage, and have been tested in numerous viral, bacterial and parasitic models of disease

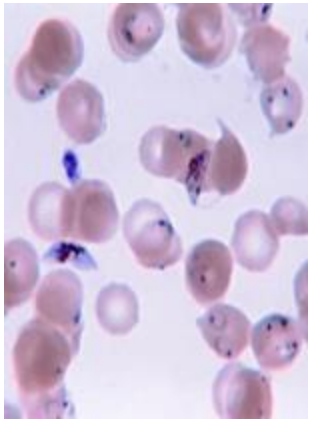
6. Peptide vaccines

- The improved knowledge of antigen recognition at molecular level has contributed to the development of rationally designed peptide vaccines. chemical approach to synthesize the identified B-cell and T-cell epitopes that are immunodominant and can induce specific immune responses. The peptide vaccines against various cancers have been developed, and entered phase I and phase II of clinical trials

Vaccines yet to developed!

HIV/AIDS Patient

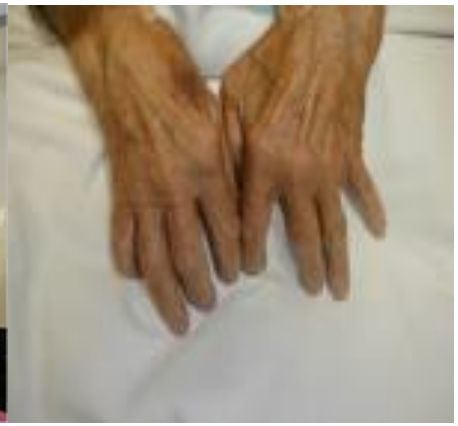
Aging



Malaria-parasitic disease

Smoking causes cancer

