PROGRAM OF SUBJECT (SYLLABUS)

RECENT DEVELOPMENTS OF BIOTECHNOLOGY IN VETERINARY MEDICINE AND ANIMAL HUSBANDRY

- **Course – 1**
  - **Semester – 1**
  - **Credits - 3**
  - **Lecture – 30 hours**
  - **Lab classes – 15 hours**
  - **Masters independent work (MIW) – 75 hours**
- **Masters independent work with tutor (MIWT) – 15 h**

Distribution of training period

Weeks	1	2	3	4	5	6	7		8	9	10	11	12	13	14	15		To-tal
Lectures	2	2	2	2	2	2	2	In-ter me-dia-te con-trol №1	2	2	2	2	2	2	2	2	In-ter me-dia-te con-trol №2	30
Lab classes	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1		15
MIW	5	5	5	5	5	5	5		5	5	5	5	5	5	5	5		75
MIWT	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1		15
Total	9	9	9	9	9	9	9		9	9	9	9	9	9	9	9		135

- ***Course objectives is:***
- **to familiarize Masters with new developments and achievements of modern Biotechnology in the field of diagnosis and prevention of infectious and parasitic diseases as well as reproduction of animals with useful properties.**
-

As a result of studying this subject,

masters must know:

- **the latest achievements of world science as well as scientists of S.Seifullin Kazakh Agro-Technical University on improvement methods of diagnosis, treatment and prevention of infectious and parasitic diseases that cause economic and social damage to the country;**
  • Modern approaches to the creation of strains of prokaryotic and eukaryotic microorganisms and mammals - the producers of biologically active substances;
  • state, problems and tendencies of development of the cellular and genetic engineering in veterinary medicine and animal husbandry

masters should be able to:

• use modern laboratory equipment;
• conduct research on the diagnosis of infectious and parasitic diseases using a variety of variants of ELISA, LFA and PCR;
• use the achievements of cell and genetic engineering techniques to improve disease diagnosis, obtaining medical preparations and vaccines as well as improving productivity and sustainability of the animals;
• to determine the actual problem of modern biotechnology and to develop an application for participation in the competition of scientific projects in the field of veterinary medicine and animal husbandry.

COURSE CONTENT

Themes	Hours
1. Preparation and use of monoclonal antibodies in Medicine and Veterinary Medicine	4
2. Serological methods of diagnosis in Medicine and Veterinary Medicine	4
3. Improvement of diagnostics of infectious and parasitic diseases on the basis of modern biotechnology approaches	8
4. Biotechnology of vaccines	4
5. Micromanipulation with embryos of domestic animals	4
6. Problems of genetic engineering in the creation of transgenic animals	2
7. Biotechnology of forage products	4
Total	30

List of Practical Classes

№	Themes	Hours
1	Enzyme linked immunesorbent assay (ELISA)	2
2	Immunochromatographic assays (Lateral flow test)	2
3	Polymerase chain reaction (PCR)	4
4	Transplantation of embryos Visiting the Republican breeding center «Asil-tүlik» (the end of March, 2015)	3
5	Sequencing of DNA samples using the CEQ ™ 8000 analyzer	2
6	Visiting Scientific and Analytical Center «Biomedpreparat» of the National Center for Biotechnology of the Republic of Kazakhstan (Mid-April, 2015) Total	2 15

SCHEDULE OF ACCEPTANCE

MIW's themes on discipline "Recent developments of Biotechnology in Veterinary Medicine and Animal Husbandry»

№	Weeks of accep-ta nce	The names of topics	Number of hours
		1st module	
1	4	Improving technology for vaccine production	8
2	5	New methods for the production of monoclonal antibodies and their fragments	8
3	6	Modern approaches in biotechnology for fodder and veterinary medicine preparations	8
4	7	Developing the technology of animal embryo transfer	8
5	8	Status and development prospects of Veterinary Biotechnology in Kazakhstan	8
		2nd module	
6	9-15	Preparation of a literature review on the topic of dissertation	35
	TOTAL:		75 hours

REFERENCE

Basic Literature:

The Basic literature of the discipline are articles and reviews published in scientific journals and proceedings of the symposiums (conferences) on current issues of Biotechnology in Veterinary Medicine and Animal Husbandry from the databases of *Elsevier, Springer Science, Thomson Reuters, Pub Med* and other publishing houses.

- **Supplementary Literature:**
- Kreuzep H. and A.Massey. Molecular Biology and Biotechnology.- Washington.-ASM PRESS, 2008.-485 p.
- Clark D.P.and N.J.Pazdernik. Cell Biotechnology. – Elsevier Inc., 2012.- 750 p.
- Chauhan A.K.and A.A.Varma. Molecular Biotechnology. –I.K.International Publishity House Pvt.Ltd., 2009.- 1337 p.
- Kun L.Y. Microbial Biotechnology.- Word Scientific Publishing, 2006.- 794 p.
- Crommelin D., R.Sindelar and B.Meibohn. Pharmaceutical Biotechnology. – N.Y. London: Informa healthcare,___.-490 p.
- Croves M.. Pharmaceutical Biotechnology. – Taylor &Francis Group, 2006.- 411 p.
- Shetty K., G.Paliiyath, A.Pometto and R.Levin. Food Biotechnology.- Taylor &Francis Group, 2006.- 1982 p.
- **Bulashev A.K. Educational-methodical complex (EMC) on discipline "Recent developments of biotechnology in veterinary medicine and animal husbandry".- Publishing house of Seifullin KazATU: Astana, 2012.-115 P.**
- Алмагамбетов К.Х. Биотехнология микроорганизмов.- Астана: Изд-во ЕНУ им. Гумилева, 2008.- 244 с.
- Алмагамбетов К.Х. Медицинская биотехнология. – Астана: Изд-во ЕНУ им. Гумилева, 2009.- 236 с.
- Булашев А.К. Моноклональные антитела в диагностике бруцеллеза. Акмола: Изд-во Акмолинского аграрного университета, 1995.-214 с.
- Булашев А.К. Иммуноферментный анализ в диагностике бруцеллеза и туберкулеза. Астана: Изд-во Казахского аграрного университета им.С.Сейфулина, 2003.- 52 с.
- Булашев А.К., Кухарь Е.В. Ветеринарная биотехнология.- Астана: Изд-во КазАТУ им.С.Сейфуллина, 2009.- 222 с.
- Васильев Д.А. и соавт. (Электронный ресурс).- Лекций по курсу: Биотехнология.- Ульяновск, 2005.-188 с.
- Глик Б., Пастернак Дж. Молекулярная биотехнология. Принципы и применение. Пер. с англ. М.: Мир, 2002.-583 с.
- Завертяев Б.П. Биотехнология в воспроизводстве и селекции крупного рогатого скота. Л.: Агропромиздат, Ленинградское отделение, 1989.-255 с.
- Основы биотехнологии /Т.А.Егорова, С.М.Клунова, Е.А.Живухина.- М:Издательсктй центр «Академия», 2003.-208 с.
- Сельскохозяйственная биотехнология /В.С.Шевелуха, Е.А.Калашникова, Е.С.Воронин и др.; Под ред. В.С. Шевелухи – 2-е изд., перераб. и доп.- М:Высш.шк., 2003.-469 с.

HYBRIDOMA TECHNIQUE

TEACHING OBJECTIVES:

