Immunology Introduction and Overview

Brief History and Prospect of Immunology

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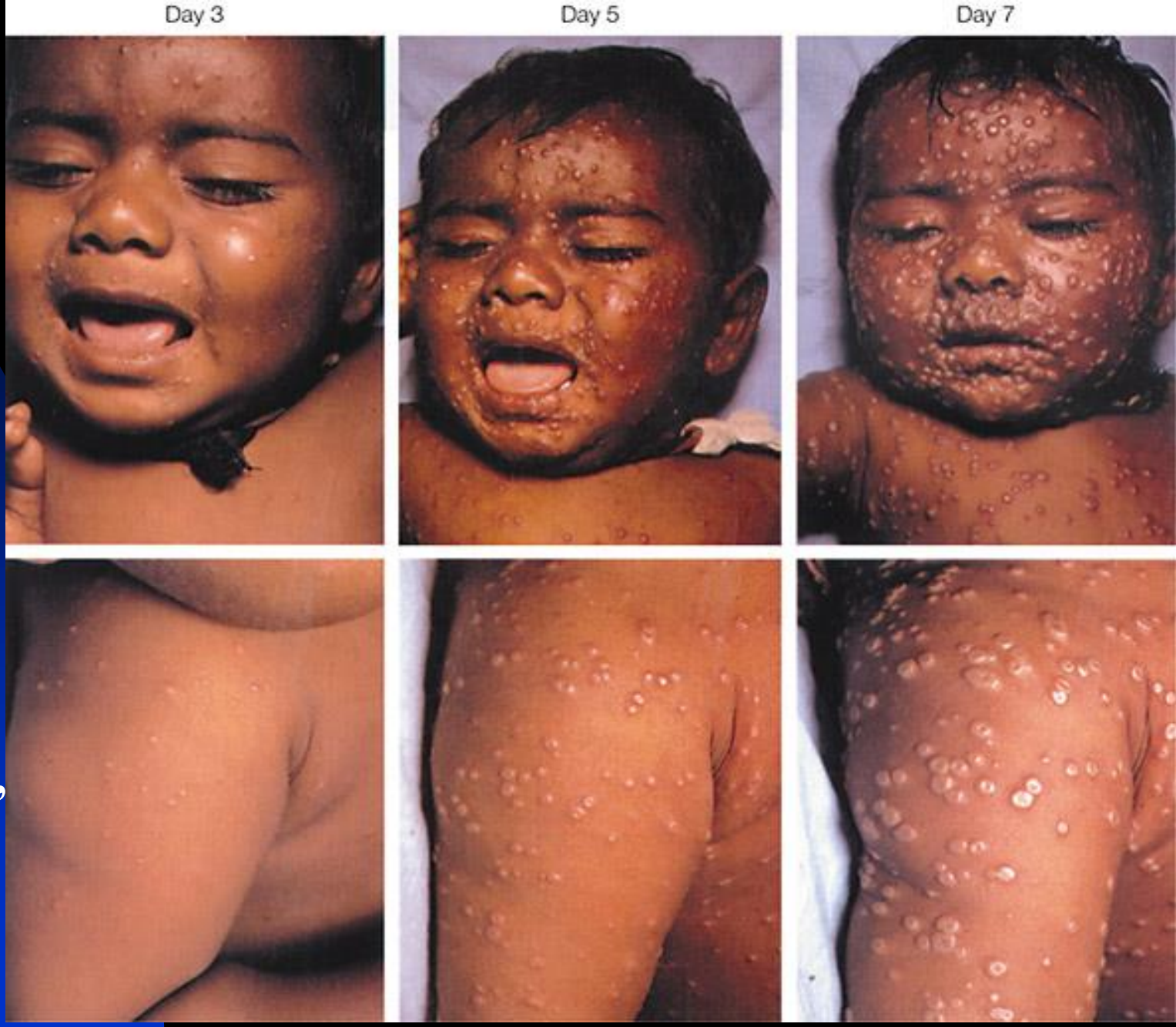
- Immunology is a relatively new science developing along with human combating against infectious diseases.
- The development of immunology has experienced 3 stages:
- Empirical immunology: from 16-17th centuries to 19th century
- Scientific Immunology: from the middle of 19th century to the middle of 20th century
- Modern Immunology: from the middle of 20th century to now.

JAMA 1999;281: 2127-37.



Smallpox

pustule

“pockmark”

variola virus

Empirical Immunology

- **Smallpox** is a contagious and often deadly disease. In the early days of immunology,
- there was no knowledge that variola is caused by variola virus.

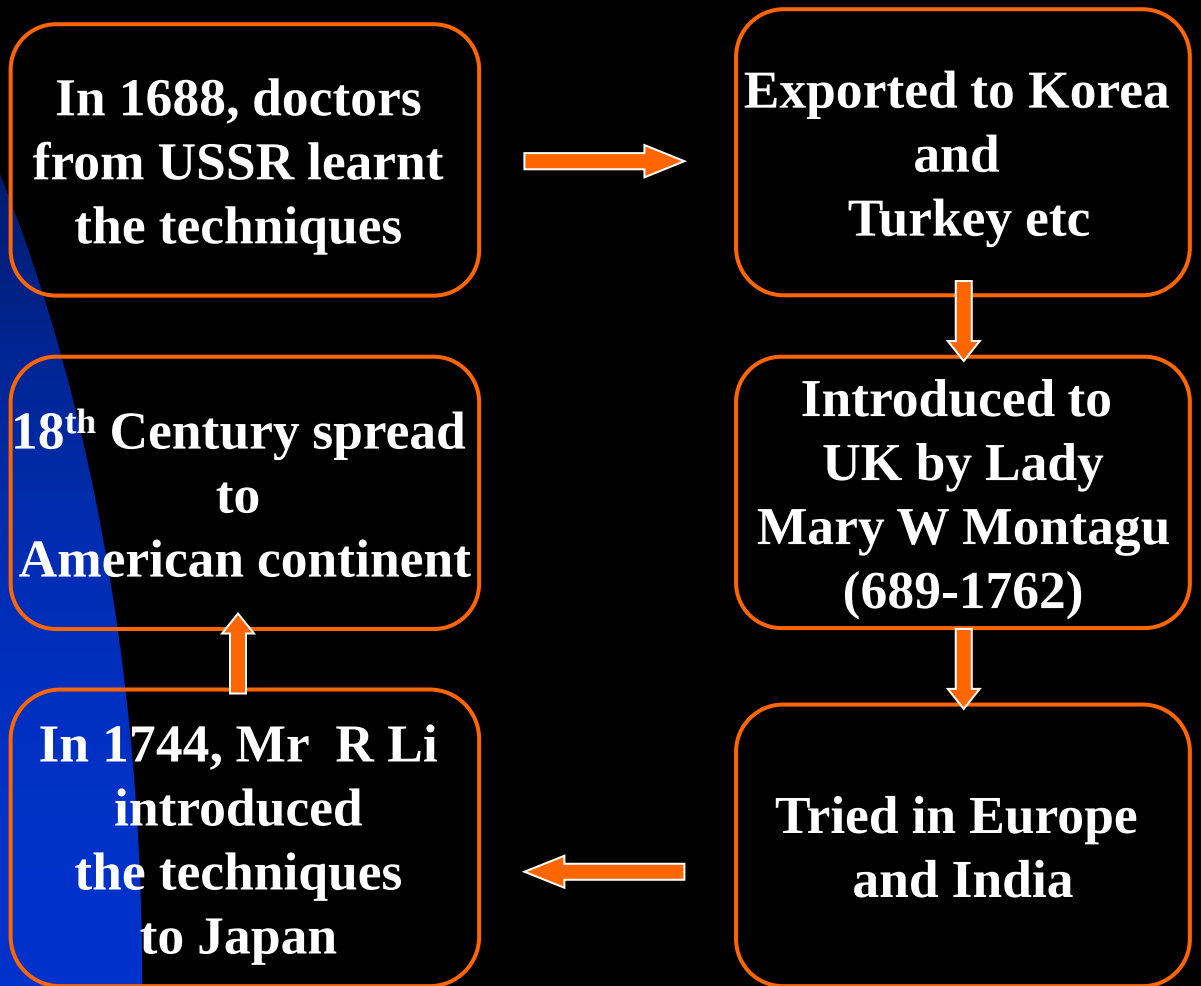
- But the ancient Chinese people observed that those who survived smallpox became immune to it and that deliberately infecting people with mild forms of smallpox could prevent infection with more deadly forms and provide life long protection.

- **Beginning around 1000 A.D, the ancient Chinese people practiced a form of immunization by inhaling dried powders derived from the crusts of smallpox lesions. The inoculation created a mild infection and resulted in immunity to smallpox.**

Chinese variolation

Eventually these methods, collectively known as variolation, reached Russia, Korea, Japan, England and other countries.

History of Immunology



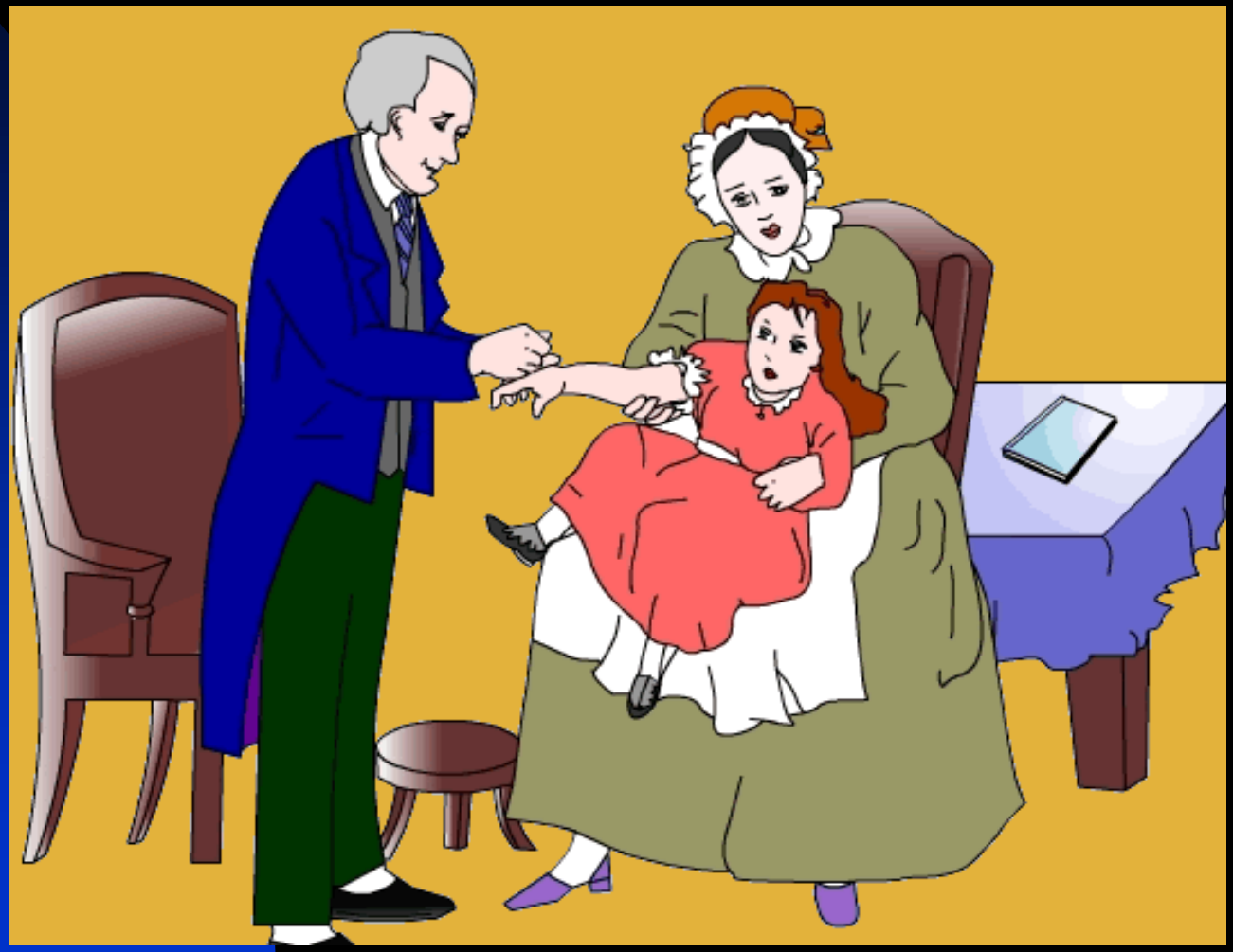
For example, the method was significantly improved by the English physician **Edward Jenner**.

In 1789, **Jenner** observed that milkmaids who contracted cowpox were thereafter resistant to smallpox. He began experiments with cowpox and

- smallpox in 1796. **Jenner** reasoned that introducing fluid from a cowpox pustule into people, that means inoculating them, might **protect them from smallpox**.

- He inoculated a boy named **James Phipps** with material obtained from a cowpox lesion and later intentionally infected the child with smallpox.

As predicted, the boy did not develop smallpox. By 1798 Jenner had published a booklet on the nature of cowpox and how if prevented variola.



Edward Jenner inoculating cowpox(1749-1823)

- After an initial few years of resistance
- and neglect, Jennerian vaccination became accepted.

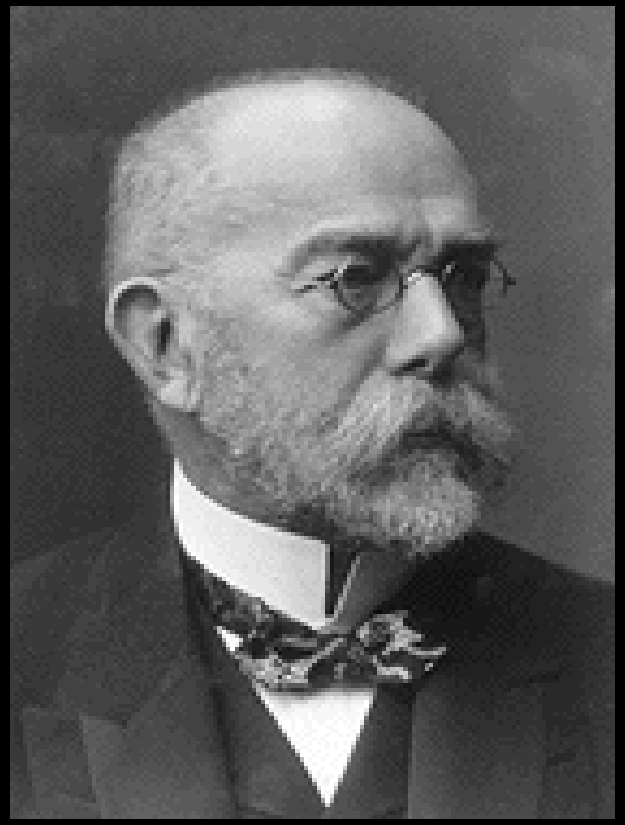
- In this stage, immunology was noted with "empirical" because the knowledge was obtained from the experiences and lacked for systematically scientific experimental supports.