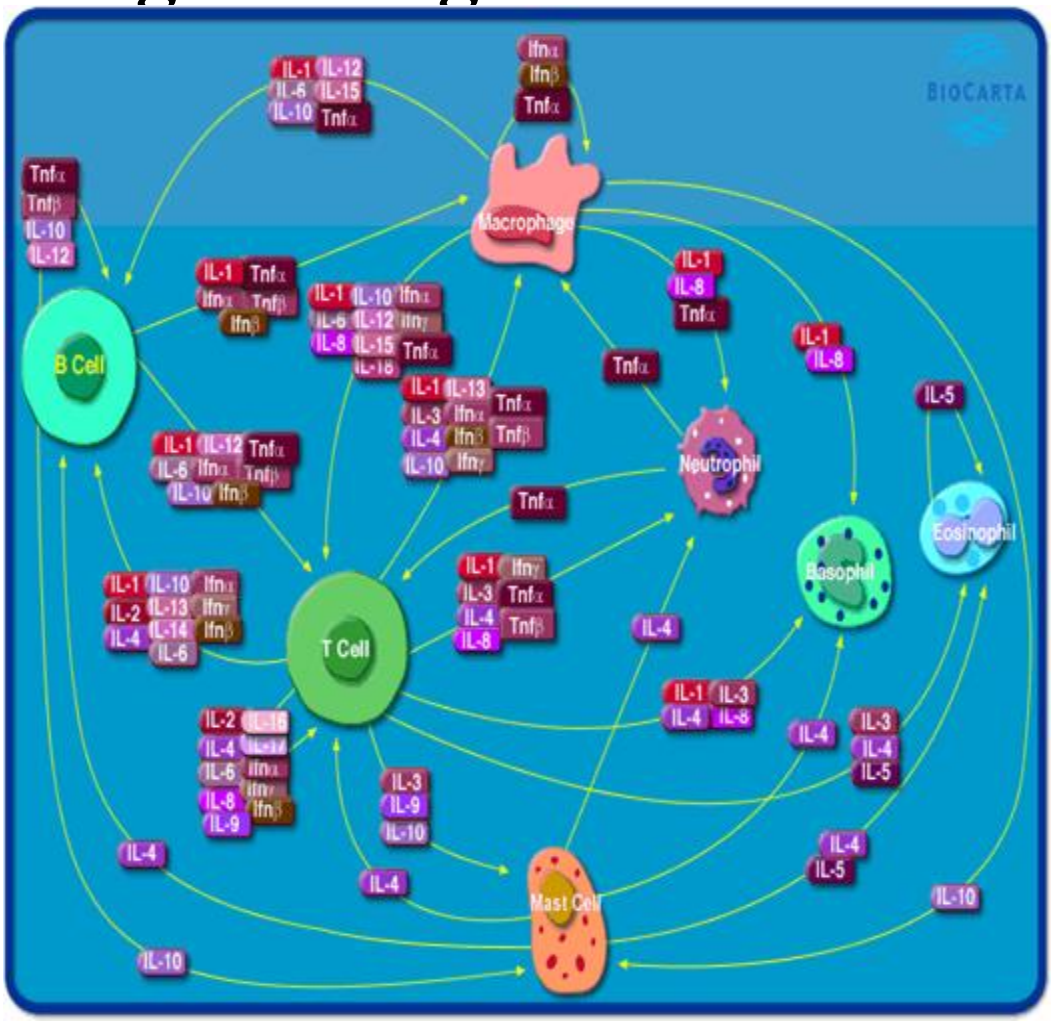
Diabetes



Rheumatoid arthritis

Immune Engineering

- The complexity of the immune system can be compared to that of the brain.
- There is a vast number of cells, molecules, and organs that compose the immune system, and these have to act in concert, and together with other vital systems, so as to promote and maintain life.
- Neither can the immune system act in isolation to maintain life, nor can a higher organism live without an immune system.



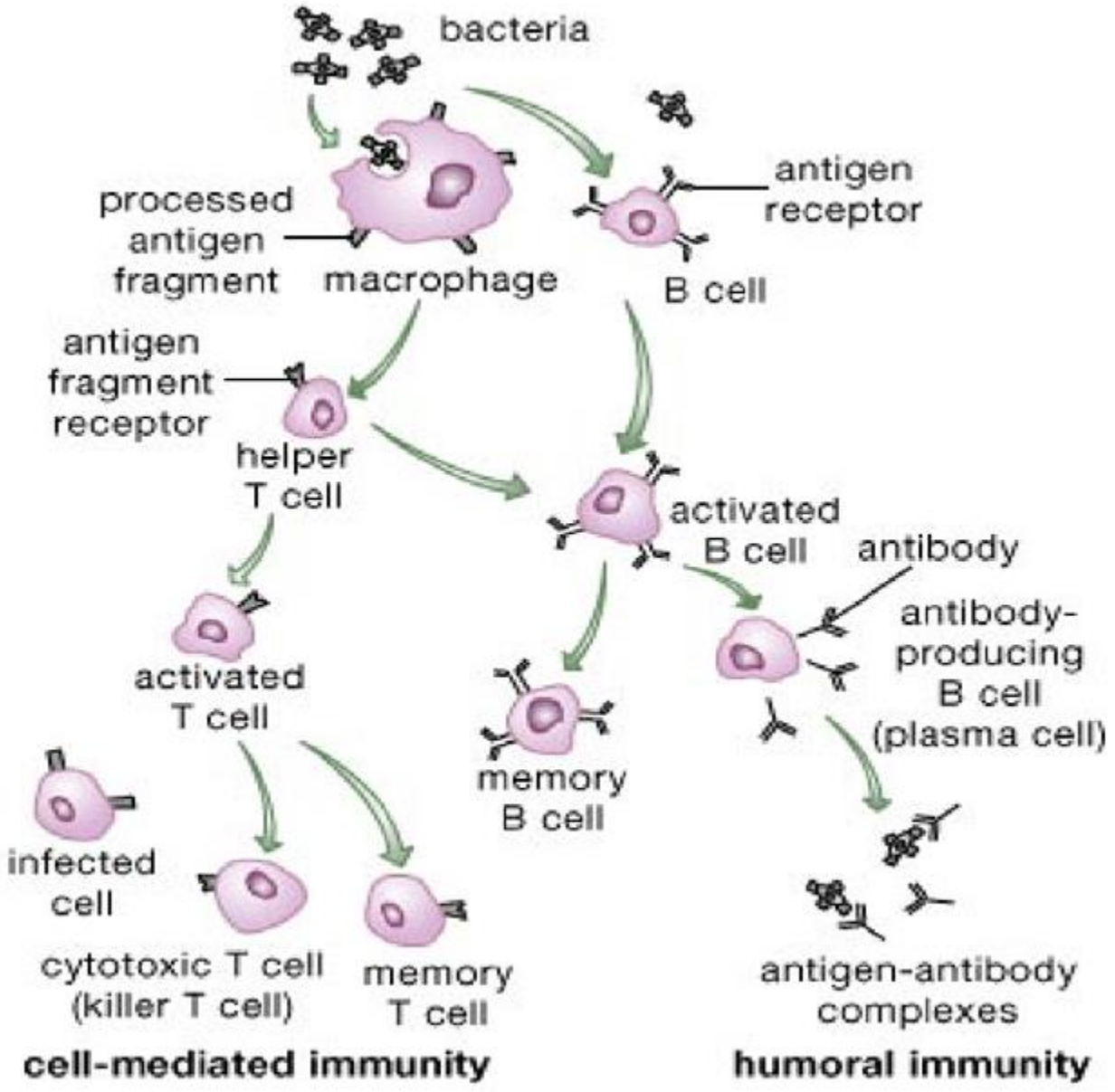
- **Artificial immune systems (AIS) compose a new computational intelligence approach inspired by theoretical and experimental immunology with applications to problem solving.**