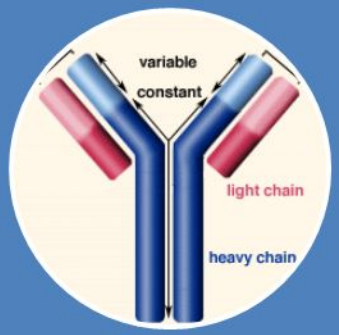
1.INTRODUCTION

2. PRINCIPLE INVOLVED IN MONOCLONAL ANTIBODIES PRODUCTION

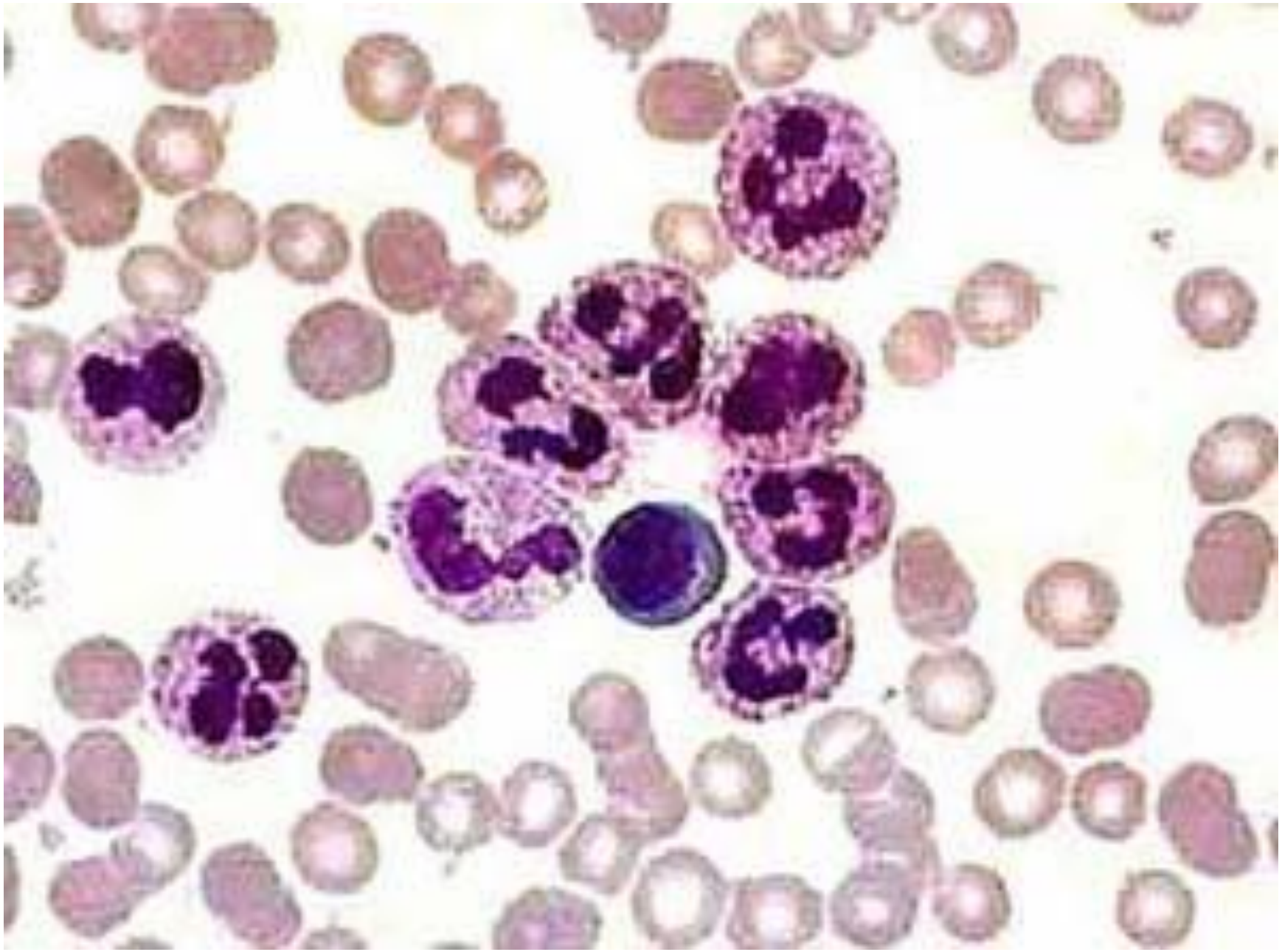
3. PRODUCTION OF MONOCLONAL ANTIBODIES

4. ENGINEERED MONOCLONAL ANTIBODIES

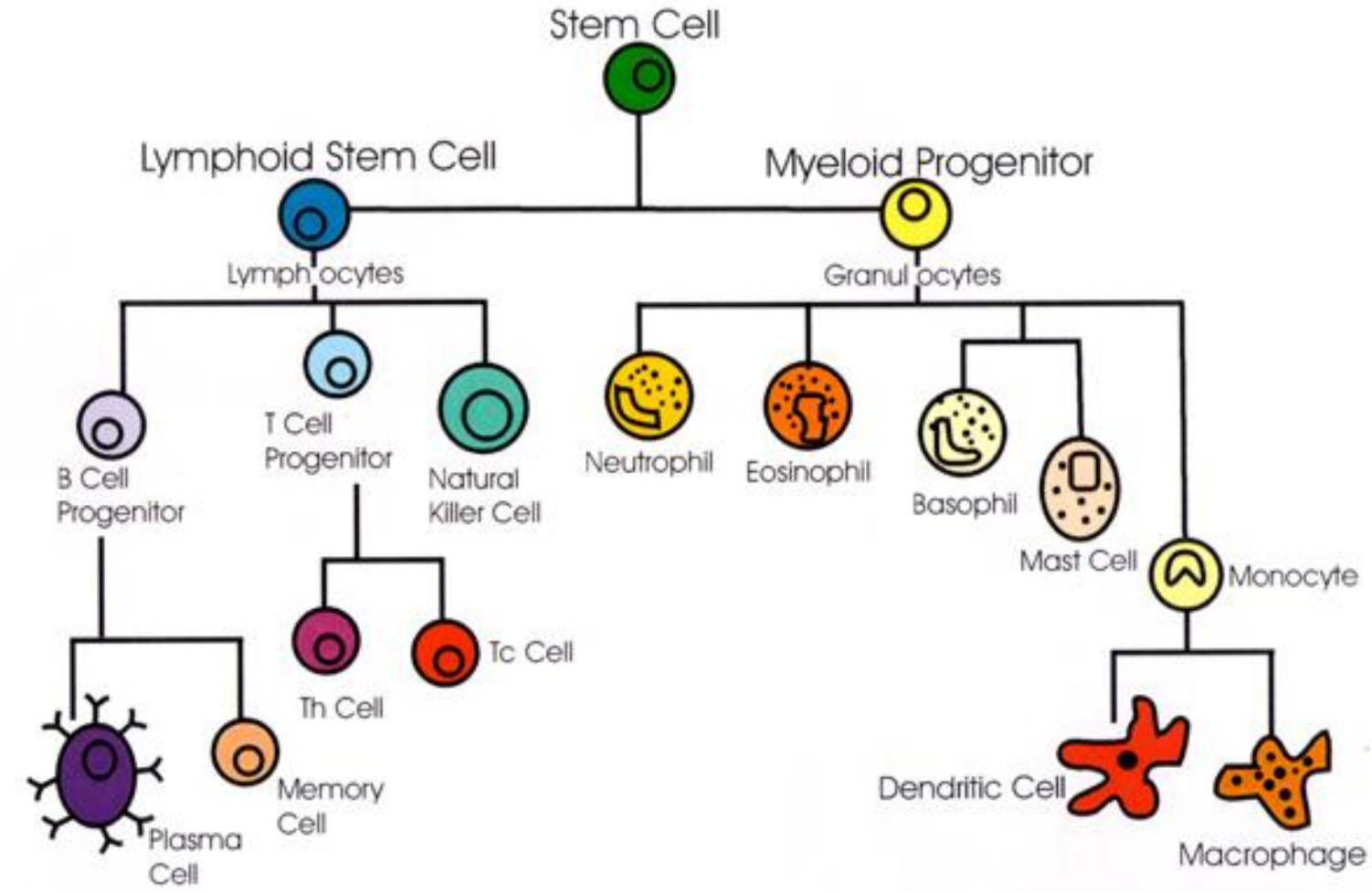
INTRODUCTION



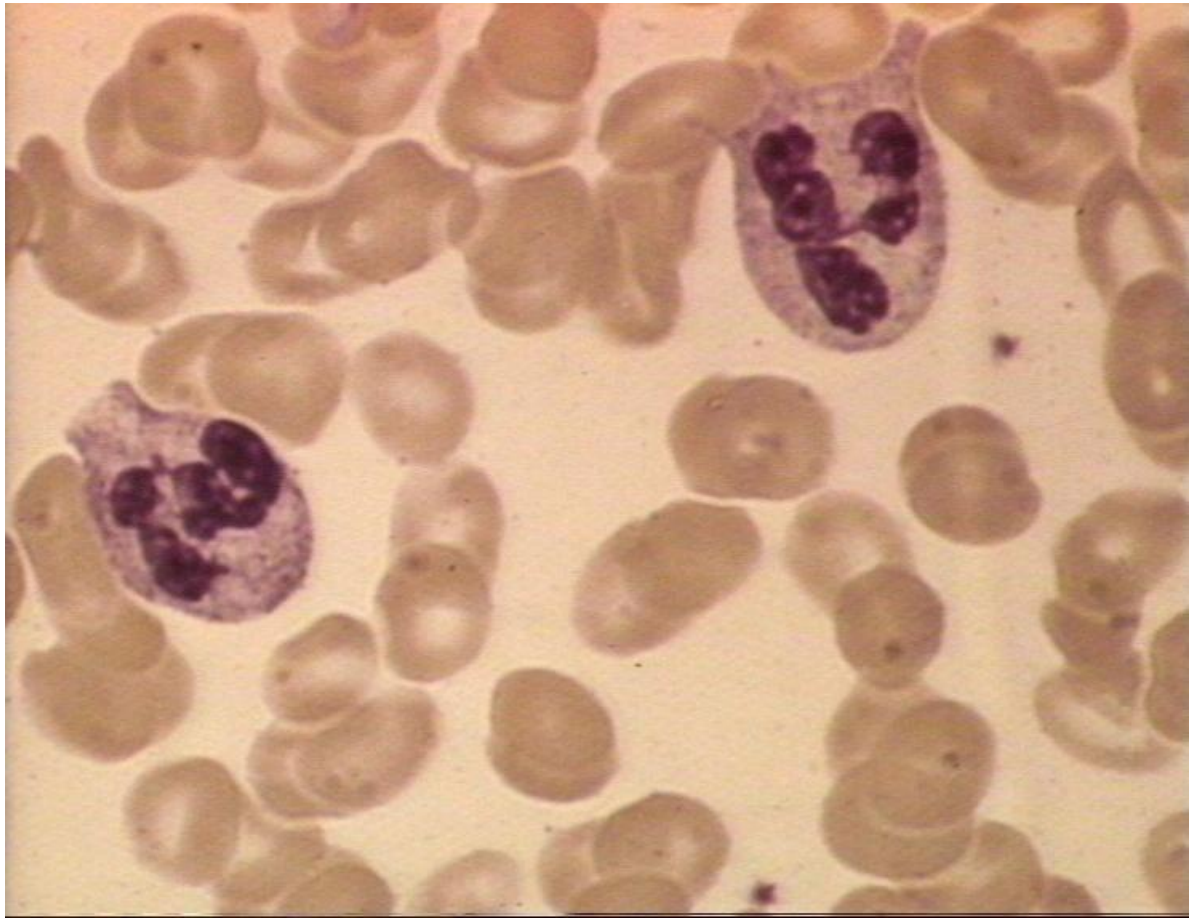
HOW THE ANTIBODIES ARE PRODUCED?