Edward Jenner

1749-1823

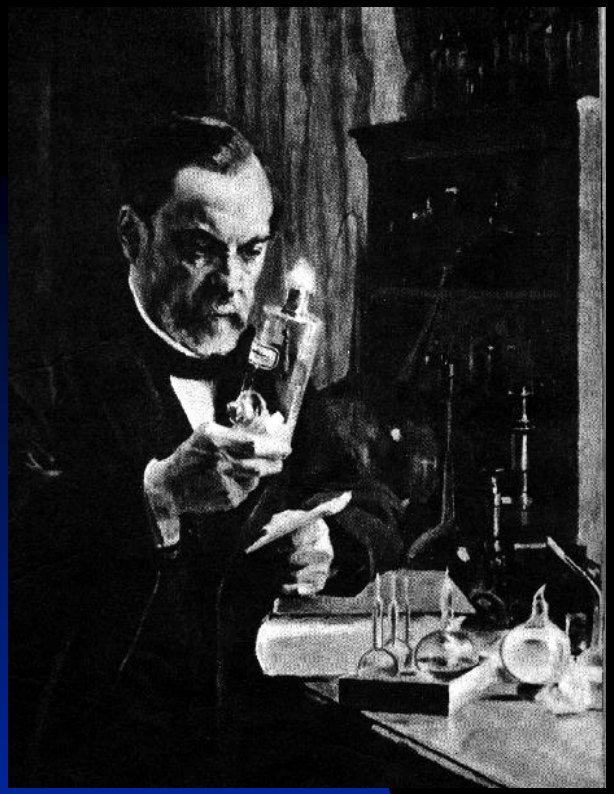
- **Scientific Immunology**

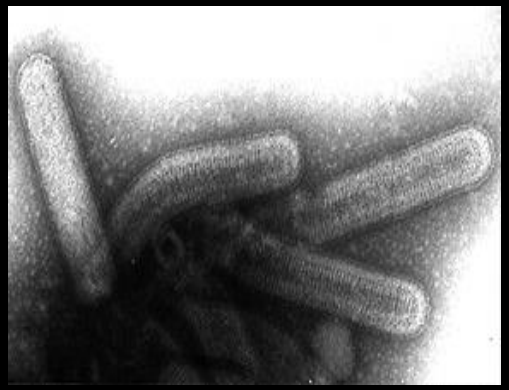
- **Although Jenner's technique of inoculation with cowpox to protect against smallpox spread quickly throughout Europe, it was nearly a hundred years before this technique was applied to other diseases.**
- **To further advance the fledgling science of immunology required the development of the germ theory of disease.**

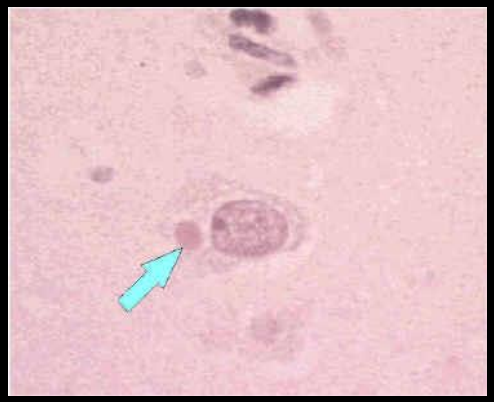


Louis Pasteur (1822-1895) **Robert Koch (1843-1910)**

Louis Pasteur, Robert Koch and other microbiologists of 19th century played a pivotal role in the evolution of the science.





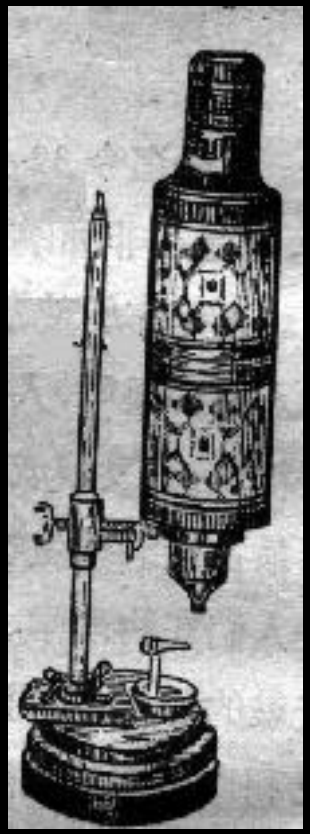


Pasteur was concerned with bacterial infectious disease, especially the prevention of disease that bacteria caused and how the human body was changed subsequent to infection so as to resist further insults. He became the first experimental immunologist.

France in 19th century, the ironsmith used the soldering iron to burn wound and “kill” the hydrophobia(rabies) ……

- **[I] Discovery of pathogens and application of vaccine**

- **Firstly, Anton van Leeuwenhock (1632-1723) developed the microscope.**
- **With microscope scientists were able to describe organisms invisible to the naked eye.**



50-300 times

Leeuwenhoek
1632-1723

Louis Pasteur was the first to isolate microorganisms from ferments and then introduce the microbes to fresh material to transfer the fermentation process.

He also demonstrated that this transfer could be stopped by heating (**pasteurization**).
(61.1~62.8 ℃ 30min or 71.7 ℃ 15-30s)

- In 1881, Pasteur first vaccinated sheep with
- heat- attenuated anthrax bacillus and then challenged the vaccinated sheep.
- All the vaccinated sheep survived and all the un-vaccinated sheep died.

- After that the most dramatic demonstration of a vaccine's effectiveness was with rabies.

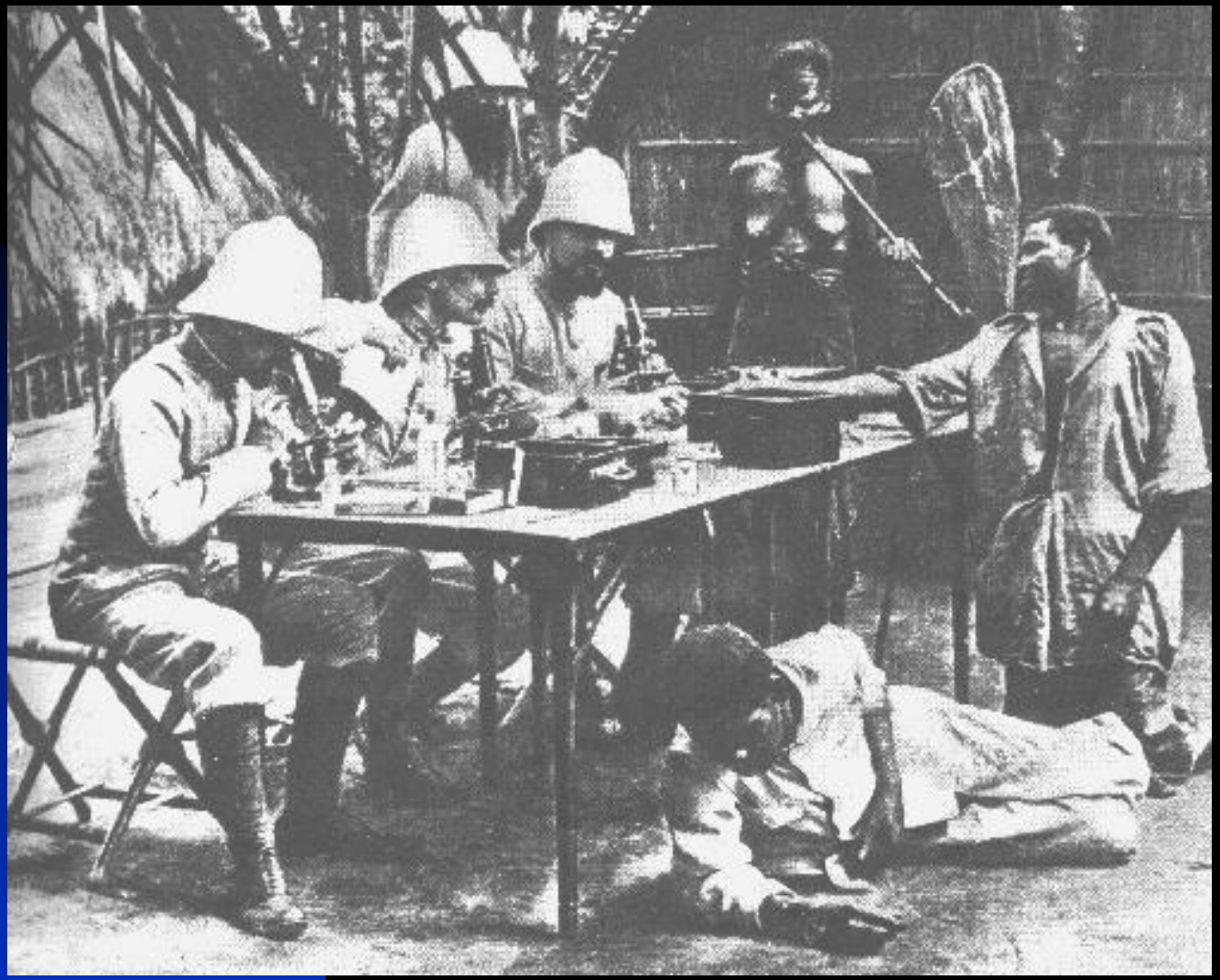
- Pasteur "selected" for variants of the virus that were less pathogenic for the fox.

- The first human trial was on July 6,
- 1885. Pasteur administered the attenuated virus into a young boy who had been bitten repeatedly by a rabid dog.

- The boy survived and Pasteur became the first experimental immunologist. These experiments marked the beginning of the discipline of immunology.

Robert Koch developed the pure culture techniques and became famous for the discovery of the tubercle bacillus and cholera bacillus.

On the whole, Pasteur and Koch were instrumental in defining microorganisms as an etiological agents of a large number of diseases.

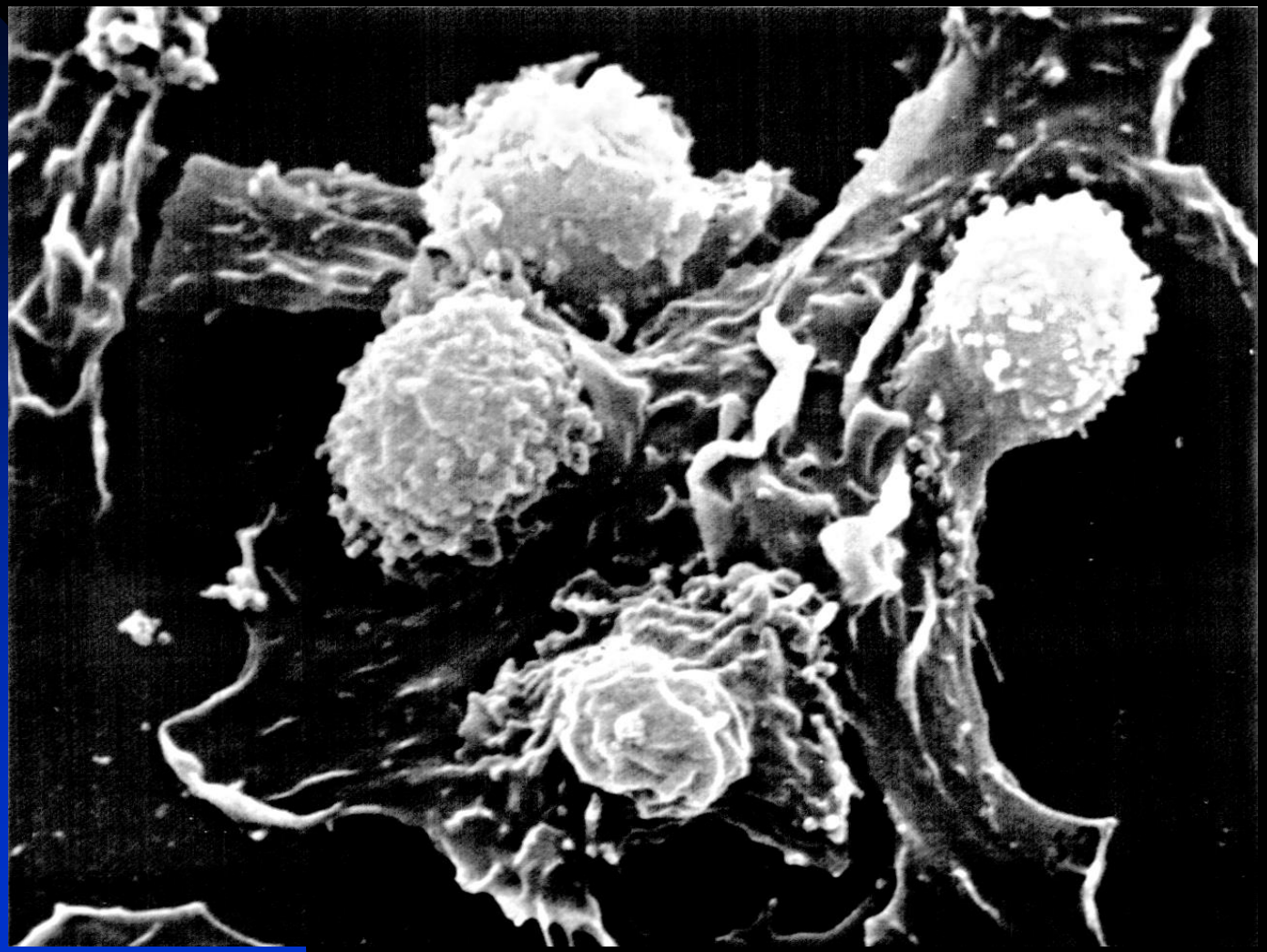


Robert Koch collecting blood from patients and diagnosing the "sleeping disease" in Eastern Africa

★ **"Trypanosomiasis"**

[II] Cellular immunity

- In 1883, **Elie Metchnikoff** demonstrated that the cells also contributed to an immune state of an animal.
- He observed that certain white blood cells, which he termed **phagocytes**, were able to ingest microorganisms and other foreign materials.



- With the emergence of improved cell culture techniques in the 1950s, the lymphocyte was identified as the cell responsible for both cellular and humoral immunity.

- Both systems are necessary for the immune response.

Humoral and Cellular immunity

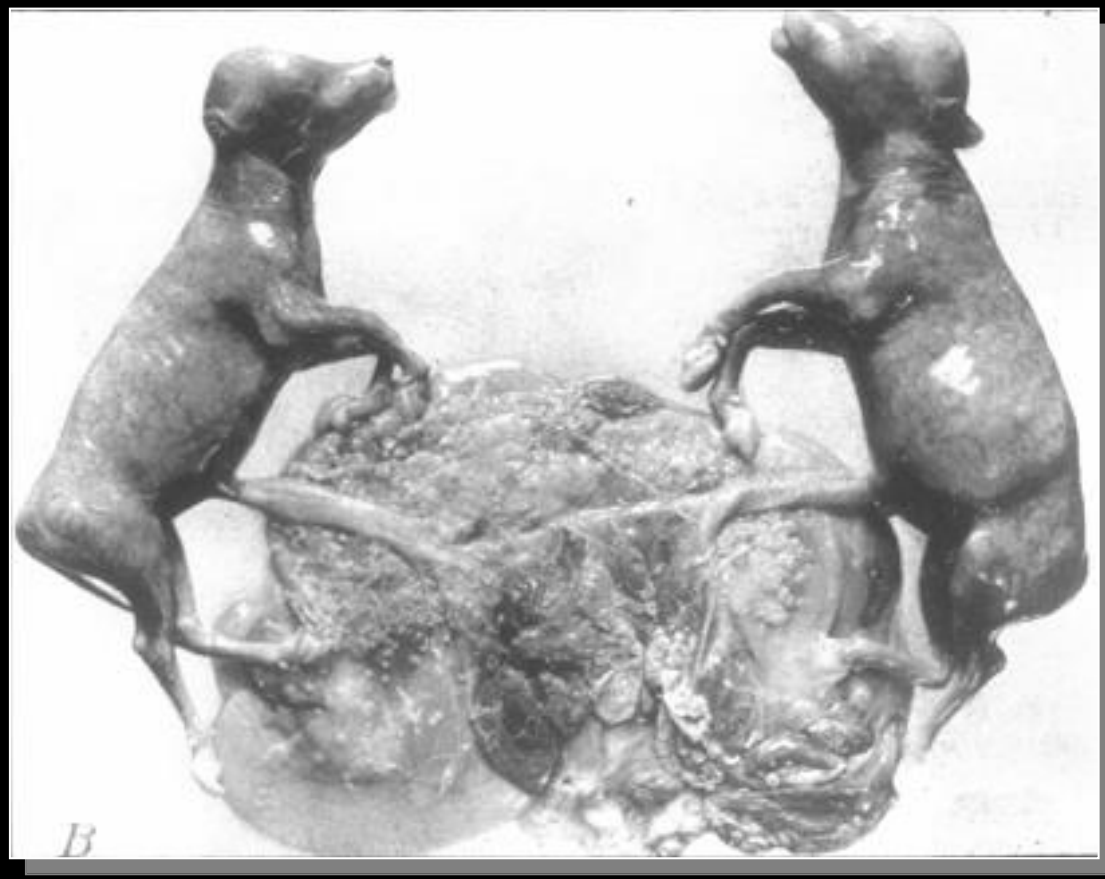
- **Humoral immunity** -
  - Secreted products of B lymphocytes
  - Antibodies or Immunoglobulins (Ig)
- **Cellular immunity** -
  - T lymphocytes. T cell receptor
  - Cytokines and cell-cell contact