- **Like all new approach, the field still lacks a more formal description and better theoretical foundations.**

- **The application of mathematical analysis and modeling to immunology may result in outcomes such as a deeper and more quantitative description of how the immune system works, a more critical analysis of hypothesis, it can assist in the prediction of behaviors and the design of experiments.**

- Immuno-informatics
- Epitope prediction tools

Summary of Acquired Immunity

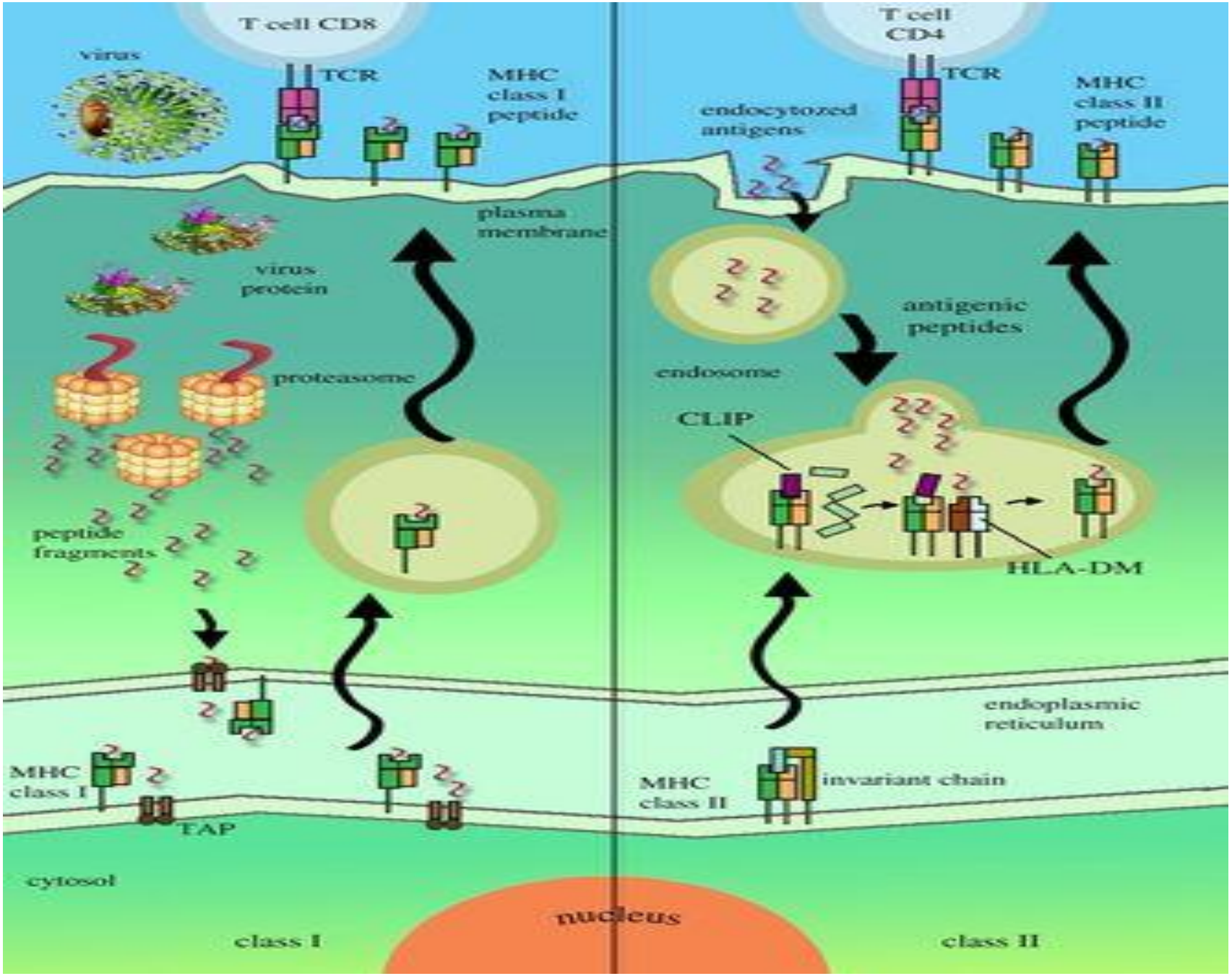


Combinatorial Science

- Immunoinformatics also known as computational immunology
- Astouding diversity of immune system components
- Complexity of the regulatory pathways
- Complexity of the network-type interactions

T-cell epitopes

- T-cell epitopes are presented on the surface of an antigen-presenting cell (APC), where they are bound to major histocompatibility (MHC) molecules in order to induce immune response
- MHC class I molecules usually present peptides between 8 and 11 amino acids in length, whereas the peptides binding to MHC class II may have length from 12 to 25 amino acids


T cell CD8
virus
TCR
MHC class I peptide
plasma membrane
virus protein
proteasome
peptide fragments
MHC class I
TAP
cytosol
class I
T cell CD4
TCR
MHC class II peptide
endocytozed antigens
antigenic peptides
endosome
CLIP
HLA-DM
endoplasmic reticulum
MHC class II
invariant chain
nucleus
class II

T cell Epitope Prediction Methods

- Binding affinity predictions for a range of MHC molecules is considered to be the most popular epitope prediction methods.
- Molecular binding between antigenic peptides and MHC molecules is necessary for recognition by cytotoxic T-cells.
- The peptides with ability to bind MHC molecules could be predicted by different methodologies including Hidden Markov model (HMM), Artificial neural networks (ANNs), Support Vector Machine (SVM), and Quantitative matrices.

Table 6. A list of reliable tools and databases for T-cell epitope prediction.