Cells of the Immune System

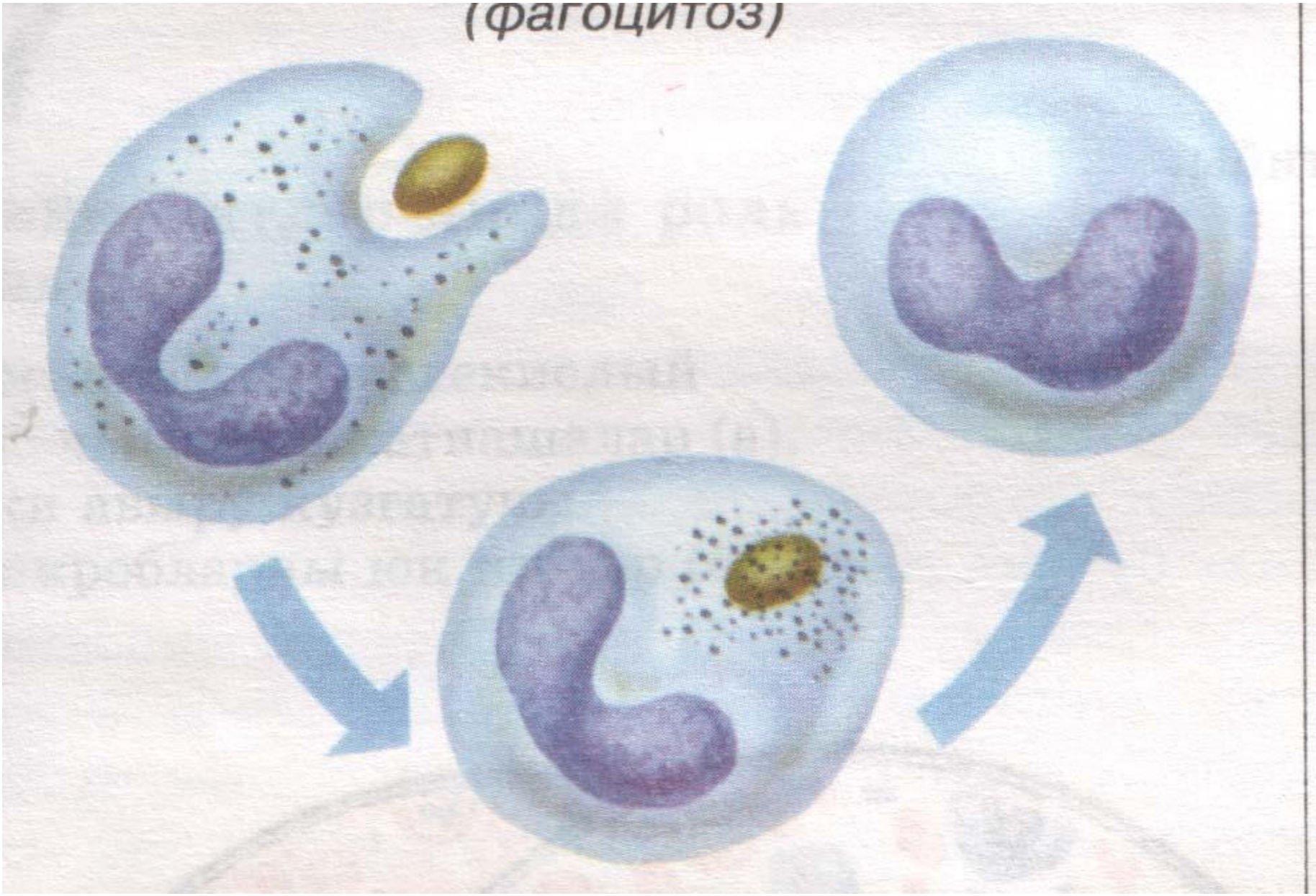


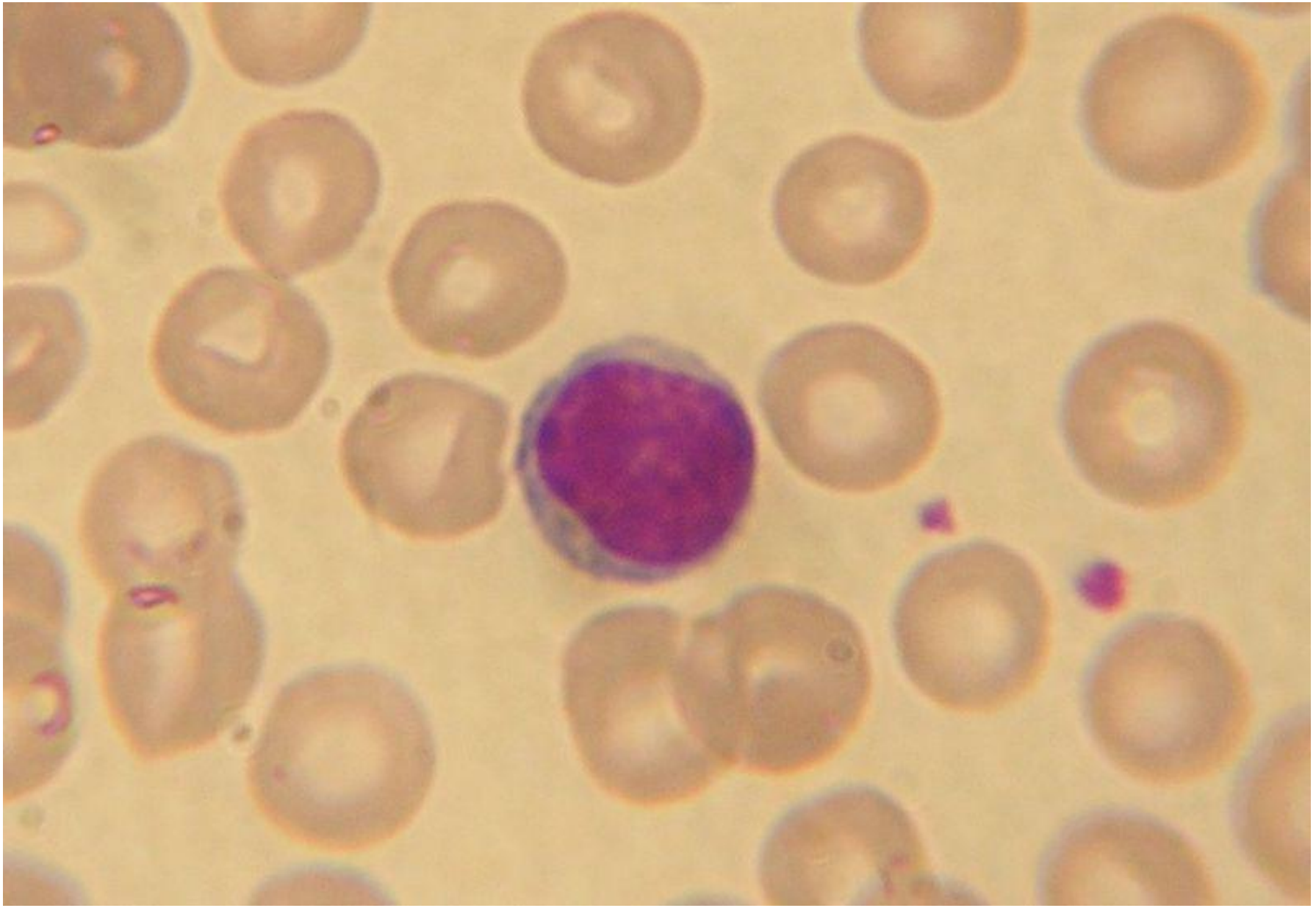
Two neutrophils in blood film

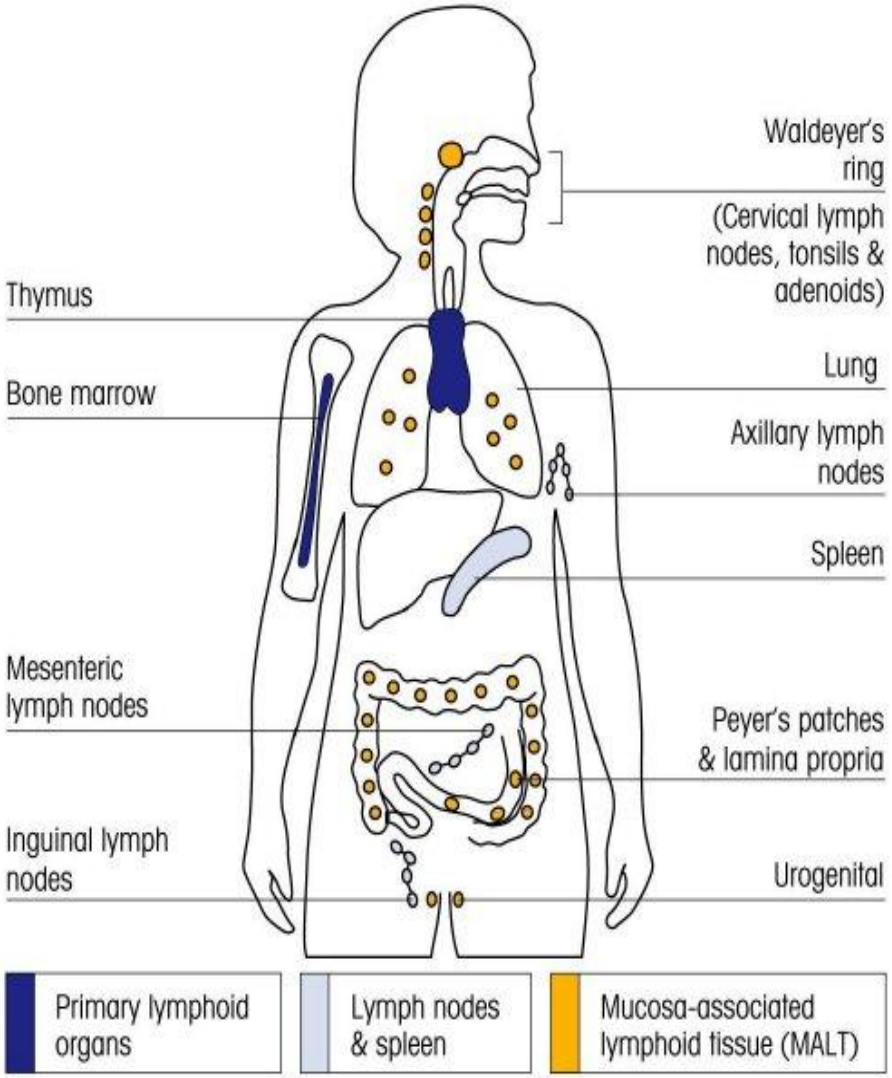


Polymorphonuclear cells are recruited to the site