[III]Discovery of immune tolerance and artificially induced immune tolerance

- Immunological tolerance is an important for several reasons. In 1945, Owen made a crucial observation, suggesting that tolerance to self-Ag occurred because the observing that adult dizygotic twin cows each contained a mixture of their own and their twin's blood cells, indicating that they were equally tolerant of their own and each other's blood cell Ag.
- "Chimera"

Discovery of immune tolerance and artificially induced immune tolerance

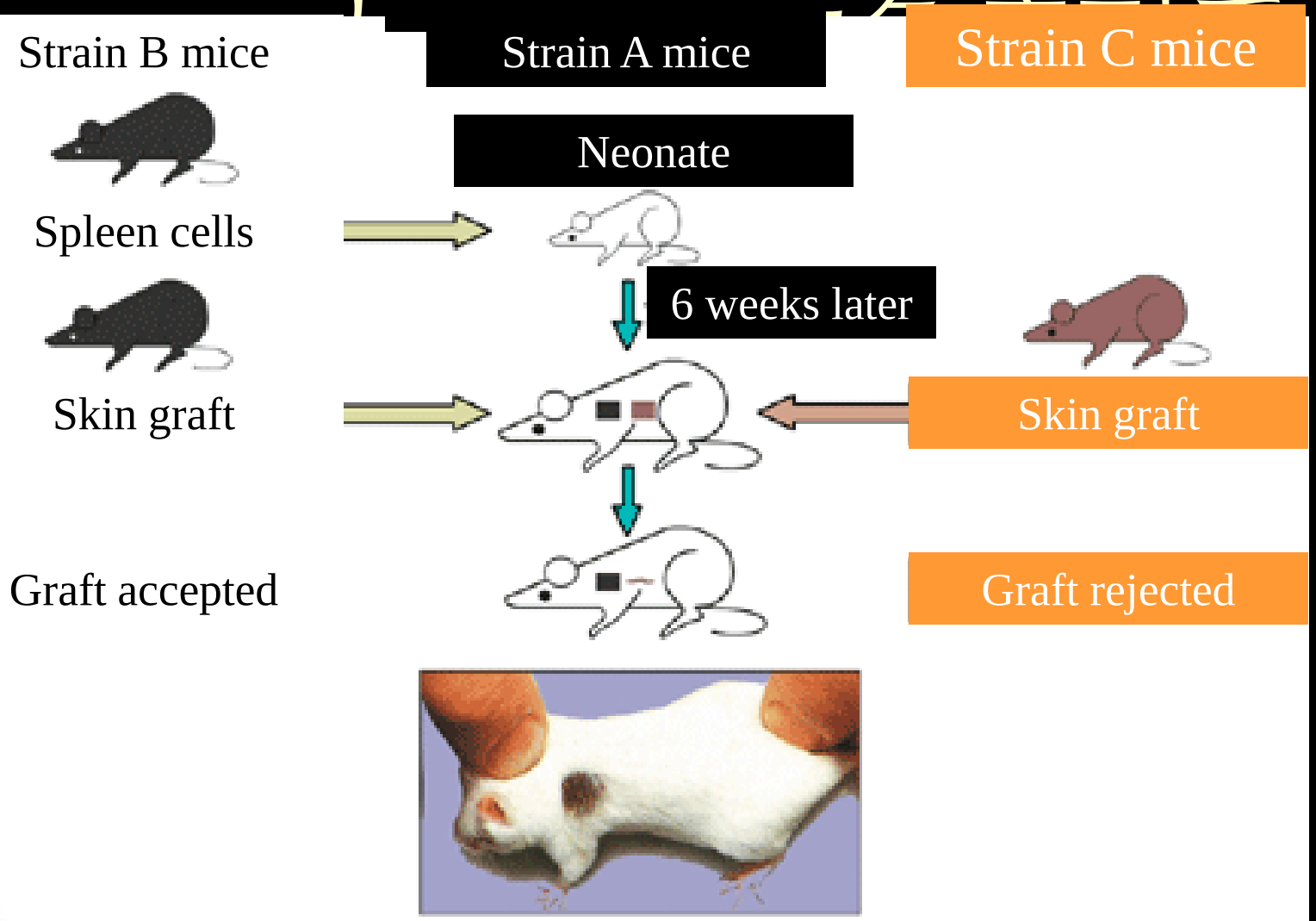
- Exposure to antigen in embryonic or neonatal period leads to immune tolerance. In 1945, Ray Owen first reported that in the embryonic exposure allotypes of Ag induced immune tolerance phenomenon.



- In 1953, Medawar carried out the first
- Lab experiments to explore the cellular basis of this immunological tolerance.

- He injected allogeneic tissues into fetal mice in uterus and found that after the animals reached maturity, they were greatly impaired in their ability to reject skin grafts from the same allogeneic mouse strain but not a third-party graft from a different allogeneic mouse strain.

Artificial induction of immune tolerance



Suggesting that tolerance easily to be induced in embryonic > neonatal period >

- This rejection deficiency could be corrected
- if the tolerant mice were given primed lymph node cell populations.

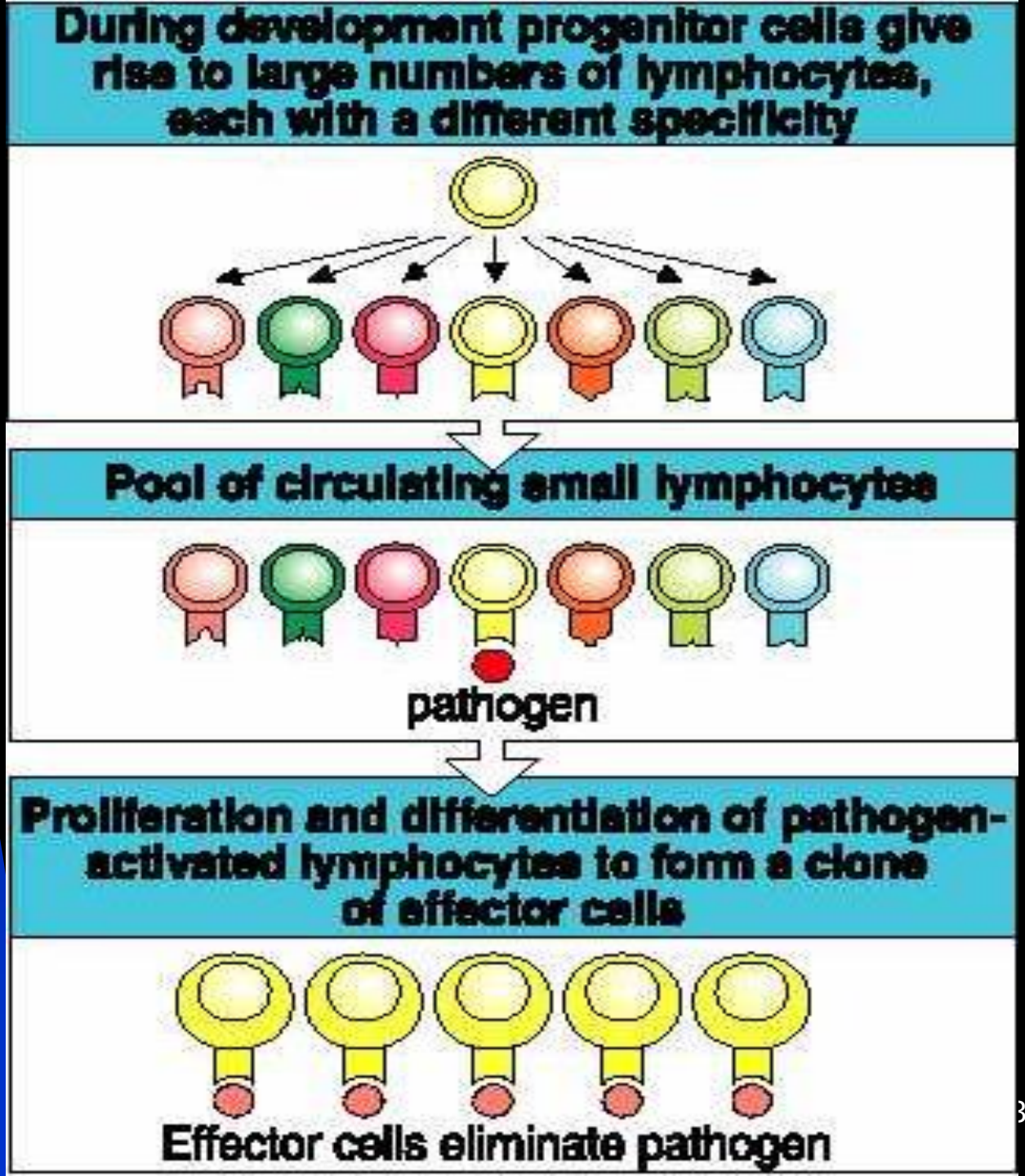
- The mechanism proposed by **Burnet** for this acquired tolerance process was **selective clonal deletion** of the lymphocytes specific for the alloantigens injected during development.

[IV] Burnet's clonal selection theory

- In 1957, Burnet enunciated the clonal selection theory, in which he explained the remarkable specificity as well as diversity of recognition of everything foreign in the environment.

- He proposed that each lymphocyte was specific for only one Ag and if a lymphocyte met this Ag during early development it would be deleted from the repertoire.

Clonal Selection theory



- **The clonal selection theory has been further refined and is now accepted as the underlying paradigm of modern immunology. It helped immunology to became a new science independent of microbiology.**

- **According to the theory, individual lymphocyte expresses membrane receptors that are specific for a distinct antigen (Ag). This unique receptor specificity is determined before the lymphocyte is exposed to the Ag.**

- Binding of Ag to its specific receptor activates
- the cell, causing it to proliferate into **a clone of cells** that have the same immunologic specificity as that of the parent cells.

- Lymphocytes with receptors against self are deleted from an early stage or became **forbid clone** and are absent from the repertoire of mature lymphocytes.

- **Modern Immunology**

- **Burnet's clonal selection theory opened a new era of immunology in the middle of 20th century .**

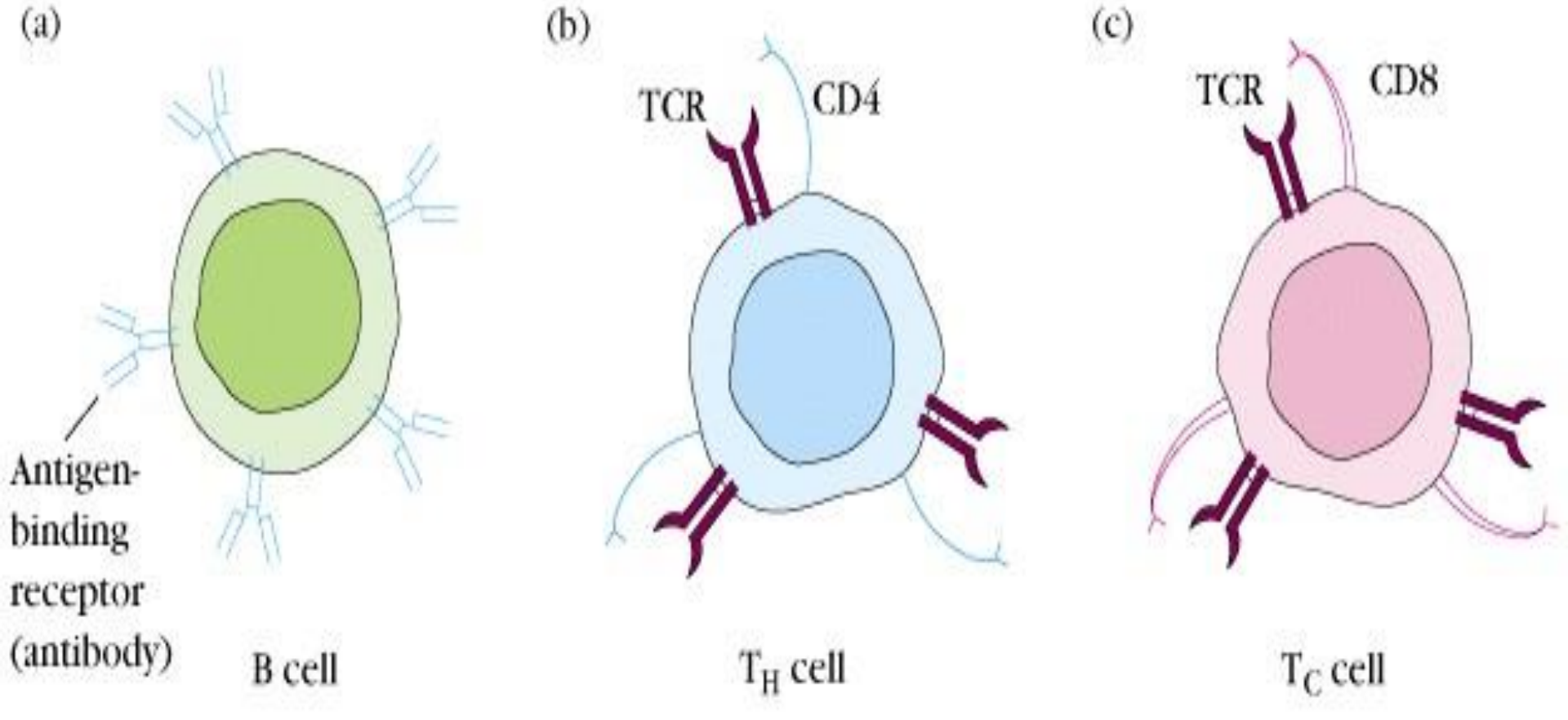
- **This theory provided a relatively correct opinion about how the immune system defends the body against non-self invaders without injuring self.**

[I] Discovery of Ag receptors

- According to **Burnet's clonal selection theory**, individual lymphocyte expresses membrane receptors that are specific for a distinct Ag. This unique receptor specificity is determined before the lymphocyte is exposed to the Ag.
- However, it has been estimated that each person is capable of producing at least 10^8 different antibody (**Ab**) molecules, each with own distinct properties.

Recognition of Antigen by Lymphocytes

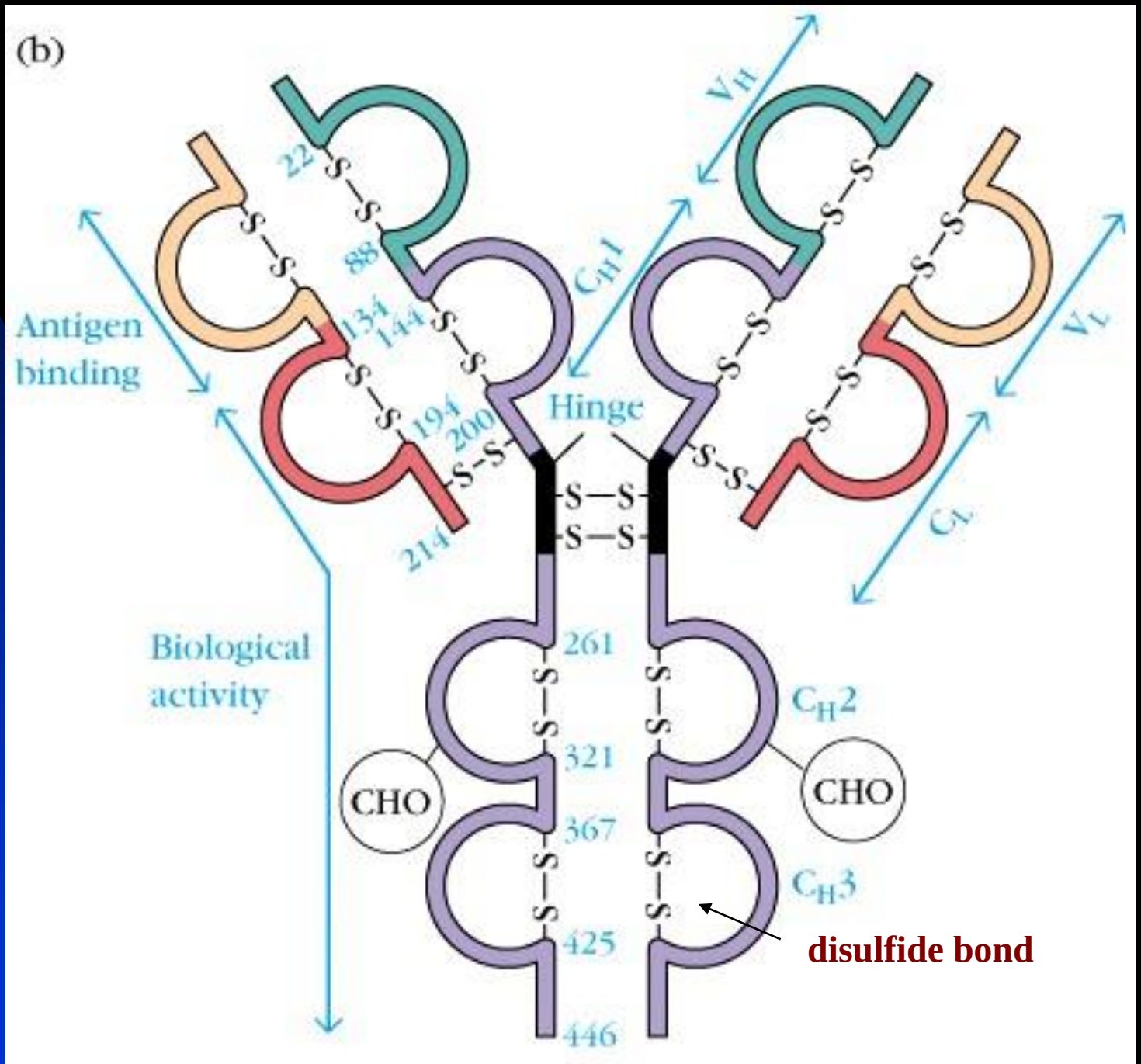
(B cells and T cells)



- How can so many different receptors be
- encoded in the genes of every human being?