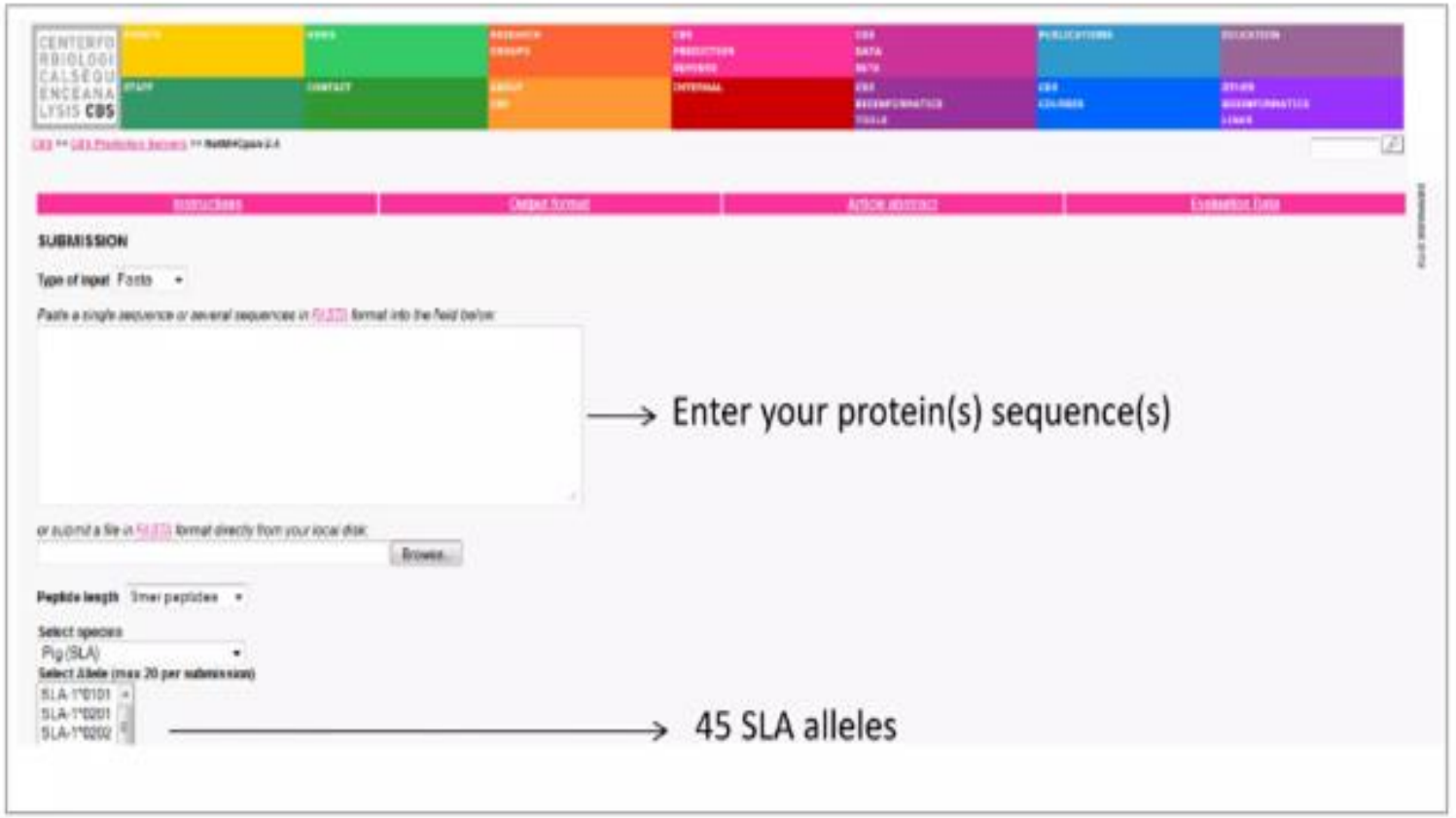
Database name	URL Link	Application
Allele frequencies	http://www.allelefrequencies.net	HLA frequencies in worldwide population and polymorphism frequencies in immunologically
MHCPred	http://www.ddg-pharmfac.net/mhcpred/MHCPred/	Quantitative prediction of peptide-MHC binding
MMBPred	http://www.imtech.res.in/raghava/mmbpred/	Prediction of atypical MHC class I binders as well as mutations that allow high-affinity binding
NetCTL	http://www.cbs.dtu.dk/services/NetCTL	Prediction of CTL/HLA supertype epitopes
NetMHC	http://www.cbs.dtu.dk/services/NetMHC	Prediction of the MHC binding propensity of peptides
NetChop	http://www.cbs.dtu.dk/services/NetChop	Prediction of proteasome/immunoproteasome cleavage
TAPPred	http://www.imtech.res.in/raghava/tappred/	Prediction of binding affinity of TAP protein
Pcleavage	http://www.imtech.res.in/raghava/pcleavage/	Prediction of proteasome/immunoproteasome cleavage
Propred	http://www.imtech.res.in/raghava/propred1/	Prediction of class II binding regions in antigenic protein sequence
ElliPro	http://www.tools.immuneepitope.org/tools/ElliPro	Prediction of linear and conformational antibody epitopes
EpiToolKit	http://www.epitoolkit.org	Prediction of MHC classes I/II ligands using several method
EpiVax	http://www.epivax.com	Prediction of classes I/II conserved and promiscuous epitopes
MAPPP	www.mpiib-berlin.mpg.de/MAPPP/cleavage.html	Prediction of proteasome cleavage sites
SYFPEITHI	http://www.syfpeithi.de	A database for MHC anchor motifs and binding specificity
IMGT®	http://www.imgt.org	A high-quality integrated resource of IG, TR, MHC, and related proteins
IMGT/HLA	http://www.ebi.ac.uk/imgt/hla/allele.html	A database with 5518 HLA class I and 1612 HLA class II alleles