of infection where they phagocytose invading organisms and kill them intracellularly. In addition, PMNs contribute to collateral tissue damage that occurs during inflammation.

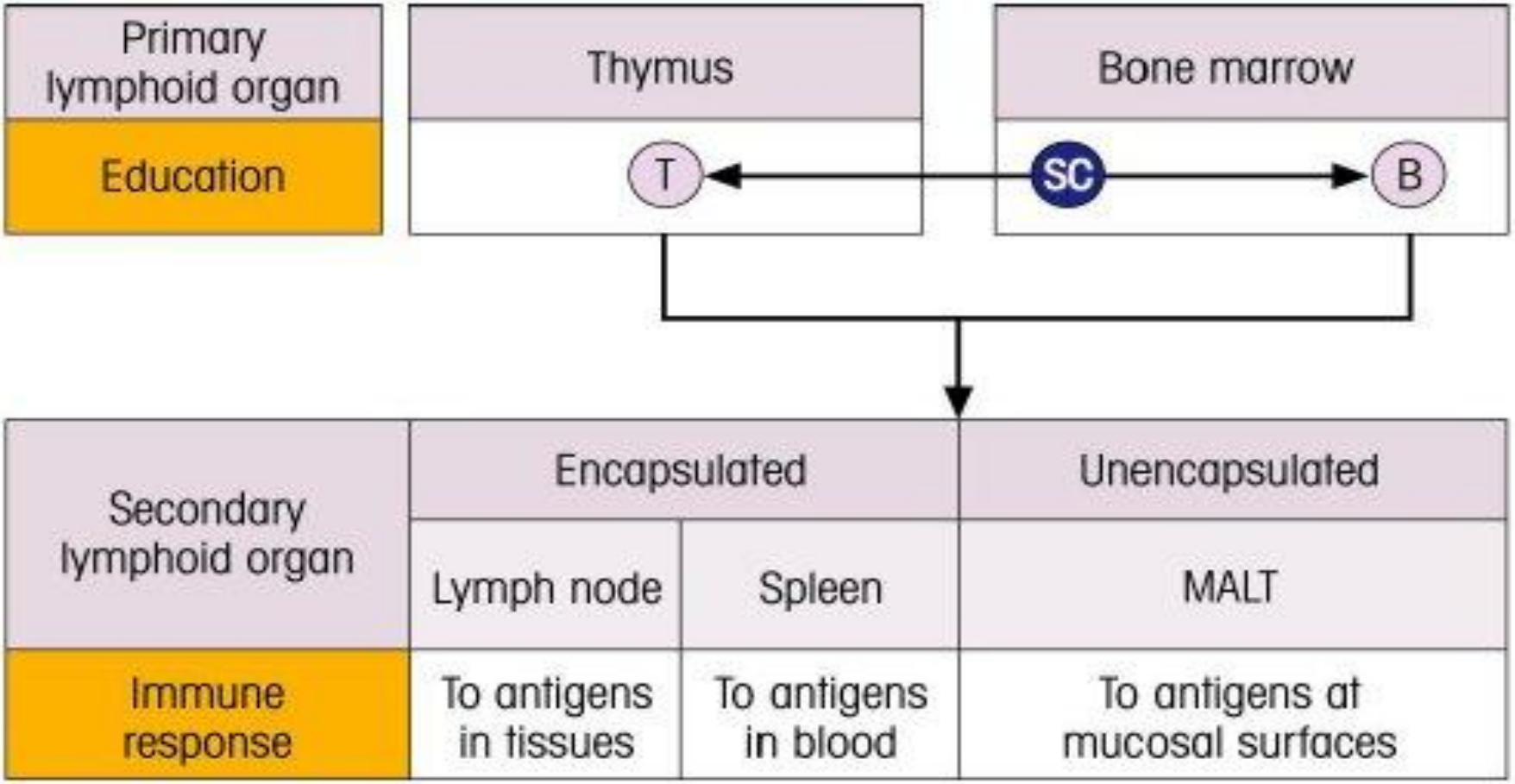
(фагоцитоз)