- In 1965, W. Dreyer and J. Bennett first suggested that two separate genes encode a single immunoglobulin heavy or light chain, one gene for the variable region, the other for constant region. They proposed that hundreds of thousands of V-region genes and only single copies of C region class and subclass were carried in the germ line.

Antibody


(b)
Antigen binding
Biological activity
Hinge
V_H
C_H1
V_L
C_L
C_H2
C_H3
CHO
CHO
22
88
134
144
194
200
214
261
321
367
425
446
disulfide bond

- **The results demonstrated that the inherited chromosomes contained no Ig genes at all, but only the building blocks from which these genes could be assembled.**

- **Because of the astonishing discovery, Susumu Tonegawa was awarded the Nobel Prize of 1987.**

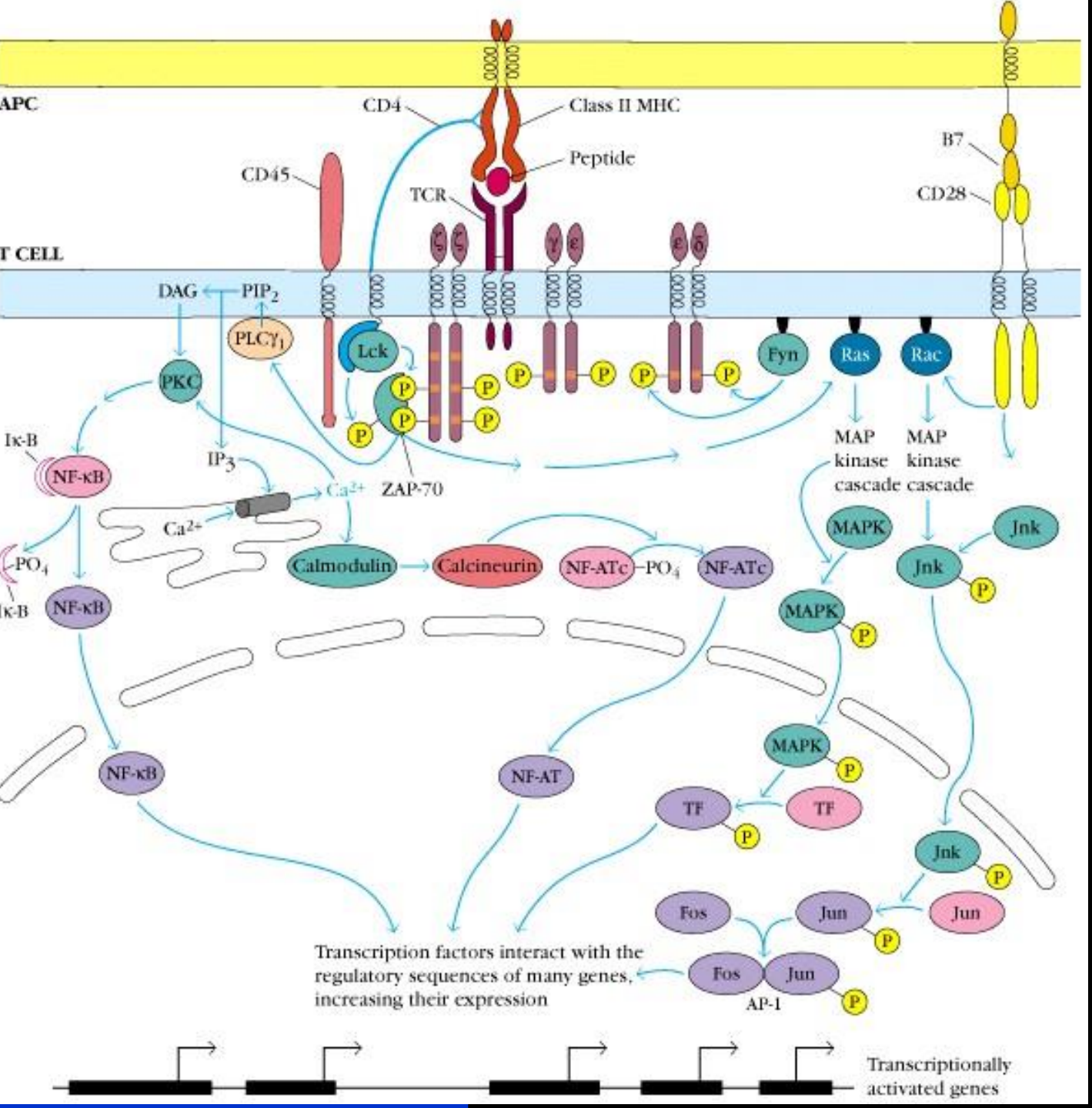
[II] Discovery of signal transduction pathway

The discovery of signal pathway provides a deeper insight into activation of lymphocytes and functions of cytokines.

Lymphocytes Ag receptor signal for cell activation uses signal transduction mechanisms common to many intracellular signal pathways.

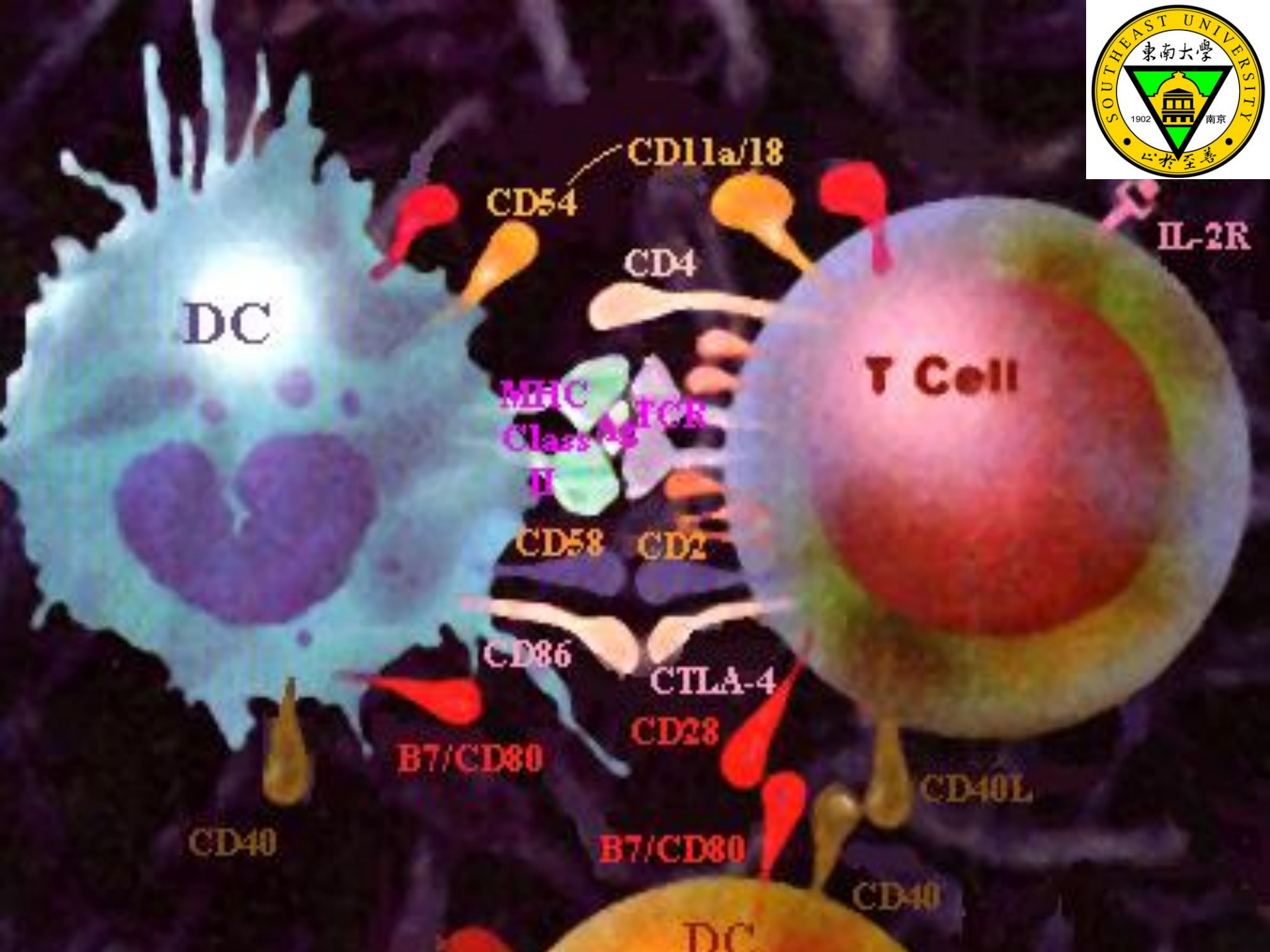
- The Ag receptors on B cells and T cells are
- multi-protein complexes made up of clonally variable Ag-binding chains that are associated with accessory molecules.

- These accessory molecules are required both for transport of the receptors to the cell surface and, most importantly, for initiating signaling when the receptors bind to an extra-cellular ligand.
- **Let's see Figures!**



Signal Transduction

- Receptor x-linking
- Synapse (rafts)
- Second Messengers
- Protein Kinases
- Protein phosphatases
- Induced Assembly Cascades
- G Proteins
- Transcription factors
- Promoter Accessibility


CD11a/18
CD54
CD4
IL-2R
DC
T Cell
MHC
Class
II
TCR
CD58
CD2
CD86
CTLA-4
CD28
B7/CD80
CD40L
CD40
B7/CD80
CD40
DC

TNF/TNFR Family

Receptor	Ligand
CD40	CD40L
OX40	OX40L
4-1BB	4-1BBL
CD30	CD30L
CD27	CD70
RANK	RANKL
CD95	CD95L
TNFR	TNF
HVEM/DcR3/LTβR	LIGHT

Immunoglobin Superfamily

Receptor	Ligand
CD28	B7-1/B7-2
CTLA-4	B7-1/B7-2
ICOS	B7RP-1/ B7-H/GL50
PD-1/?	B7-H1/B7-DC
CD2	LFA-3
LFA-1	ICAM-1/2/3
?	B7-H3
?	B7x/B7-H4
BTLA	HVEM/LIGHT

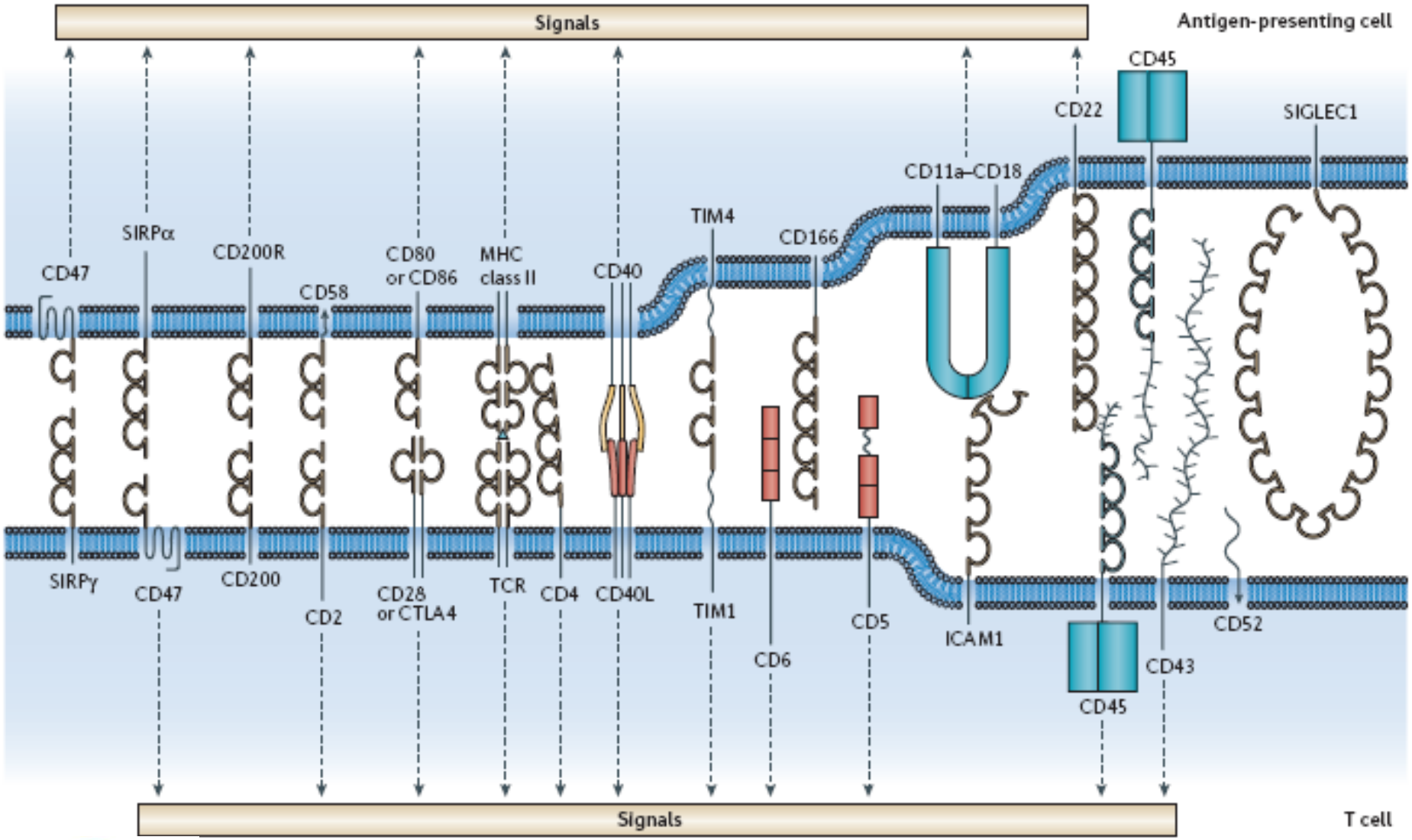
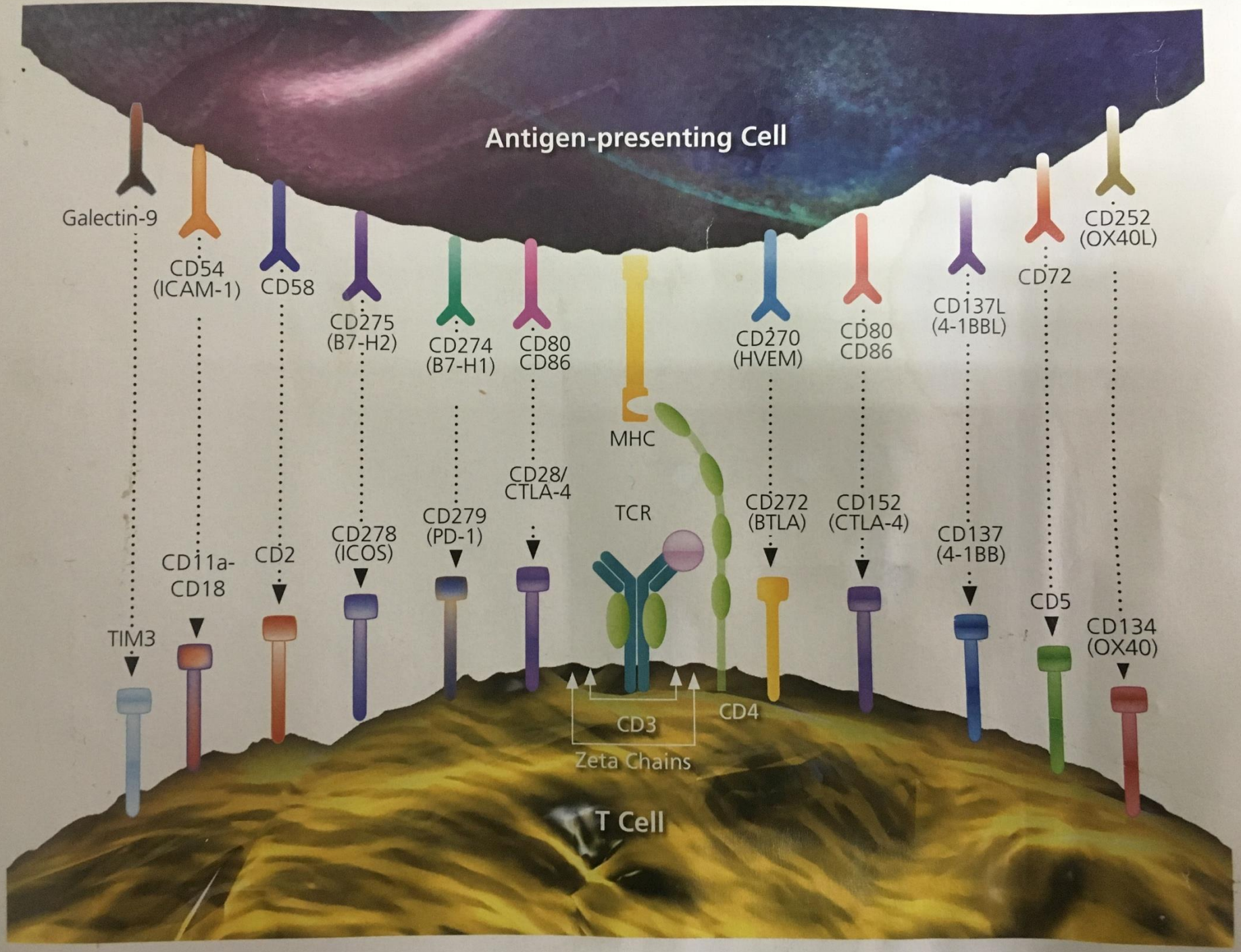