NetMHCpan

- Predicts binding of peptides to any known MHC molecule using artificial neural networks (ANN).
- Trained on more than 115,000 quantitative binding data covering more than 120 different MHC molecules.
- MHC class I: humans, non-human primates (chimpanzee, rhesus macaque, gorilla), mice, pigs, and cattle.
- Includes the newest MHC allele releases from the IMGT/HLA & IPD-MHC databases.

https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1

NetMHCpan



NetMHCpan Prediction Results

NetMHCpan Server - prediction results

Technical University of Denmark

NetMHCpan version 2.4

Input is in FSA format

Peptide length 9

SLA-1:0201 : Estimated prediction accuracy 0.416 (using nearest neighbor HLA-A80:01)

Threshold for Strong binding peptides 50.000
Threshold for weak binding peptides 500.000

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pos	HLA	peptide	Identity	1-log50k(aff)	Affinity(nM)	%Rank	BindLevel
131	SLA-1*0201	ITYLNNMGY	gi_397586_emb_C	0.604	72.62	0.05	<= WB
440	SLA-1*0201	GILSYTSLY	gi_397586_emb_C	0.502	218.19	0.20	<= WB
458	SLA-1*0201	KVCNMLIAY	gi_397586_emb_C	0.486	259.13	0.25	<= WB
39	SLA-1*0201	YGAYMLFMY	gi_397586_emb_C	0.464	331.85	0.40	<= WB
569	SLA-1*0201	NVPDKMGLY	gi_397586_emb_C	0.452	375.69	0.40	<= WB
660	SLA-1*0201	GQYNLKLVY	gi_397586_emb_C	0.445	403.38	0.40	<= WB
687	SLA-1*0201	YITCPDSLY	gi_397586_emb_C	0.423	511.67	0.50	
998	SLA-1*0201	IVHRQCYKY	gi_397586_emb_C	0.420	532.53	0.80	
98	SLA-1*0201	LTQRPVMGY	gi_397586_emb_C	0.418	542.64	0.80	
22	SLA-1*0201	RTNAPLLFM	gi_397586_emb_C	0.417	546.54	0.80	
183	SLA-1*0201	VTPEDLVSY	gi_397586_emb_C	0.410	591.08	0.80	
162	SLA-1*0201	TTWIQLQHY	gi_397586_emb_C	0.409	597.13	0.80	