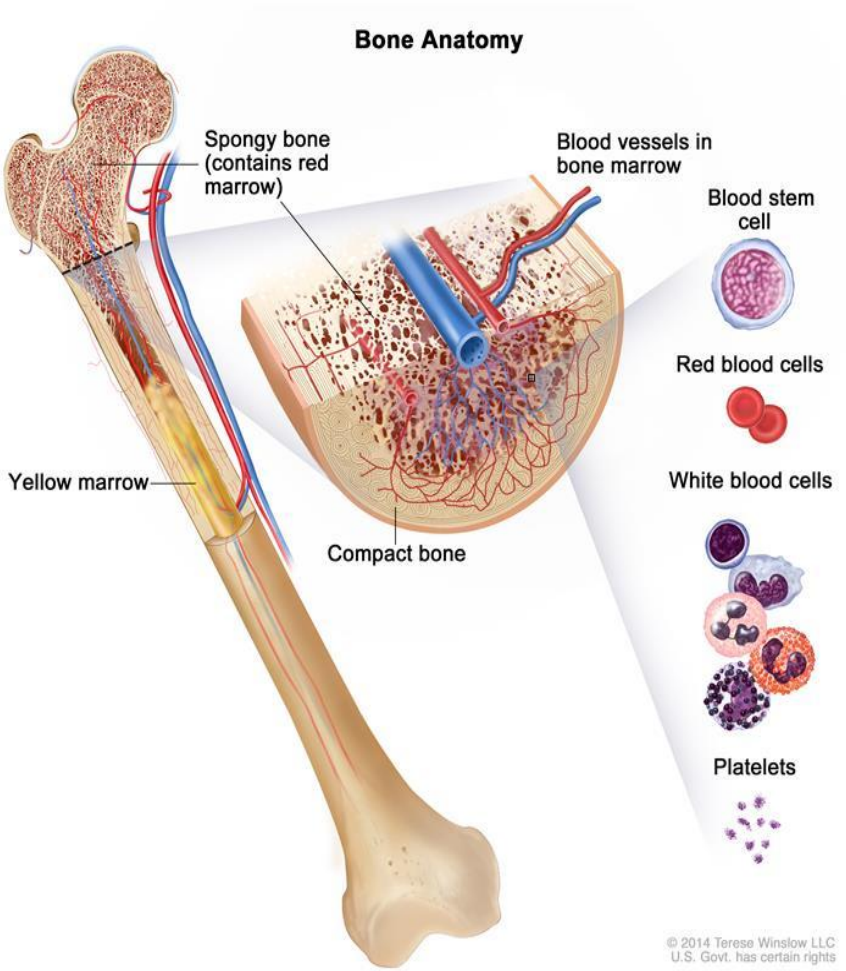




Delves *et al. Roitt's Essential Immunology, 12th ed.*



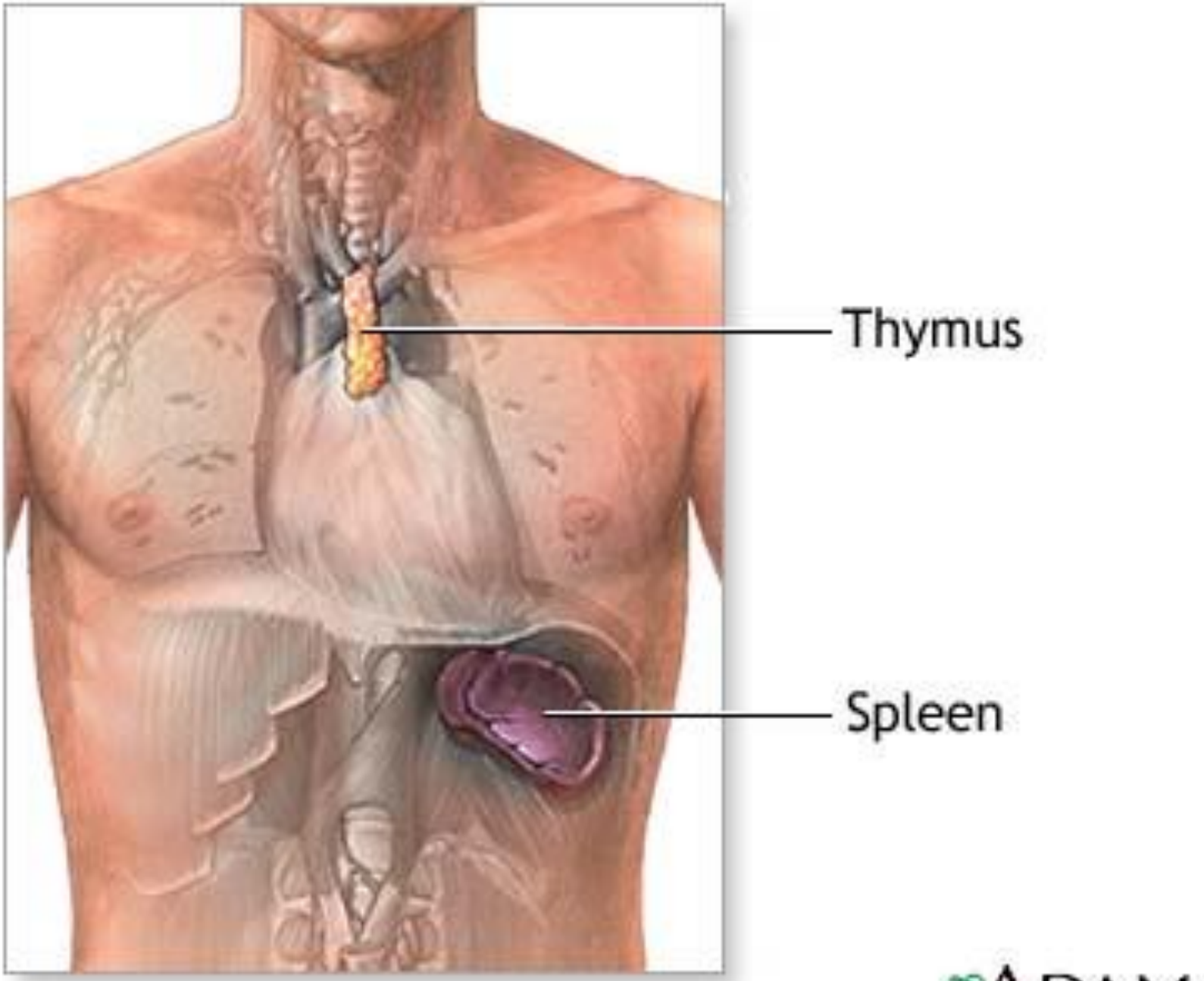
Delves *et al. Roitt's Essential Immunology, 12th ed.*

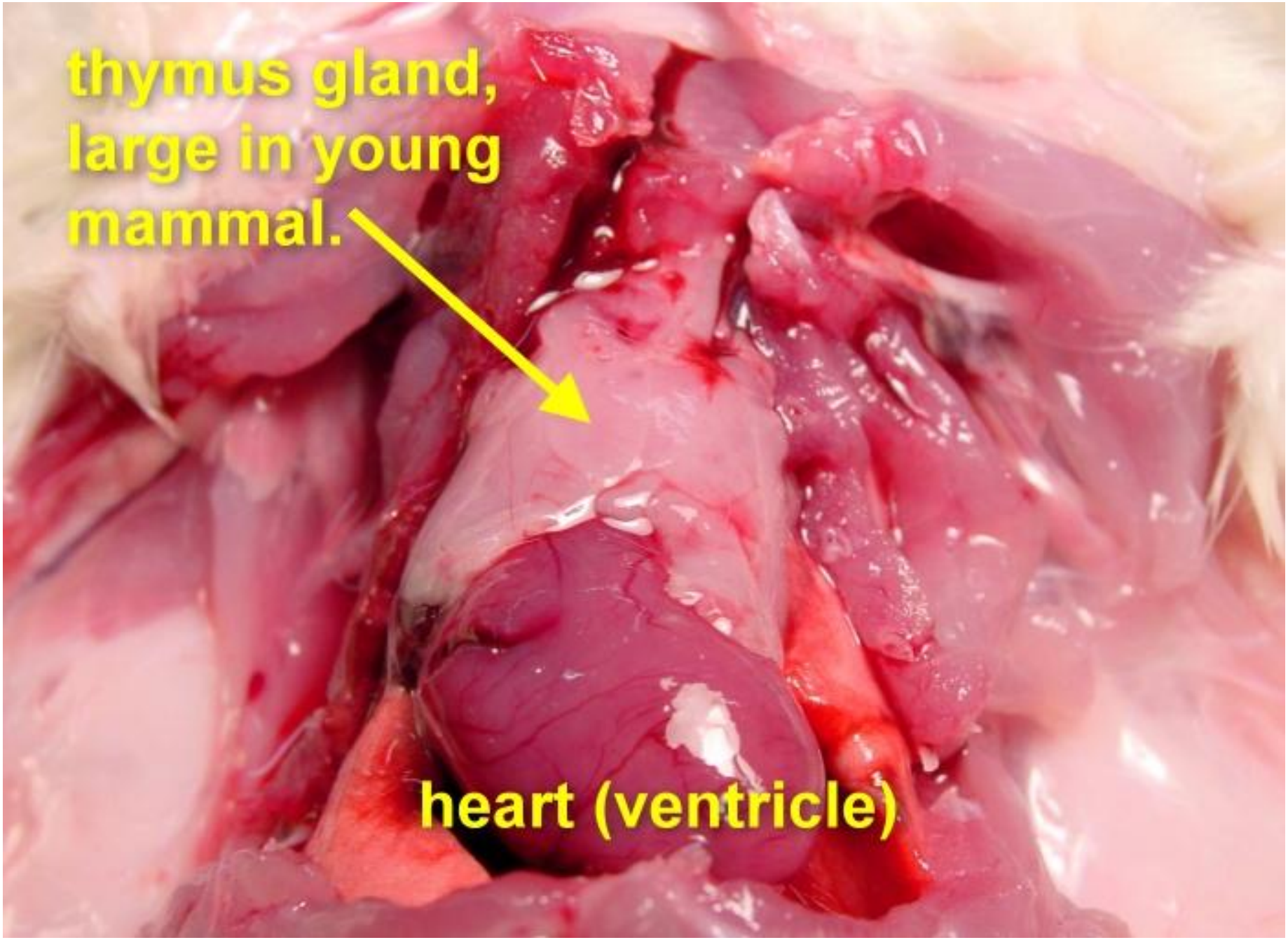


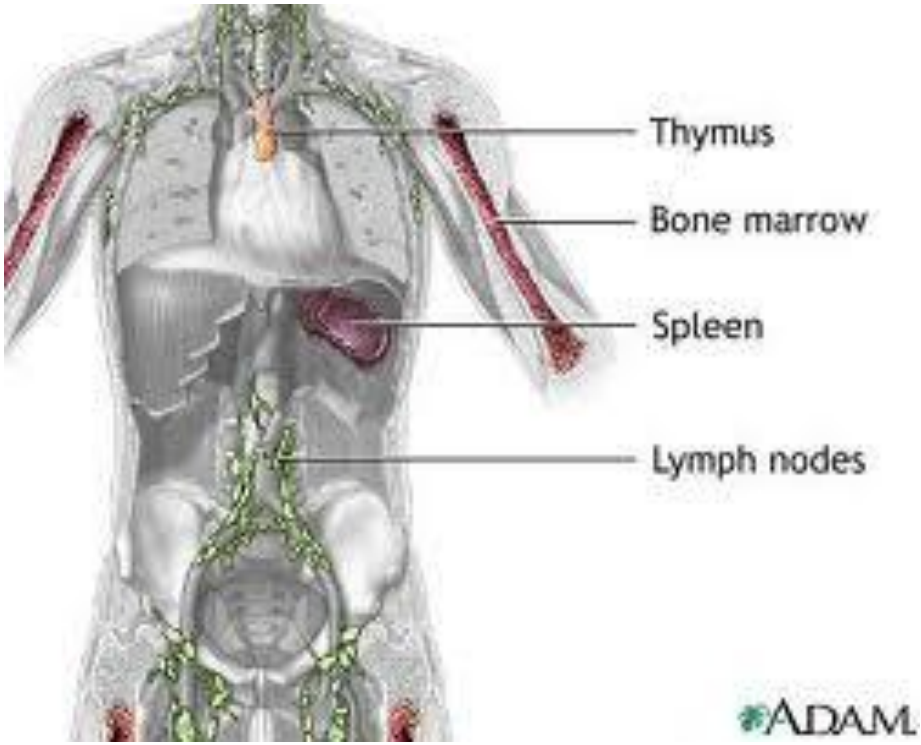
Bone Marrow

Bone marrow (medulla ossea) is the site of B cell maturation in mice and humans. B cells undergo both positive and negative selection, similar to T cell maturation in the thymus.