Figure 2 | **The topology of interactions between an antigen-presenting cell and a T cell.** Some of the interactions between plasma-membrane proteins on a T cell and an antigen-presenting cell are shown. The molecules are drawn to their relative approximate sizes based on electron microscopy and X-ray crystallography data, together with predictions from domain organization[45,46]. CD40L, CD40 ligand; CD200R, CD200 receptor; CTLA4, cytotoxic T-lymphocyte antigen 4; ICAM, intercellular adhesion molecule; SIGLEC1, sialic-acid-binding immunoglobulin-like lectin; SIRP, signal-regulatory protein; TCR, T-cell receptor; TIM, T-cell immunoglobulin domain and mucin domain.

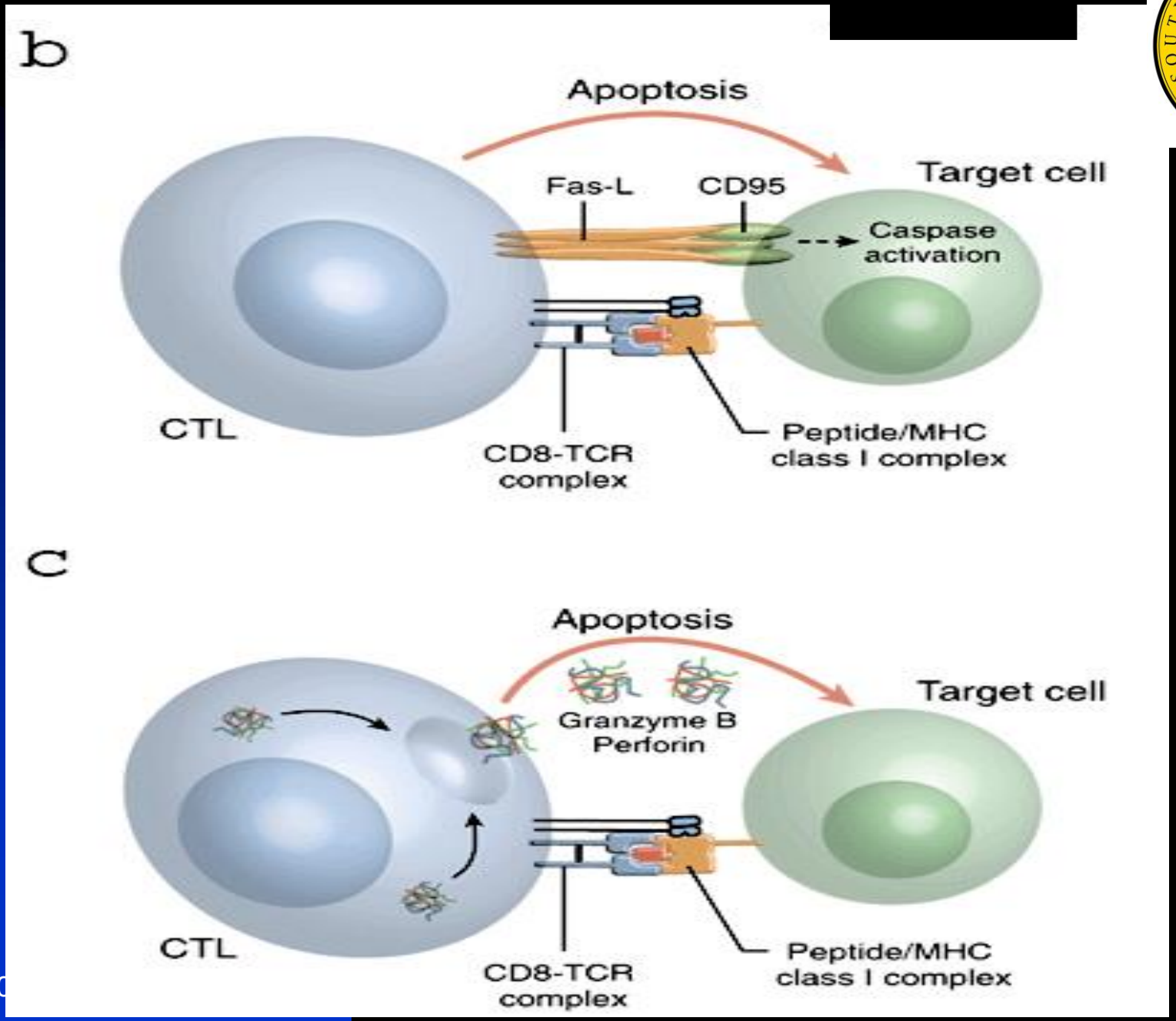

Antigen-presenting Cell
Galectin-9
CD54 (ICAM-1)
CD58
CD275 (B7-H2)
CD274 (B7-H1)
CD80 CD86
MHC
CD270 (HVEM)
CD80 CD86
CD137L (4-1BBL)
CD72
CD252 (OX40L)
TIM3
CD11a-CD18
CD2
CD278 (ICOS)
CD279 (PD-1)
CD28/ CTLA-4
TCR
CD272 (BTLA)
CD152 (CTLA-4)
CD137 (4-1BB)
CD5
CD134 (OX40)
CD3
Zeta Chains
CD4
T Cell

- **[III] Discovery of programmed cell death**
- **Under some circumstances, cells respond to environment or internal signals by committing suicide ----a phenomenon known as programmed cell death, or apoptosis.**

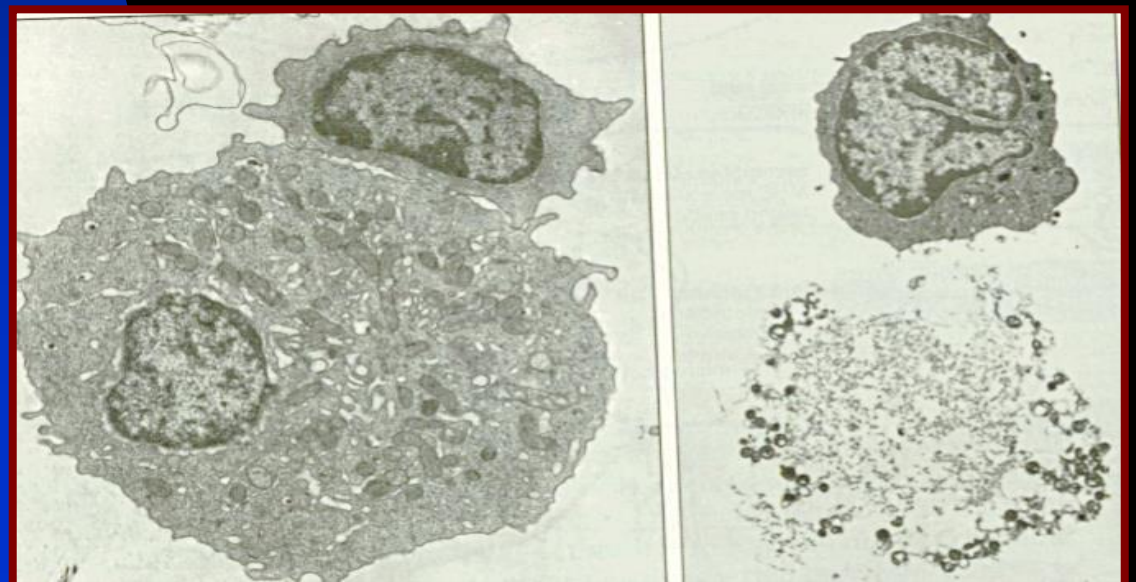
- **Such programmed deaths are extremely common in many cell types and, in fact, are essential for maintaining stable cell populations by ensuring that rate of new cell production is balanced by an equal rate of cell death.**

- The biochemical processes that occur during
- the final phase of **apoptosis** are thought to be similar in all cell types.

- One important receptor for death signal is a surface protein called **Fas**. Many cells express **Fas** when they are suicide-prone because it allows them to be killed by other cells expressing **Fas** ligand (**FasL**)


b
Apoptosis
Target cell
Fas-L
CD95
Caspase
activation
CTL
CD8-TCR
complex
Peptide/MHC
class I complex
c
Apoptosis
Target cell
Granzyme B
Perforin
CTL
CD8-TCR
complex
Peptide/MHC
class I complex

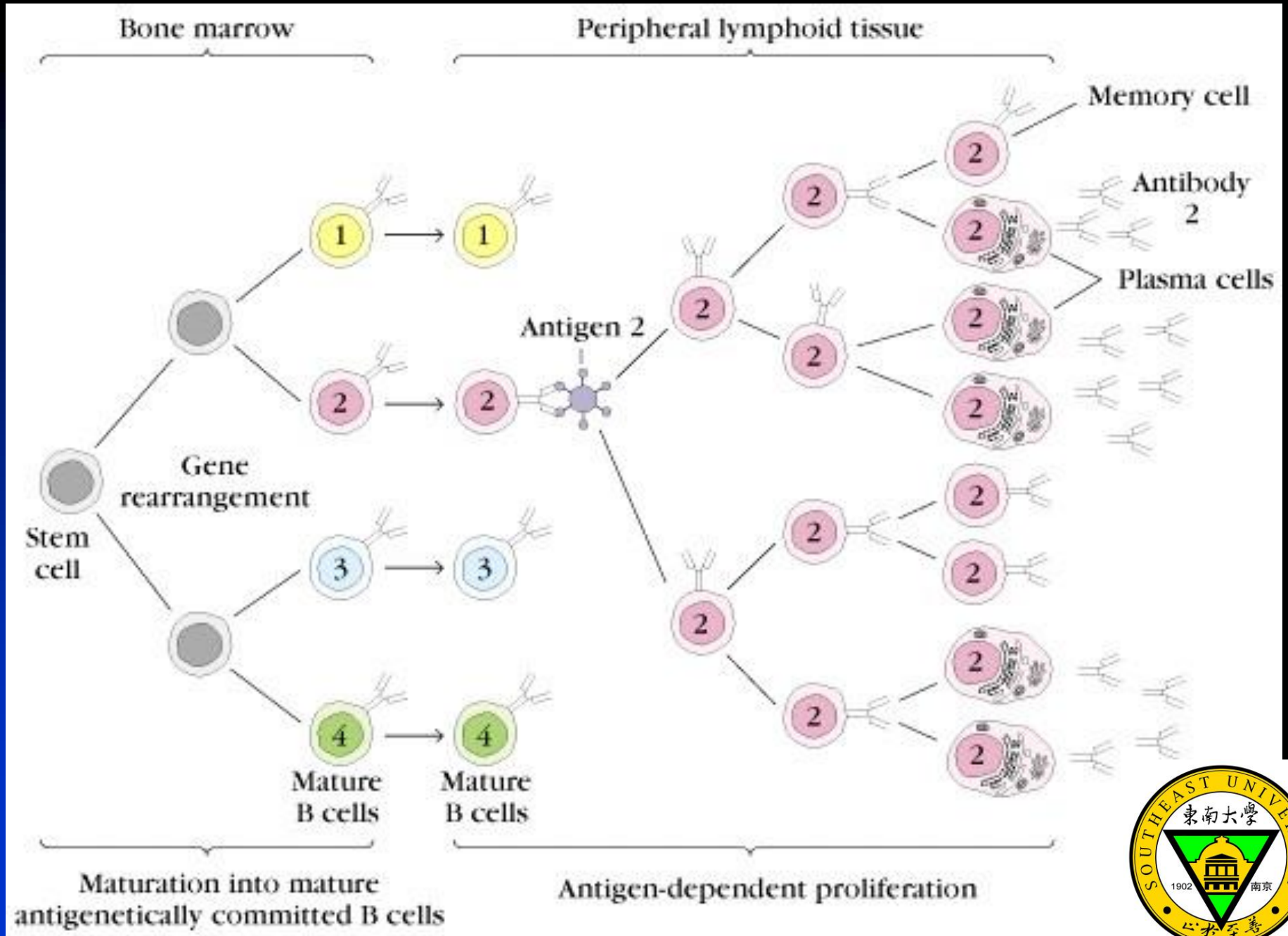
- When Fas binds FasL, Fas associated protein
- with death domain (FADD) is recruited and binds Fas, followed by recruitment of procaspase 8. The association FADD with procaspase 8 results in the proteolytic cleavage of procaspase 8 into its active form; caspase 8 then initiates a proteolytic cascade that lead to the death of the cell.
-