B-cell epitope prediction

- Mapping B-cell epitopes within the protein sequence of antigens plays a crucial role in vaccine design, immunodiagnostic design and immunogen design for antibody production efforts
- B-cell epitopes are determinant regions of the antigen with the ability to bind immunoglobulins and B-cell receptors (BCRs).
- Identification of B-cell epitopes capable of invoking strong immune responses plays a pivotal role in effective vaccine designing efforts

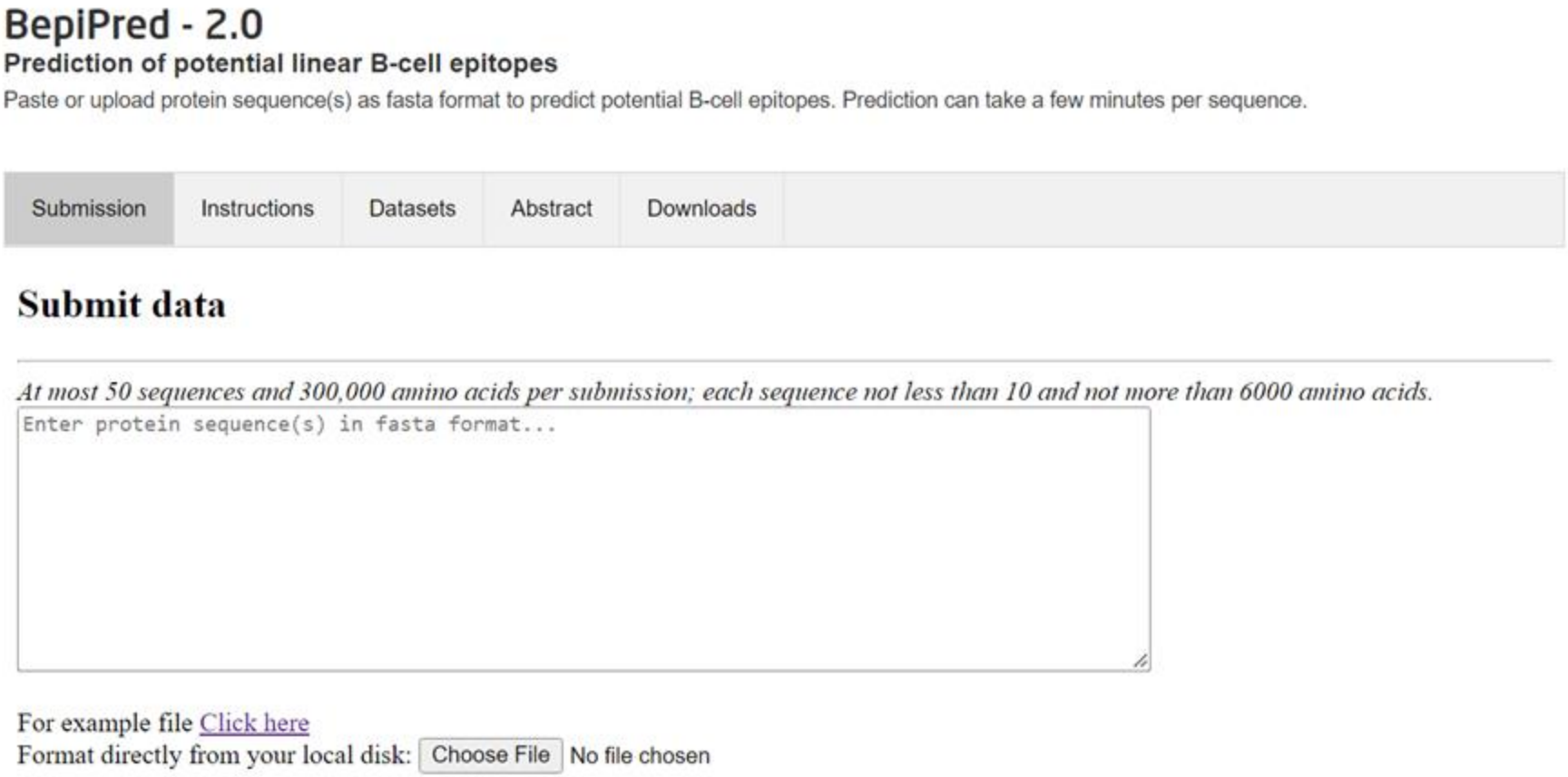
Table 7. A list of reliable tools and databases for B-cell epitope prediction.