Bone marrow is also the site of hematopoiesis, the development of the myriad blood cells from progenitor cells. The site of B cell maturation in birds is the bursa of Fabricius, after which B cells are named. The tissue of bone marrow where leukocytes, red blood cells, and platelets develop (i.e., the site of hematopoiesis) is known as myeloid tissue.

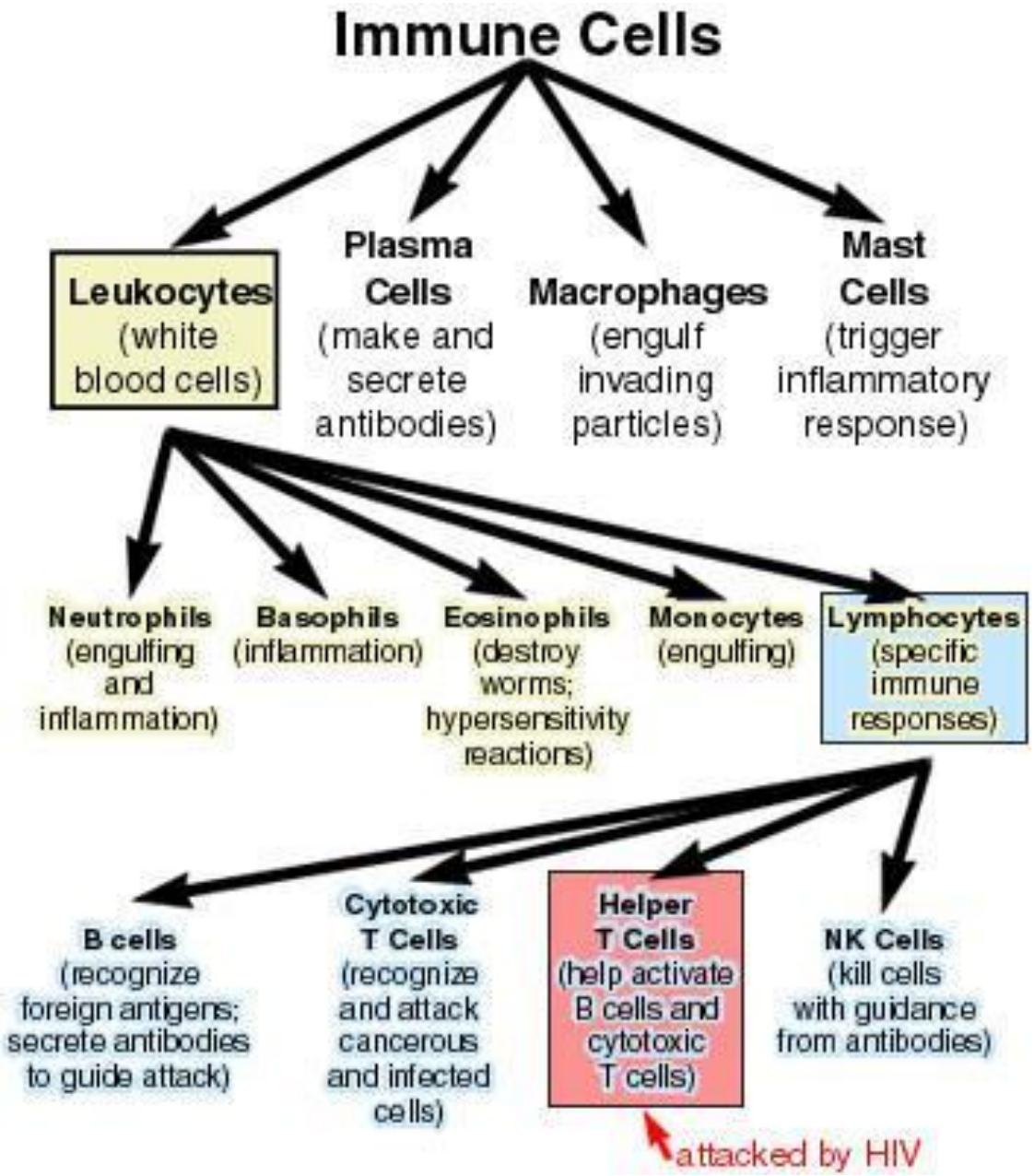

Thymus
Spleen
A.D.A.M.


thymus gland,
large in young
mammal.
heart (ventricle)



The thymus is a two-lobed organ overlying the upper part of the heart. It is large in children. Lymphopoietic cells are modified here to form T lymphocytes (d2; T for thymus; also called T cells).

T lymphocytes make up about 75% of the blood lymphocytes. They have different receptor sites than B cells, and they do not produce antibodies. T lymphocytes are responsible for cell-mediated immunity; that is, immunity associated with cellular interactions.


Immune Cells
Leukocytes (white blood cells)
Plasma Cells (make and secrete antibodies)
Macrophages (engulf invading particles)
Mast Cells (trigger inflammatory response)
Neutrophils (engulfing and inflammation)
Basophils (inflammation)
Eosinophils (destroy worms; hypersensitivity reactions)
Monocytes (engulfing)
Lymphocytes (specific immune responses)
B cells (recognize foreign antigens; secrete antibodies to guide attack)
Cytotoxic T Cells (recognize and attack cancerous and infected cells)
Helper T Cells (help activate B cells and cytotoxic T cells)
NK Cells (kill cells with guidance from antibodies)
attacked by HIV

Key Cells & Overview of their Function in Immune Defense

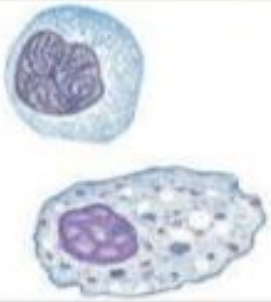
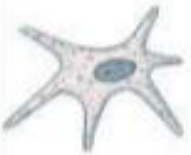
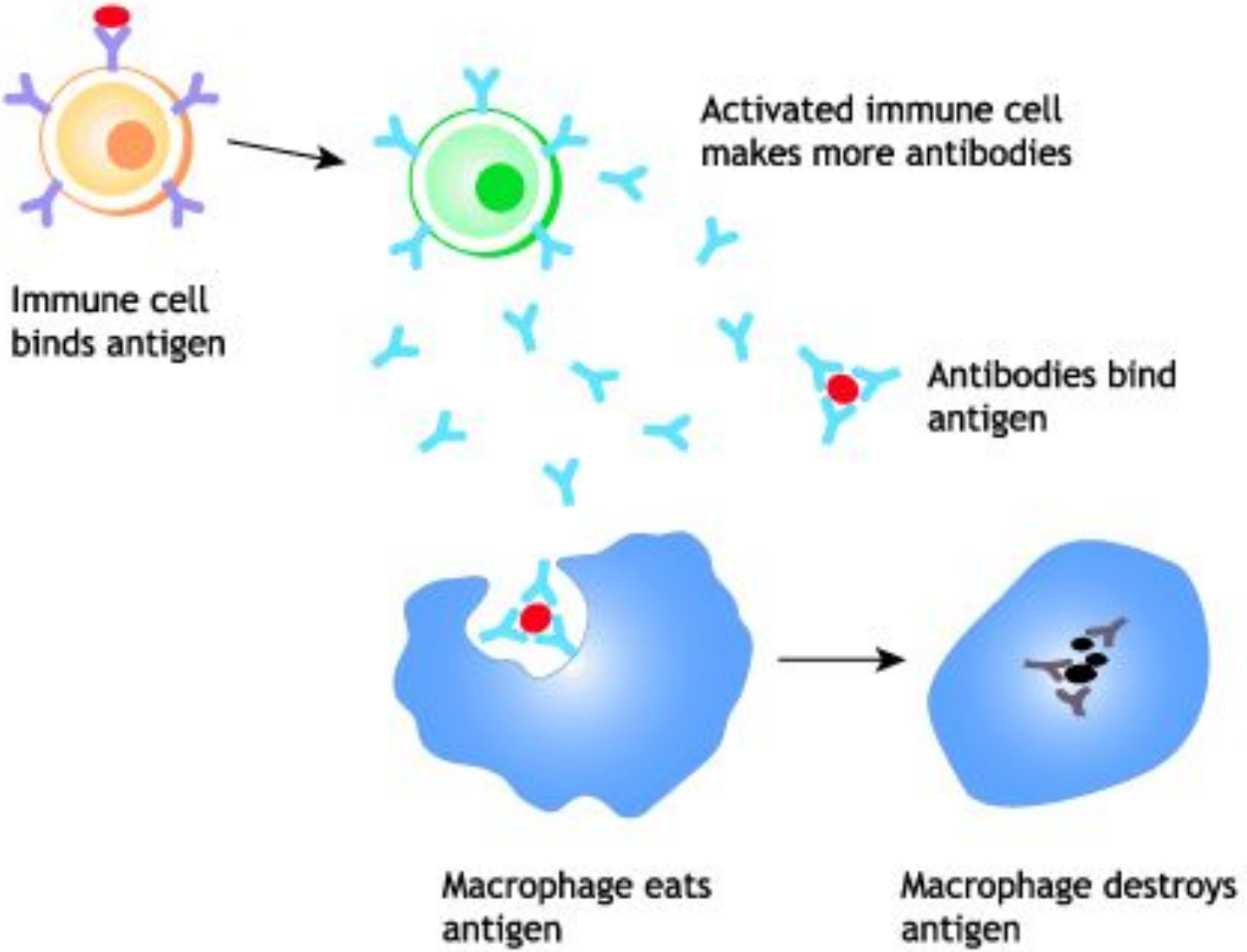
	Basophils and Mast Cells	*Neutrophils*	*Eosinophils*	*Monocytes and Macrophages*	*Lymphocytes and Plasma Cells*	*Dendritic Cells*
						