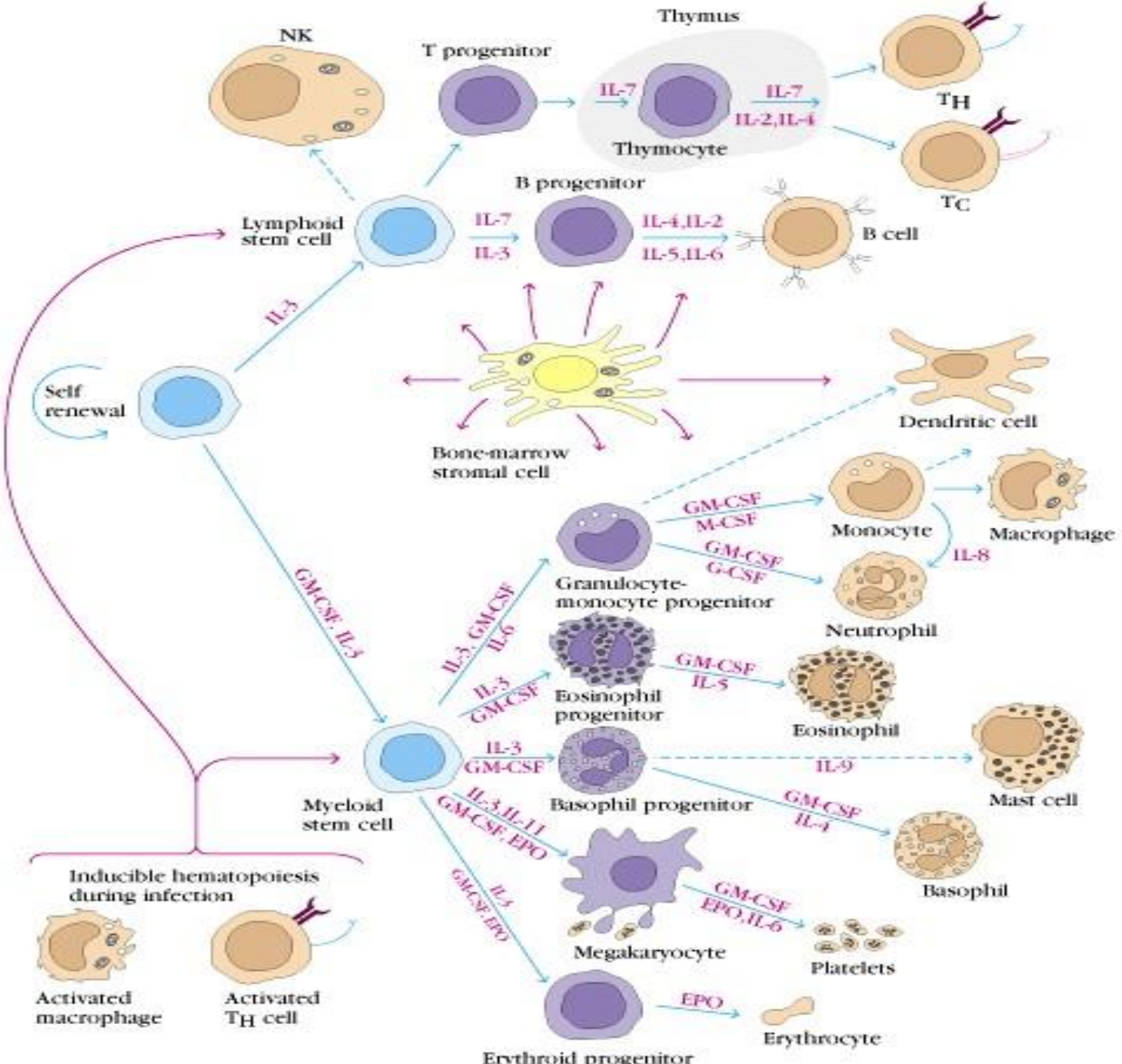


[IV] Hematogenesis and development of immune cells

All immune cells are ultimately derived from a type of cells called hematopoietic stem cells (HSCs).

A HSCs is multipotent, or pluripotent, able to differentiate in various ways and thereby generate all of the blood cell types.

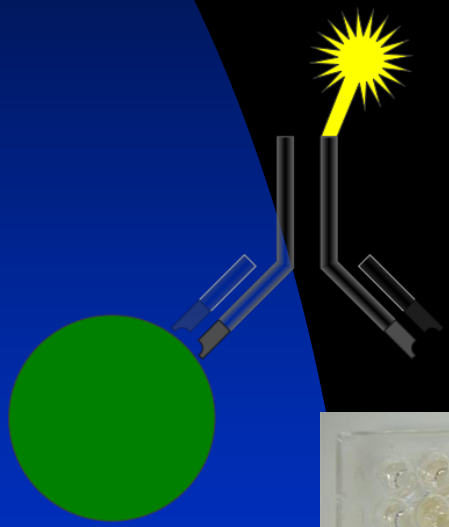

Bone marrow
Peripheral lymphoid tissue
Memory cell
Antibody 2
Plasma cells
Antigen 2
Gene rearrangement
Stem cell
Mature B cells
Mature B cells
Maturation into mature antigenetically committed B cells
Antigen-dependent proliferation

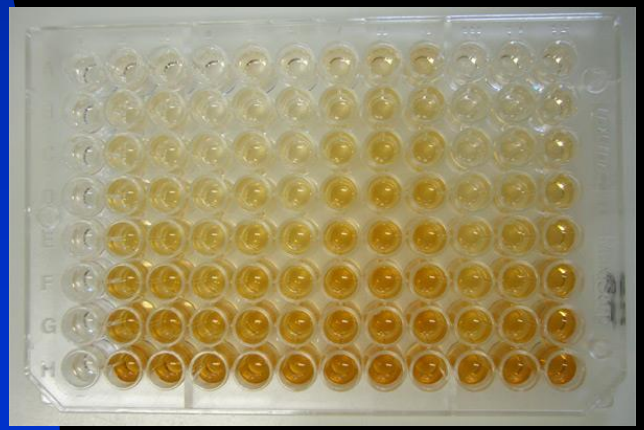

NK
T progenitor
Thymus
IL-7
Thymocyte
IL-7
IL-2,IL-4
TH
TC
B progenitor
Lymphoid stem cell
IL-7
IL-3
IL-4,IL-2
IL-5,IL-6
B cell
IL-3
Self renewal
Bone-marrow stromal cell
Dendritic cell
GM-CSF, IL-3
IL-3, GM-CSF IL-6
GM-CSF M-CSF
GM-CSF G-CSF
Granulocyte-monocyte progenitor
Monocyte
Macrophage
IL-8
Neutrophil
IL-3 GM-CSF
Eosinophil progenitor
GM-CSF IL-5
Eosinophil
IL-3 GM-CSF
Basophil progenitor
IL-9
Mast cell
GM-CSF IL-4
Basophil
Myeloid stem cell
IL-3,IL-11 GM-CSF,EPO
IL-3 GM-CSF,EPO
GM-CSF EPO,IL-6
Megakaryocyte
Platelets
Inducible hematopoiesis during infection
Activated macrophage
Activated TH cell
EPO
Erythrocyte
Erythroid progenitor

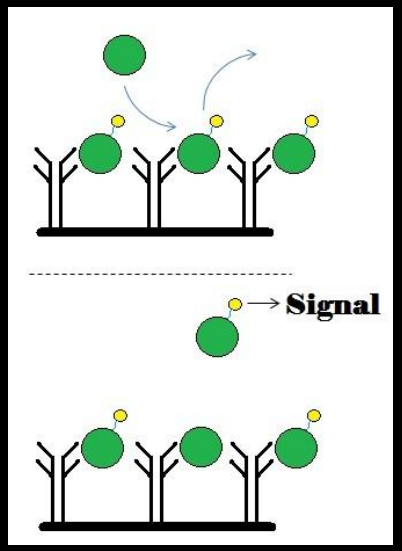
Immunological application

1. Application of the Ag-Ab interaction

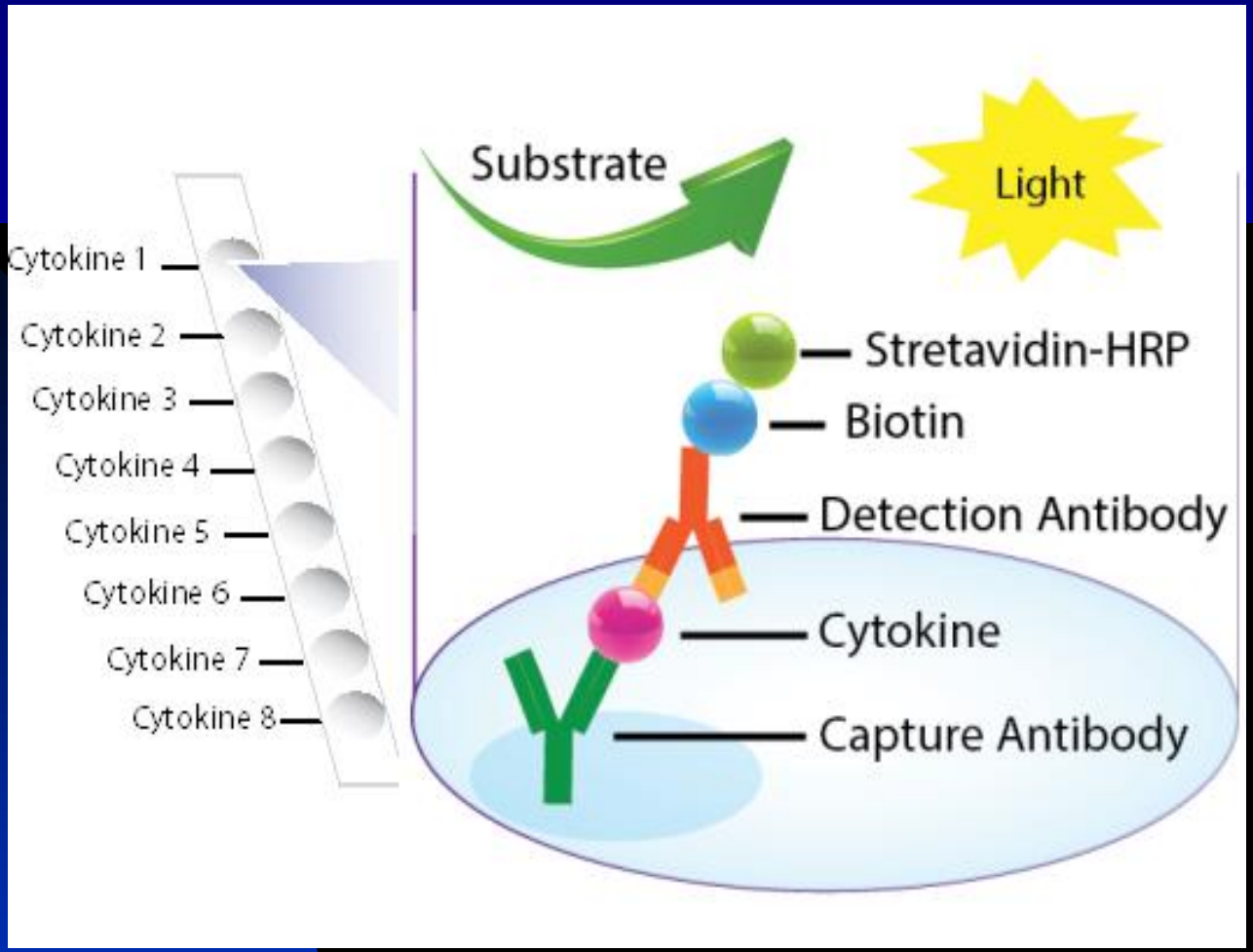
Several techniques based on immunological principles are also widely applied.



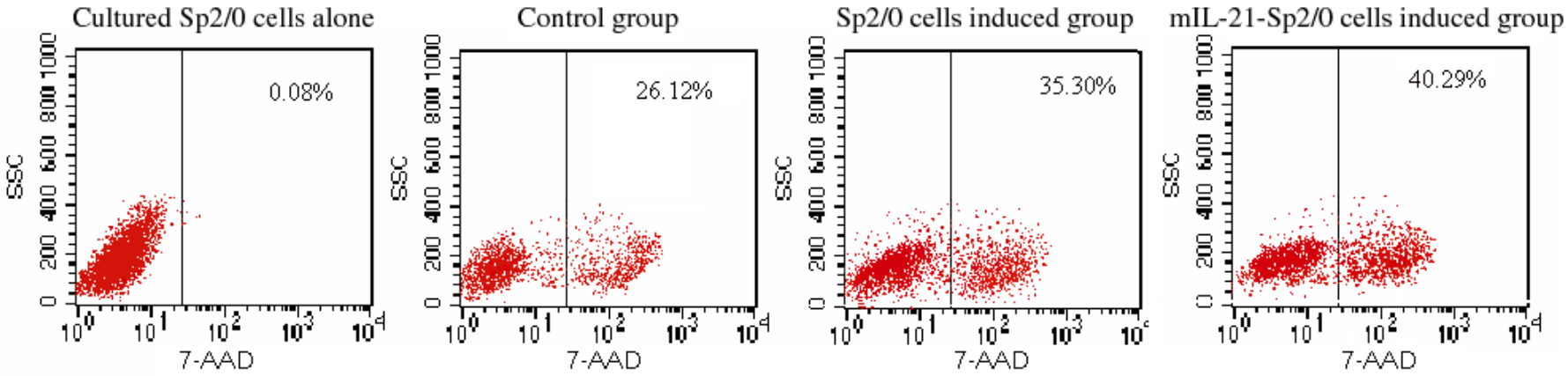




Enzyme linked immunosorbent assay (**ELISA**)



ELISPOT allows the quantitative determination of the number of cells in a population that are producing Ab specific for a given Ag or an Ag for which one has a specific Ab.



Cytotoxic activity of different effective cells on Sp2/0 tumor cells tested by 7-AAD assay with FCM

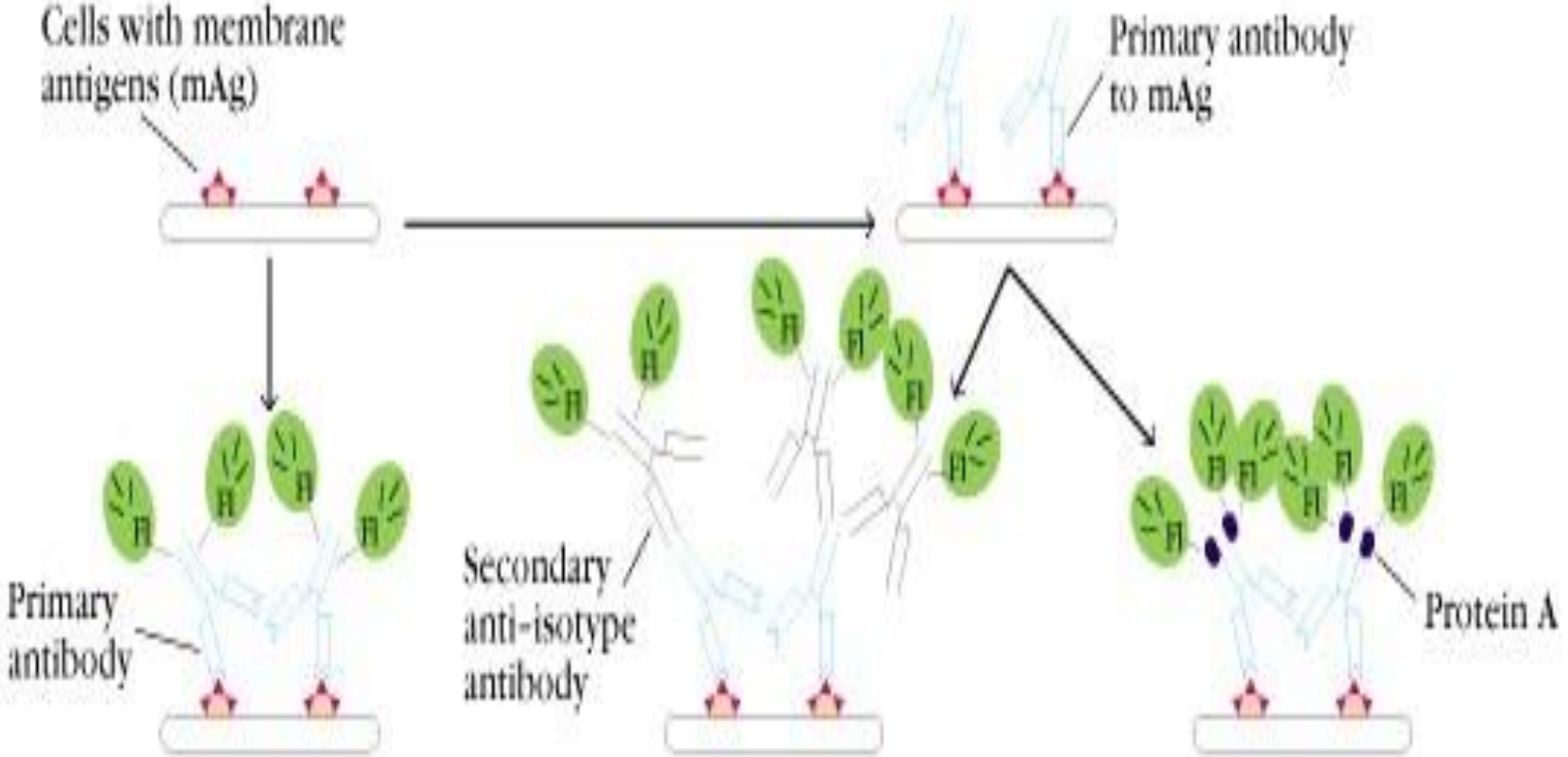
Comparison of numbers of effective cells producing IFN- γ by ELISPOT