Database name	Web address	Application
ABCpred	http://www.imtech.res.in/raghava/abcpred	Prediction of linear B-cell epitopes based on artificial neural net
BCIPEP	http://www.imtech.res.in/raghava/bcipep	B-cell epitope database
Bepipred	http://www.cbs.dtu.dk/services/BepiPred	Prediction of B-cell epitopes using HMM method
IMGT ®	http://www.imgt.org	A high-quality integrated resource of IG, TR, MHC, and related proteins
Bcepred	http://www.imtech.res.in/raghava/bcepred/	Prediction of epitopes with 58.7% accuracy
BEPITOPE	jlpellequer@cea.fr	Prediction of linear epitopes location and pattern
DiscoTope	http://www.cbs.dtu.dk/services/DiscoTope/	Prediction of conformational B-cell epitopes based on 3D structure
COBEpro	http://scratch.proteomics.ics.uci.edu	A two-stage system to predict linear B-cell epitopes, associated with the SCRATCH database
CEP	http://bioinfo.ernet.in/cep.htm	Prediction of B-cell epitopes
AgAbDb	http://www.115.111.37.206:8080/agabdb2/home.jsp	Antigen-antibody interaction database
MIMOP	Request from franck.molina@cpbs.univ-montp1.fr	prediction of 3D epitopic region from the mimotope peptide sequences
MIMOX	http://www.immunet.cn/mimox/	Epitope mapping based on phage display method
Pepitope	http://www.pepitope.tau.ac.il/	Epitope mapping based on affinity-selected peptides
3DEX	http://www.schreiber-abc.com/3dex/	Conformational epitopes mapping in 3D protein structures
IEDB	http://www.immuneepitope.org	Epitope prediction database
AntiJen	http://www.jenner.ac.uk/antijen/	Quantitative binding data for B-cell epitopes
CED	http://immunet.cn/ced	**C**onformational **E**pitope **D**atabase

BepiPred

- BepiPred-2.0 is based on a random forest algorithm trained on epitopes annotated from antibody-antigen protein structures.
- This new method was found to outperform other available tools for sequence-based epitope prediction both on epitope data derived from solved 3D structures, and on a large collection of linear epitopes downloaded from the IEDB database

https://services.healthtech.dtu.dk/service.php?BepiPred-2.0



Sequence Markup Types

- **Epitopes:** Positions above epitope threshold
- **Predictions:** The protein sequence displayed with orange gradient, illustrating BepiPred-2.0 predictions

Name	Sequence Markup
5C0N_A	Epitopes : .....EEEEEEEEEEEEEEEEEEEEEEE.....EEEE.....EEEEEEEEE..... Predictions: CDAFVGTWKLVSSENFDDYMKEVGVGFATRKVAGMAKPNMIISVNGDLVTIRSESTFKNTEISFKLGVEFDEITADDRKVKSIITLDGGA 1--------10--------20--------30--------40--------50--------60--------70--------80--------9

Discussion

- Immunoinformatics accelerate the knowledge acquisition in clinical immunology
- Bioinformatics has broad applicability to immunology
- Development of *in-silico* models of entire systems – towards a virtual immune system

THANK YOU