% of WBCs in blood	*Rare*	*50–70%*	*1–3%*	*1–6%*	*20–35%*	*NA*
Subtypes and nicknames		Called "polys" or "segs" Immature forms called "bands" or "stabs"		Called the mononuclear phagocyte system	B lymphocytes, Plasma cells T lymphocytes Cytotoxic T cells Helper T cells Natural killer cells Memory cells	Also called Langerhans cells, veiled cells
Primary function(s)	Release chemicals that mediate inflammation and allergic responses	Ingest and destroy invaders	Destroy invaders, particularly antibody-coated parasites	Ingest and destroy invaders Antigen presentation	Specific responses to invaders, including antibody production	Recognize pathogens and activate other immune cells by antigen presentation in lymph nodes
Classifications		*Phagocytes*				
		Granulocytes				
			Cytotoxic cells		*Cytotoxic cells (some types)*	
				Antigen-presenting cells		

Figure 24-4: Cells of the immune system



Some immune cells are activated to produce antibodies (such as IgE) against the food toxin.

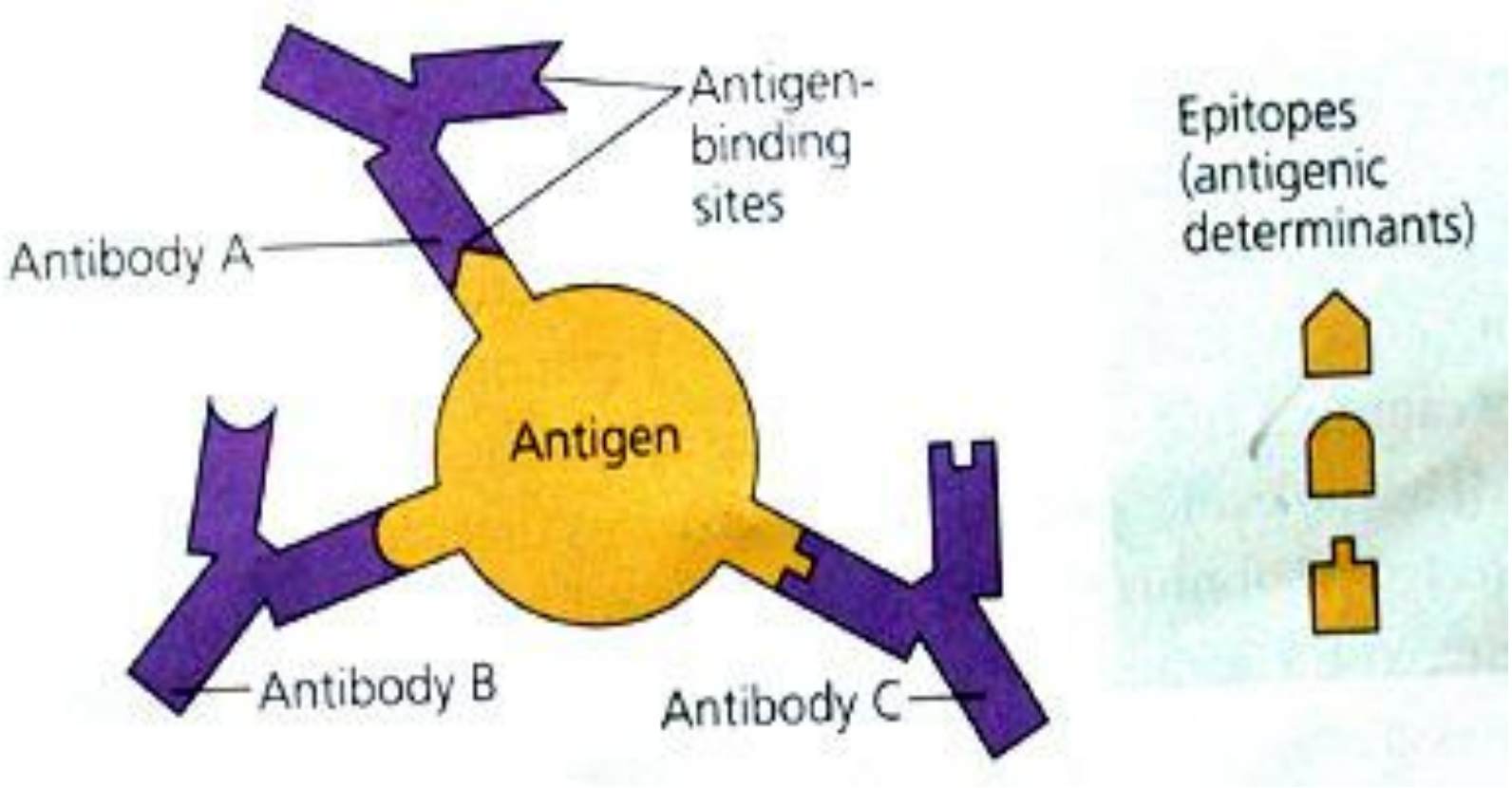
- Antibodies are produced by a specialized group of cells called B-Lymphocytes.

- When an foreign antigen enters the body due immune response B-Lymphocytes develops into plasma cells and liberates antibodies or immunoglobulins of various types(Ig A, Ig D, Ig E, Ig G, Ig M).

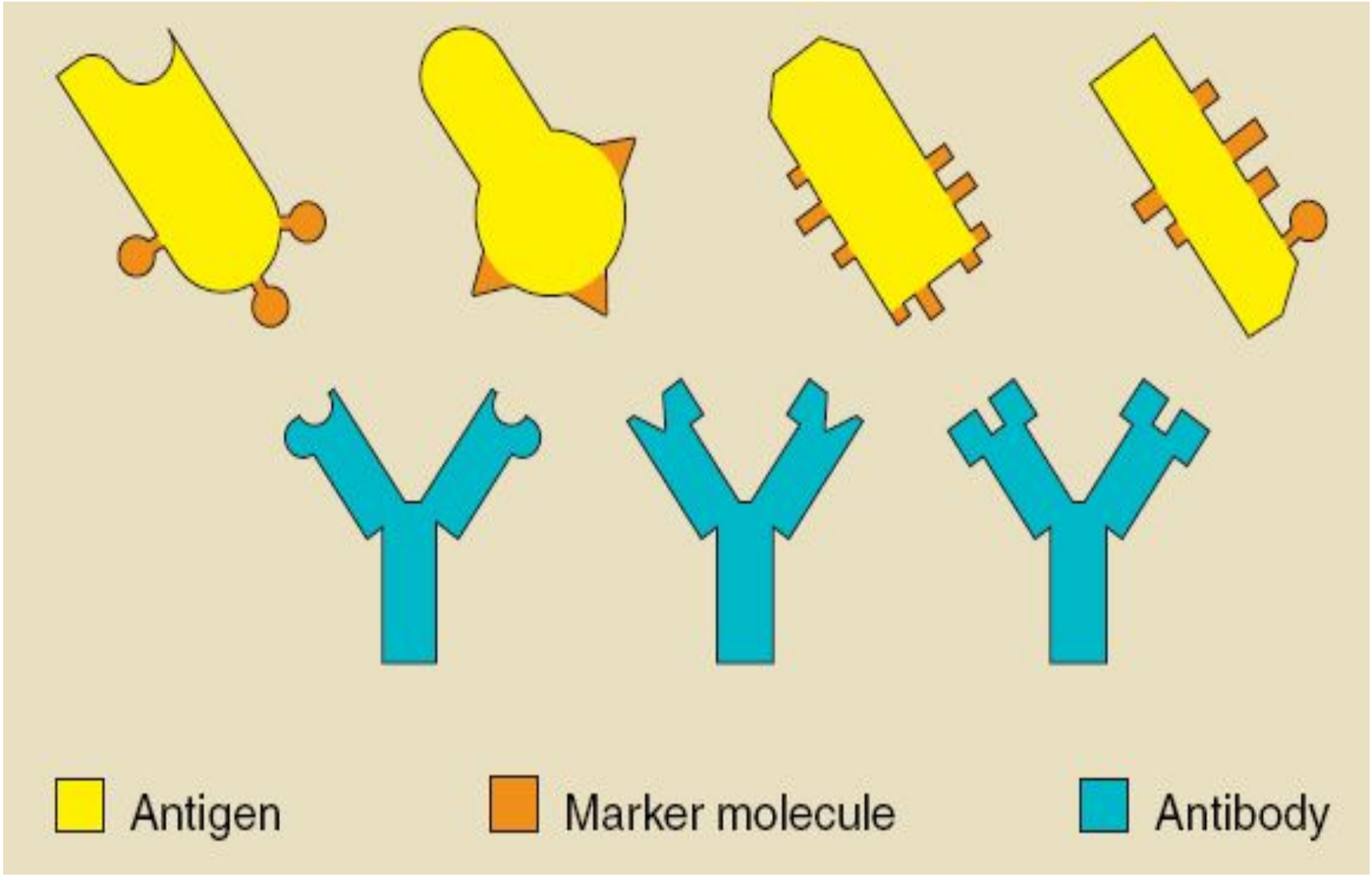
WHAT'S THE ROLE OF ANTIBODY IN IMMUNE SYSTEM?