J Dou, et al. Cancer Gene Ther. 2010 Oct;17(10):675-83

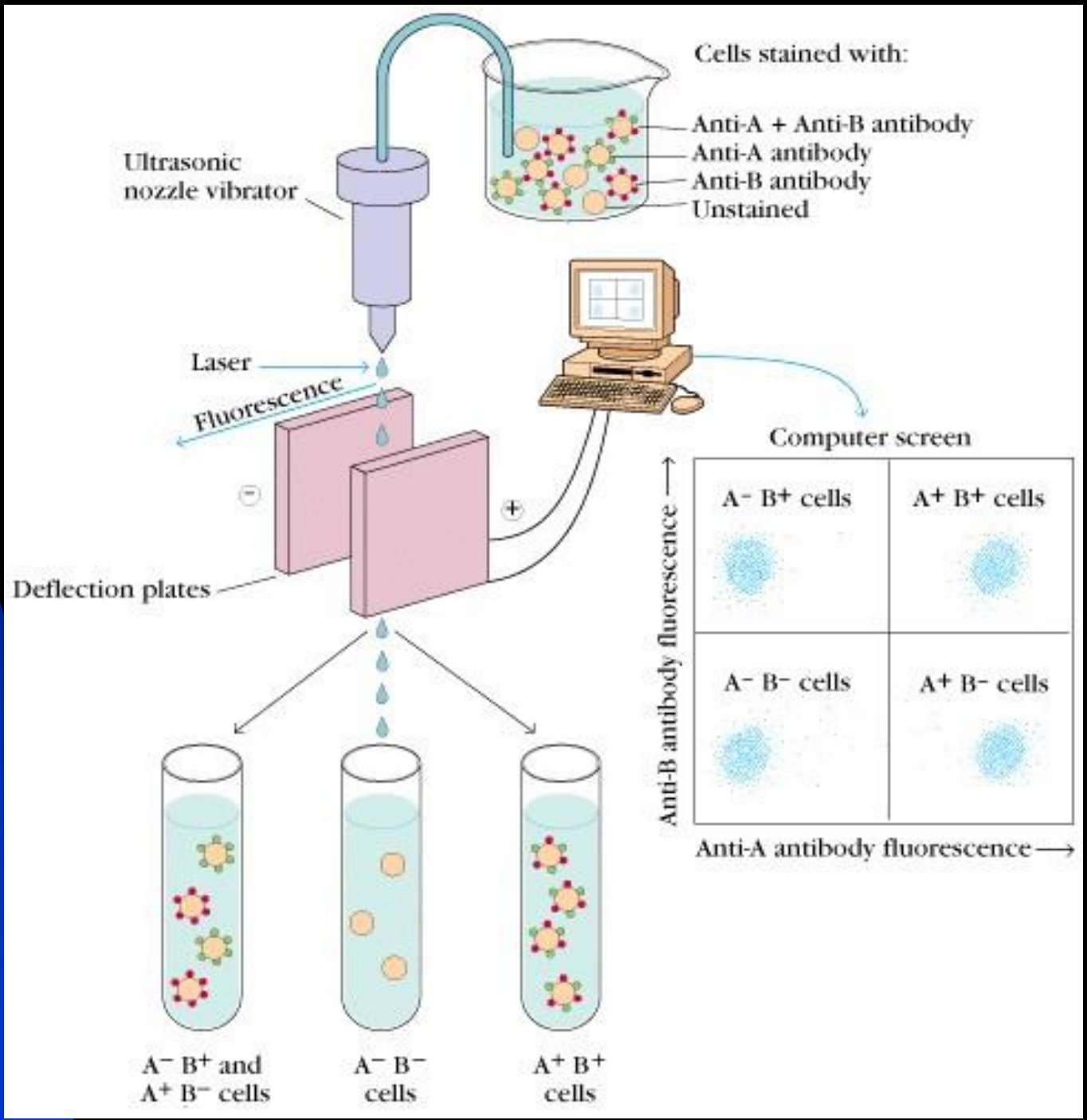
- The **immunofluorescence** technique,
- this technique is combined with immuno-fluorescence microscope or flow cytometry (FCM) to analyze and separate cells with different membrane markers in the field of cellular biology and immunology.

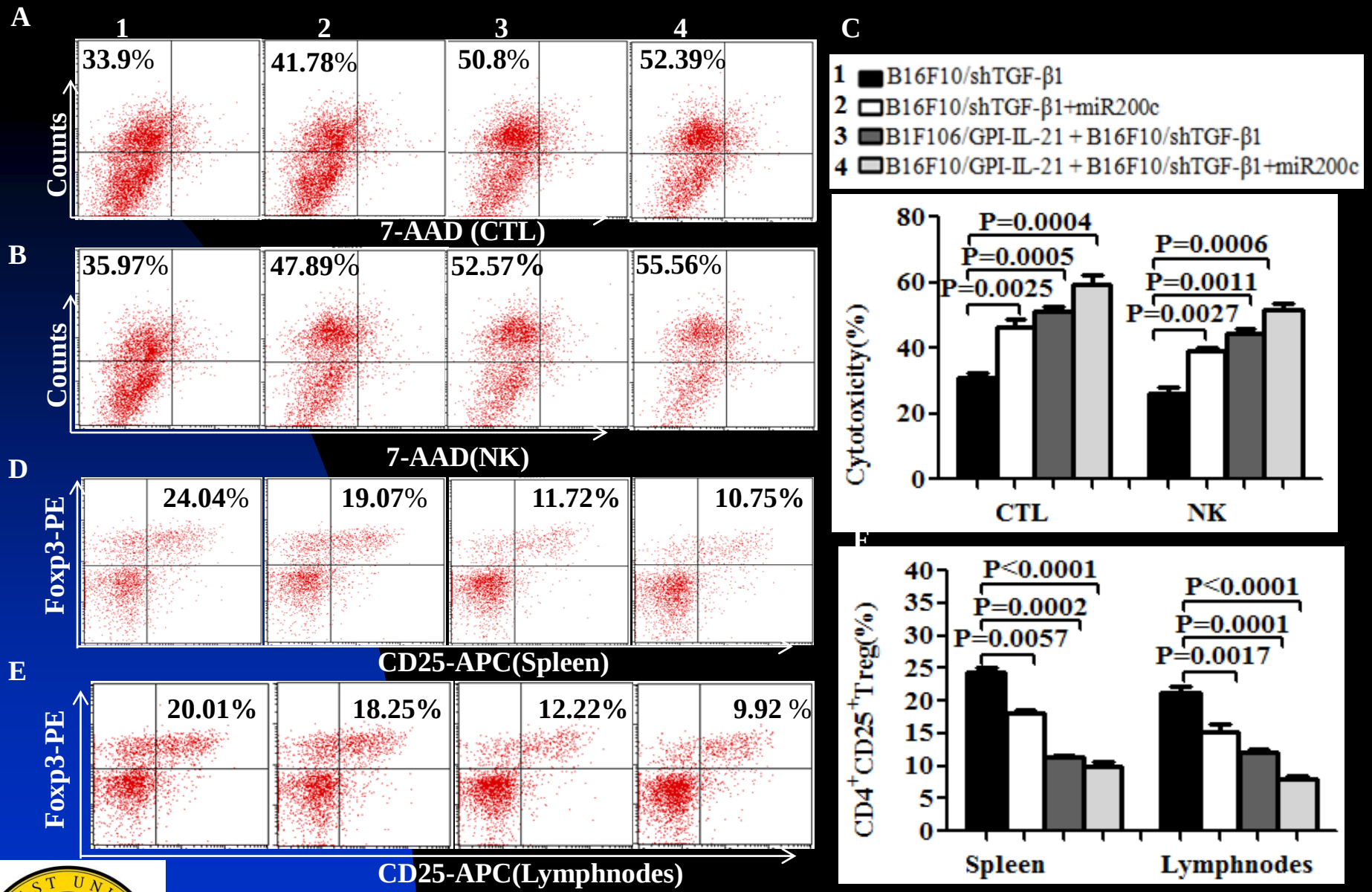
- **Western Blotting** can identify a specific protein in a complex mixture of proteins.

Immunoflourescence



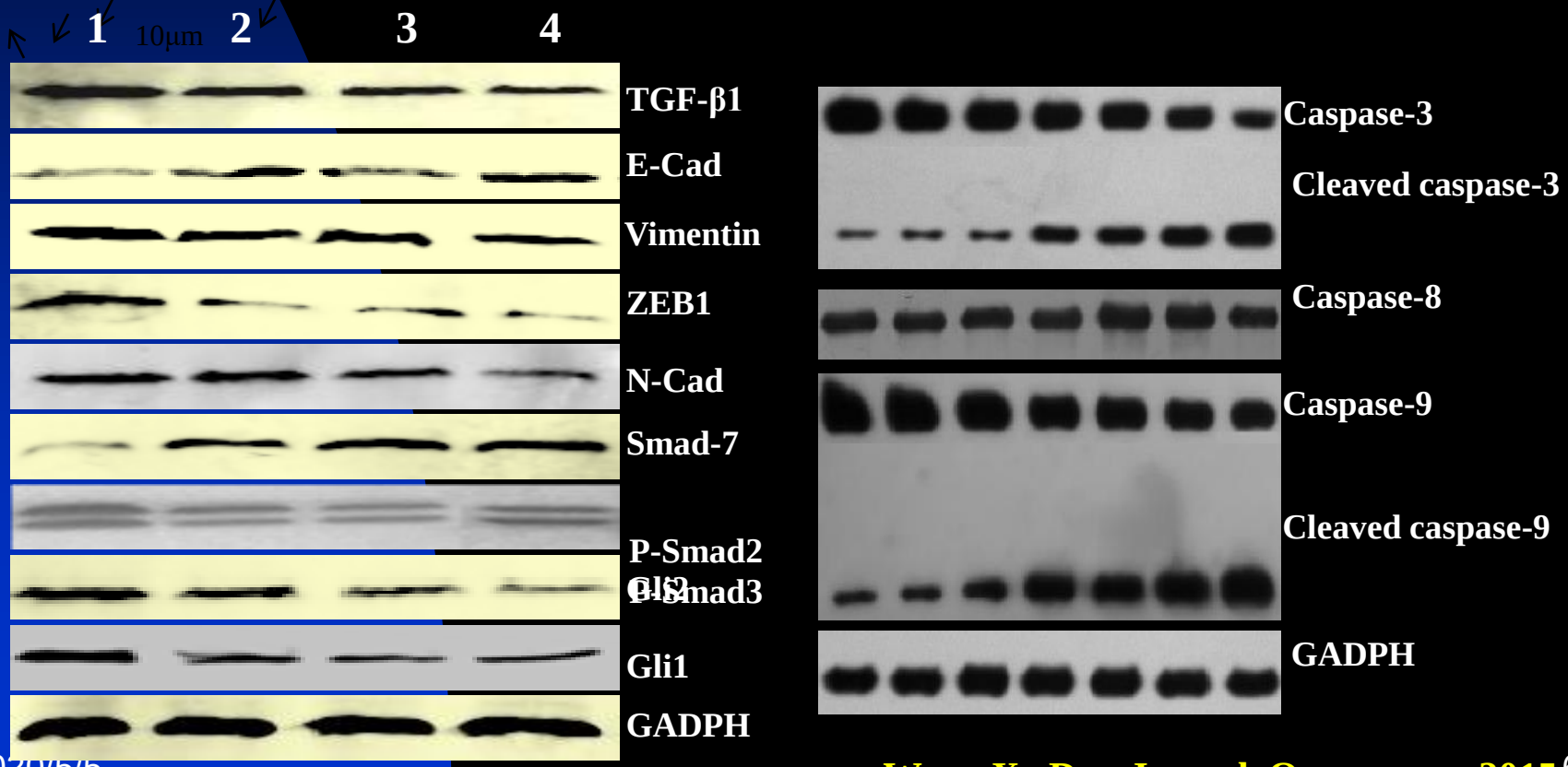
FACS Or Flow Cytometry





Wang X, Dou J , et al. Oncotarget. 2015

- **Western Blot** can identify a specific
- protein in a complex mixture of proteins.

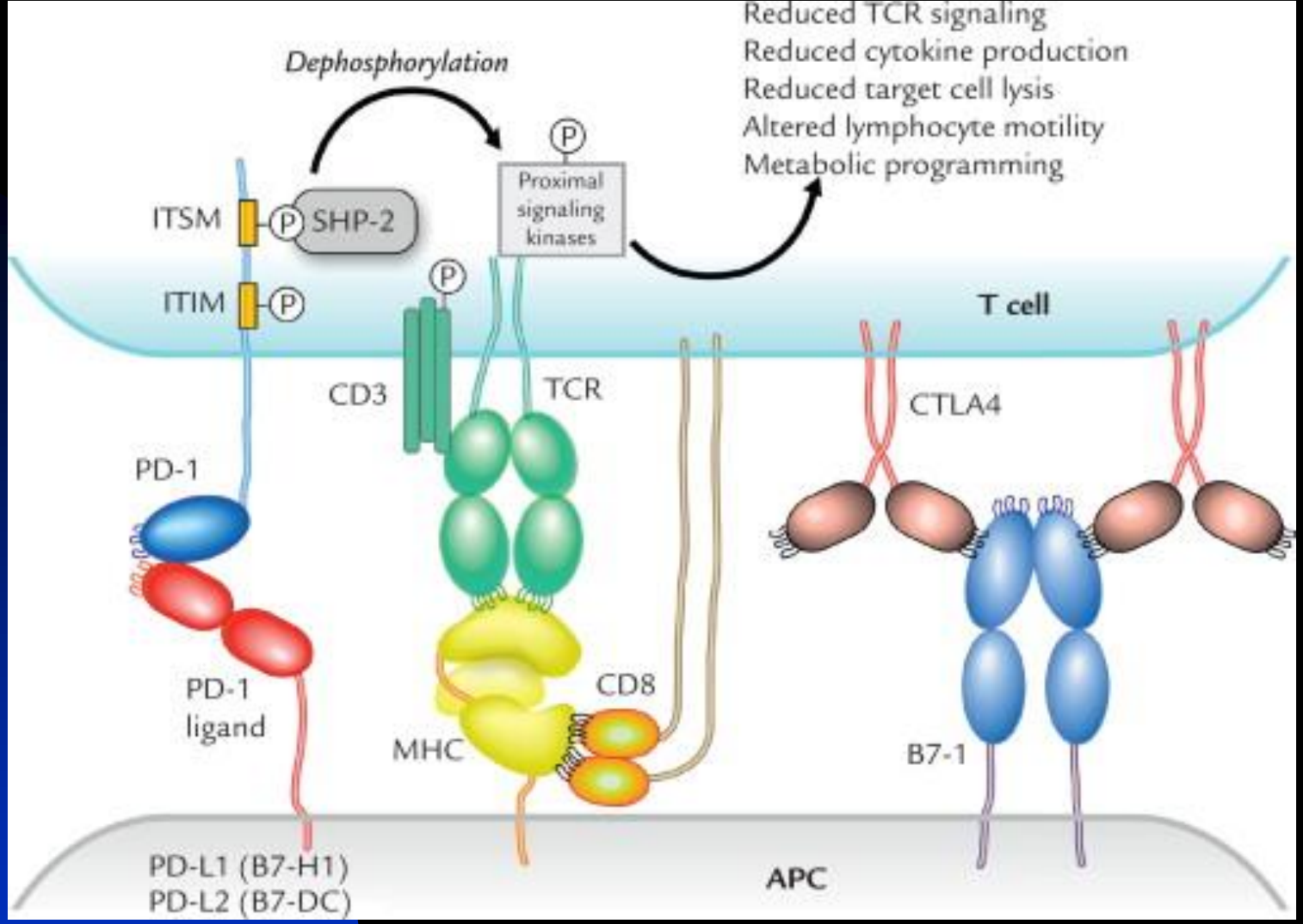


Wang X, Dou J , et al. Oncotarget. 2015

The Nobel Assembly at Karolinska Institutet has decided to award
the 2018 Nobel Prize in Physiology or Medicine jointly to

James P. Allison
Tasuku Honjo

for their discovery of cancer therapy by inhibition of negative immune regulation



The interaction of PD-1 and PD-L1 reduces T-lymphocyte function. ITIM = immunoreceptor tyrosine-based inhibitory motif; ITSM = immunoreceptor tyrosine-based switch motif; P = phosphoryation site; PD = programmed cell death protein; SHP = Src homology 2 domain–containing phosphatase.

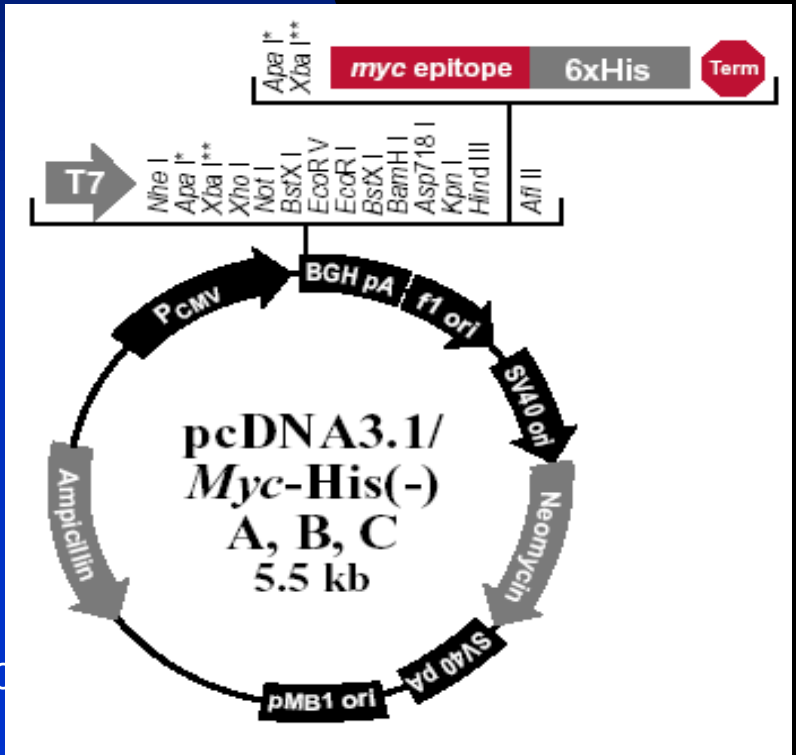
Clin Ther. 2015;37(4):764-82.

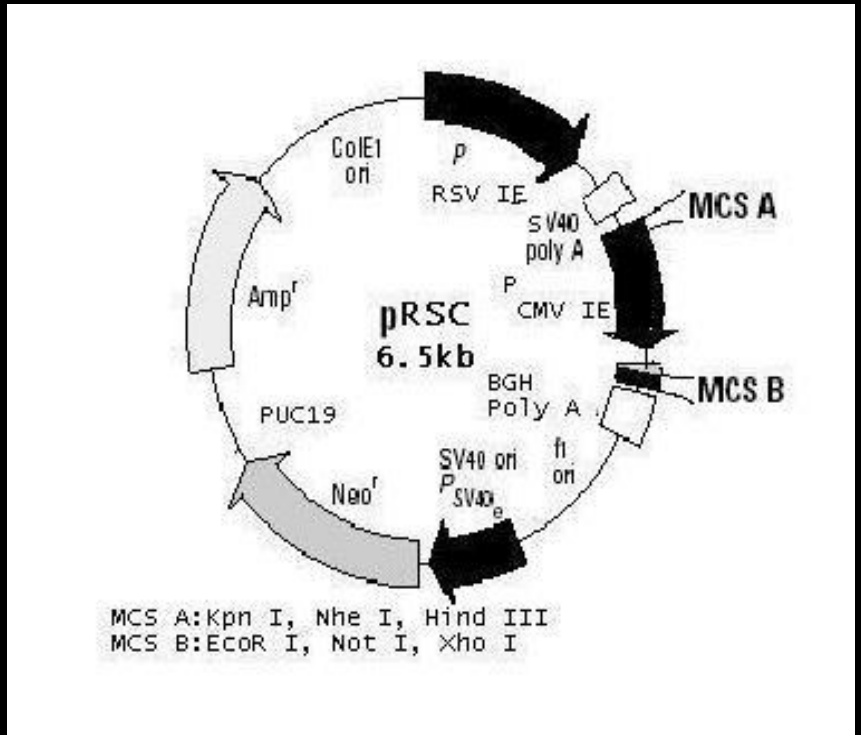
2. New Vaccines

- In a recently developed vaccination strategy, plasmid encoding antigenic proteins is injected directly into the muscle of the recipient. Muscle cells take up the DNA and the encoded protein Ag is expressed, leading to both a humoral Ab and cell-mediated response.

- DNA vaccine has many advantages over conventional vaccine. It is stable, easily manufactured and purified. Only a single injection may suffice.

- Tests of DNA vaccines in animal models have shown that it can induce protective immunity against several pathogens.
- Some DNA vaccines have entered into clinical trials for a number of disease such as HBV, influenza, HIV, malaria, and cancer.





全书分两篇。

总论篇，介绍疫苗研究历史、对人类的贡献和面临的挑战，疫苗研制的理论、技术、流程、应用、计划免疫、市场管理及疫苗相应法规等，并提供了新疫苗研究和开发的新技术和新信息。

各论篇，按细菌类疫苗、病毒类疫苗、真菌类疫苗、寄生虫类疫苗、肿瘤疫苗等，分别介绍对预防艾滋病、肝炎、结核病、疟疾、SARS、禽流感等传染病的新疫苗的研究进展，特别是对肿瘤疫苗、结核疫苗、治疗性疫苗、寄生虫类疫苗等的最新研究成果即时更新。

全书体现新颖性和可读性。

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江苏省重点教材

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窦骏，教授、博士生导师，东南大学医学院病原生物学和免疫学系主任。

1997年获浙江大学传染病学博士学位，1999年赴德国乌尔姆大学病毒学研究所高访；2001–2004年赴美国CDC高访，2006年赴美国佐治亚州州立大学生物系高访，2014年赴美国耶鲁大学医学院高访。

现任东南大学生命科学与医学学部委员、江苏免疫学会常务理事、《生物技术通讯》杂志常务理事、美国Frontiers in Bioscience 杂志编委、《东南大学学报》（医学版）编委、《河北中西医结合杂志》特邀编委、国家自然科学基金和国家863计划同行评议专家、教育部学位与研究生教育发展中心学位论文评审专家、江苏省第二批六大人才高峰(医药行业)培养对象。

主要研究领域有DNA疫苗、肿瘤免疫、肿瘤干细胞、感染免疫及中药免疫调节，先后主持或参加国家、省、部、厅级科研项目18项，教学研究项目6项，共发表论文210篇，其中SCI论文50篇。主编《肿瘤干细胞》、《疫苗工程学》（第1版），参编Advance in Cancer Stem Cells和Cancer Stem Cells –The Cutting Edge等。

Email：njdoujun@yahoo.com.cn

- **3. Recombinant cytokines**
- **As a result of advances in recombinant DNA**
- **technology, recombinant cytokines, such as interferon, interleukin, etc, are available as therapeutic agents. They have been used for the adjuvant therapy of infectious disease, cancer, immuno-deficiency, and anemia or leukemia.**

- **Genetic engineering makes it possible to get high-yielding and low-cost cytokines and prevent blood transmitted diseases when expensive blood-derived cytokines is used.**

- **4. Immune cells therapy**
- **Stem cell transplantation is useful for gene therapy, the introduction of a normal gene to correct a disorder caused by a defective gene. Rapid advances in genetic engineering may soon make gene therapy a realistic treatment for genetic disorders of blood cells, and hematopietic cells are attractive vehicles for such an approach.**

- **Another important immune cells applied in therapy are dentritic cells (DCs). DCs are highly specialized professional APCs with potent capacity to elicit primary immune response.**

Prospect of Immunology

The technology of **microarray**, **proteomics**, as well as **bioinformation** will help to study the mechanisms of immune responses.

Various **transgenic** and **knockout** mouse strains can be used to evaluate immune function *in vivo*.

Immunology deals mostly with acute **infectious diseases**. We have learnt that most of these diseases are readily taken care of by vaccines.

But, there are many diseases that eventually became debilitating , however, which can't be controlled, particularly **autoimmune diseases**, **tumors** and **graft rejections**, etc.

It is an important to devise clever methods to use immunity against autoimmune diseases, tumors , graft rejections , **hypersensitivity** and so on.

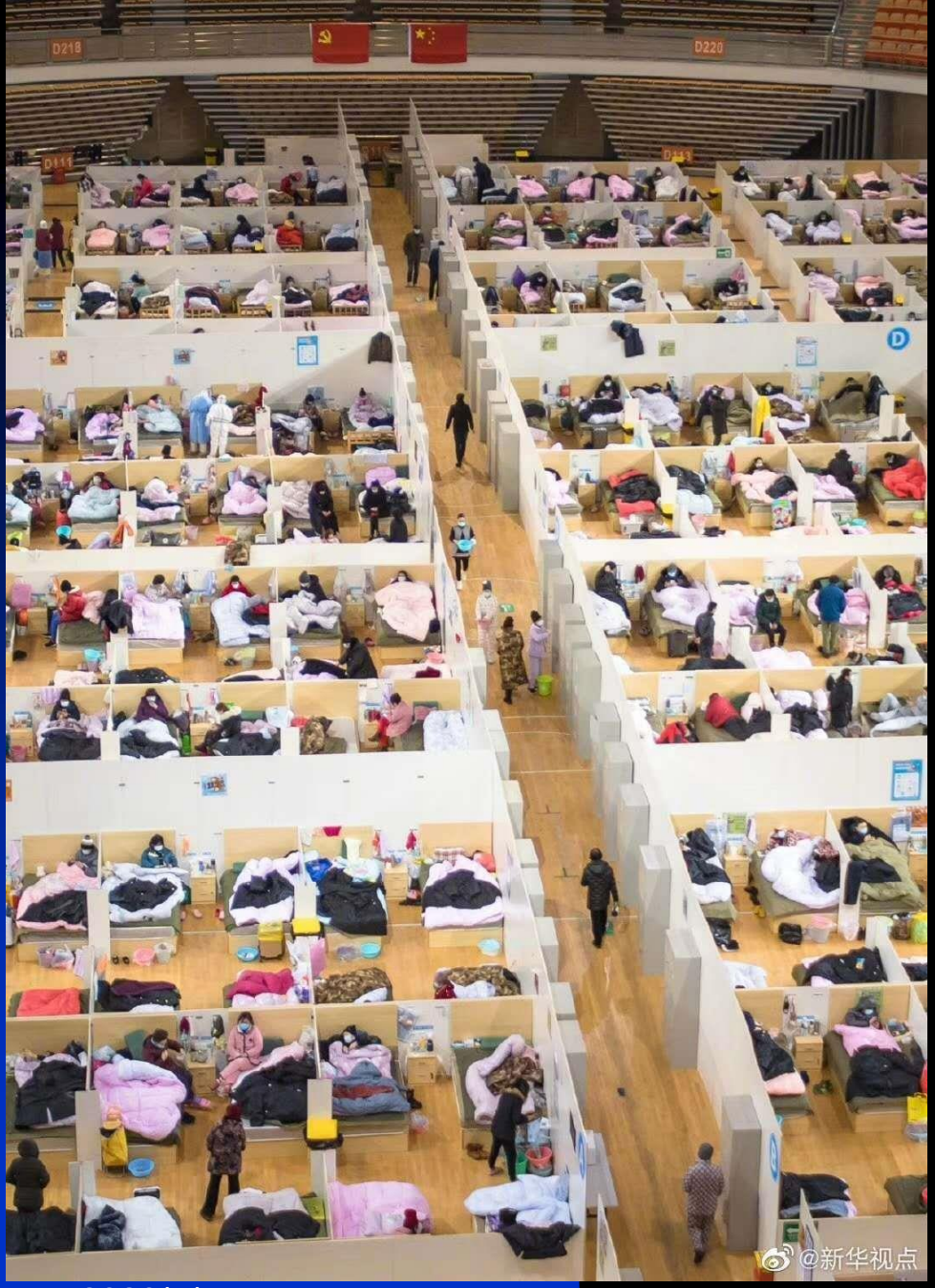
Recent developments in immunology are opening

up a huge range of potential new approaches of prevention and therapy of diseases.

Emergency data demonstrate that we develop the vaccines that fight not only against HIV, tuberculosis, but also fight SARS-CoV，MERS-CoV，2019-nCoV/SARS-CoV-2，H7N9‘bird flu’, Ebola virus, Zika Virus, other infectious diseases.

In addition, we need the vaccines that fight against cancer, autoimmune diseases such as diabetes, systemic lupus erythematosus, etc.

- Therefore, we have a long way to go.



2020/5/5

COVID-19， Wuhan

Milestones of Immunology (I)

713-1000: Variolation, inhalation of live smallpox virus from dried pustule (China)

1500: Variolation, inoculation of live smallpox virus from dried pustule (15th century middle East) made popular by Lady Mary Wortley Montagu (wife of the British ambassador in Istanbul) in Great Britain in 1717

1798: 1st vaccine against smallpox (Edward Jenner)

1879: Attenuated chicken cholera vaccine or vaccination (Louis Pasteur)

1885: Rabies vaccination (Louis Pasteur)

1891: Delayed type hypersensitivity (Robert Koch)

1900: Antibody formation theory (Paul Ehrlich)

1901: Serum therapy against diphtheria (Von Behring, Nobel Price)

1905: Cellular immunity to tuberculosis (Koch, Nobel Price)

1909: BCG vaccine (Bacille de Calmette et Guérin or BCG)

Milestones of Immunology (II)

Brazil Australia	Peter Medawar Macfarlane Burnet	Acquired tolerance Clonal selection theory	1960
UK USA	Rodney Porter Gerald Edelman	Antibody structure	1972
USA	Rosalyn Yalow	Radioimmunoassay	1977
Venezuela USA USA	Baruj Benacerraf Jean Dausset, George Snell	Histocompatibility antigens	1980
Germany UK UK	George Köhler Cesar Milstein Niels Jerne	Monoclonal antibody Network theory	1984
Japan	Susumi Tonegawa	Gene rearrangement	1987
USA USA	E. Donnall Thomas Joseph Murray	Transplantation immunology	1990
Switzerland Australia	Rolf Zinkernagel, Peter Doherty	MHC restriction	1996 2011,2018

Further readings

- Medical Immunology, by Yunqing An and Zhi Yao. 2017-2. ISBN: 978-7-5659-0750-0.
- Immunology, 7th Edition, by David Male, Jonathan Brostoff, David Roth and Ivan Roitt. 2006-05-09. ISBN: 97803233992.
- http://immuneweb.xxmc.edu.cn/
- http://en.wikipedia.org/wiki/

Concepts:

1. Smallpox
2. Humoral Immunity and 3. Cellular Immunity
3. ELISA and ELISPOT
4. 2019-nCoV/SARS-CoV-2, COVID-19

Questions:

1. What is the Burnet's clonal selection theory?
2. What is the Immunological Tolerance ?
3. What is the challenges of the immune system?