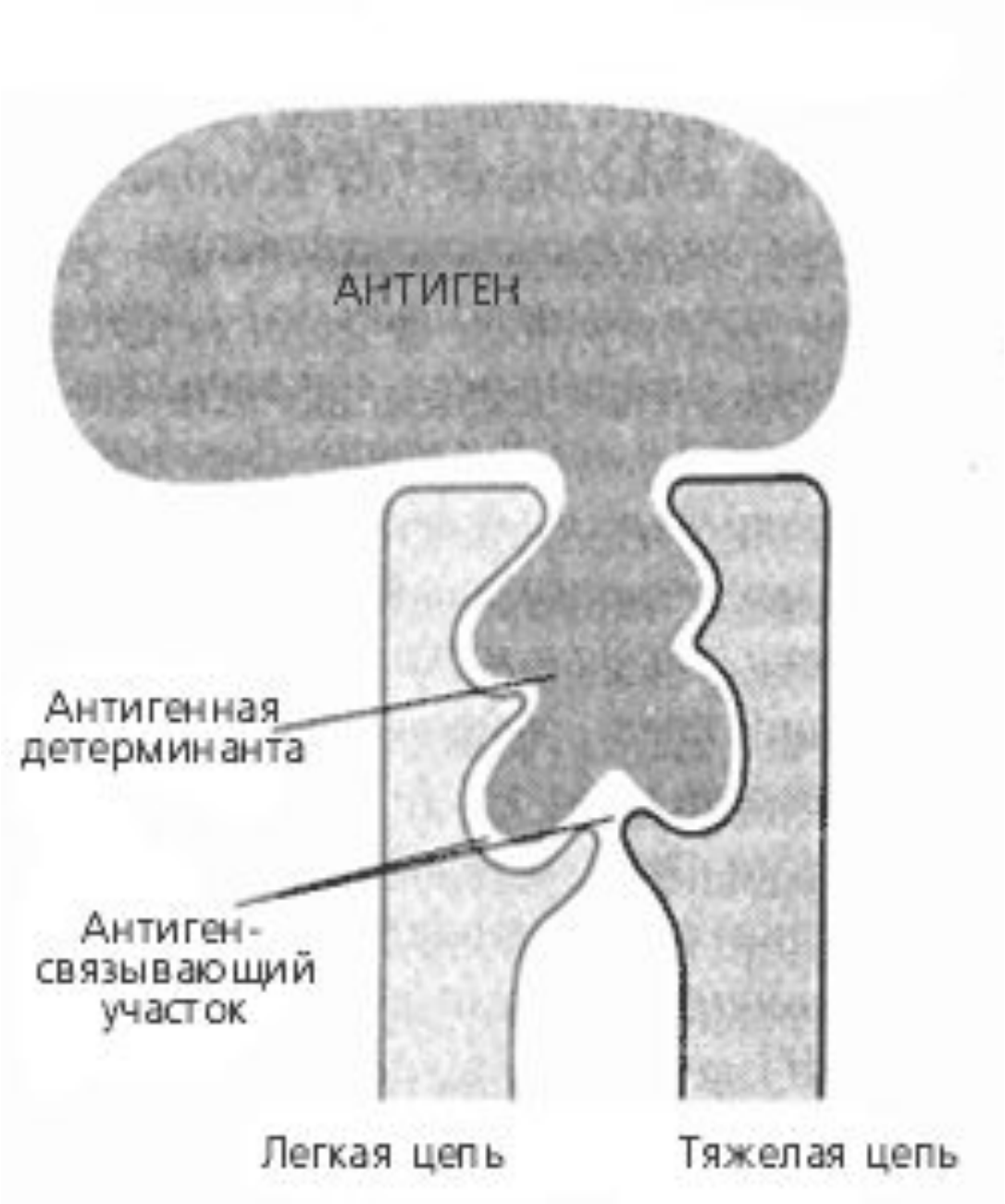
- Each Antigen has specific antigen determinants (epitopes) located on it. The antibodies have complementary determining regions (CDRs). These are mainly responsible for the antibody specificity.
- Each antigen has several different epitopes on it. They are recognised by many different antibodies. All these antibodies thus produced act on the same antigen. Hence these are designated as polyclonal antibodies.

Поликлональность антител при традиционной технологии

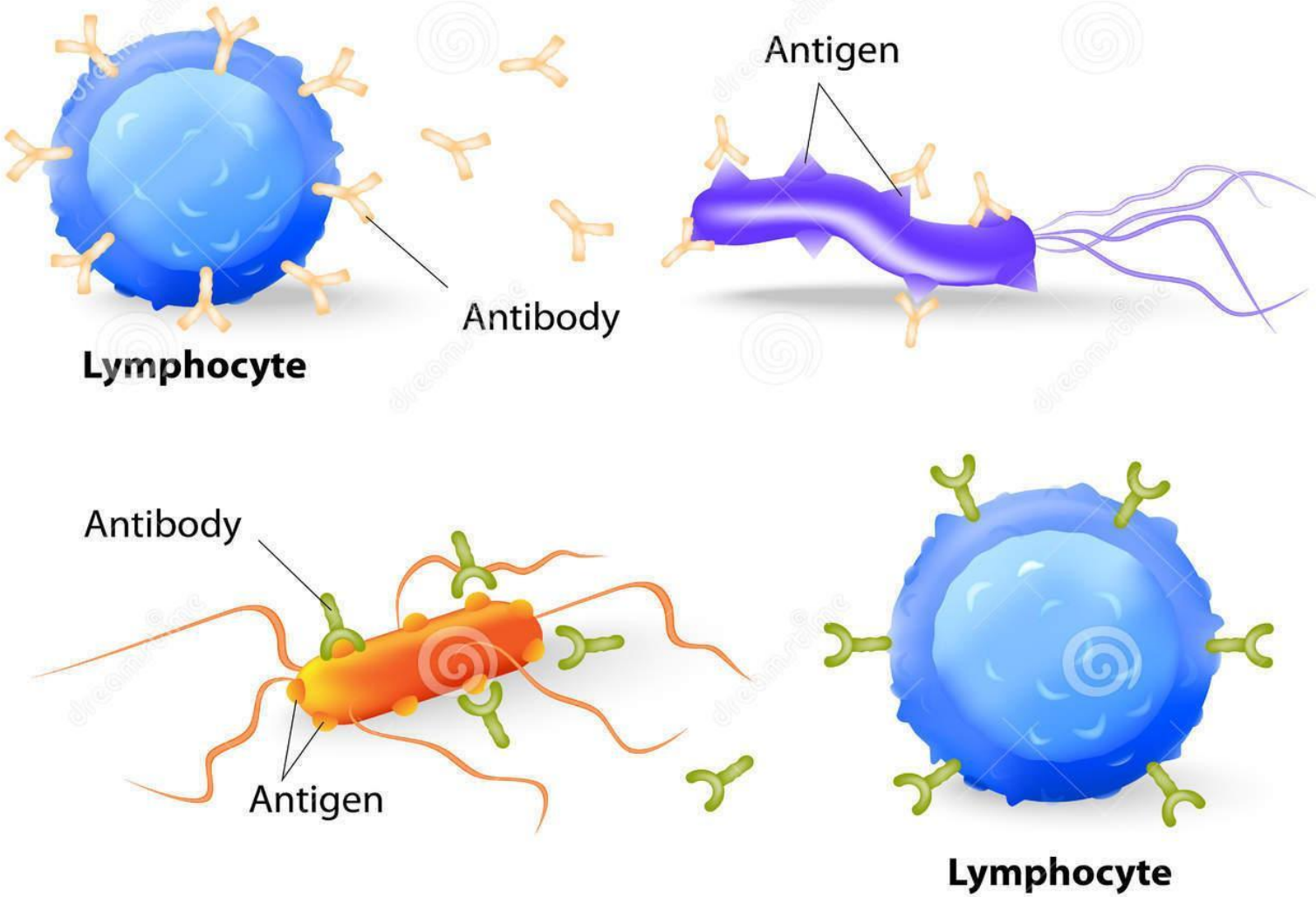


Общие эпитопы гетерогенных антигенов




АНТИГЕН
Антигенная детерминанта
Антиген-связывающий участок
Легкая цепь
Тяжелая цепь

HUMORAL IMMUNITY



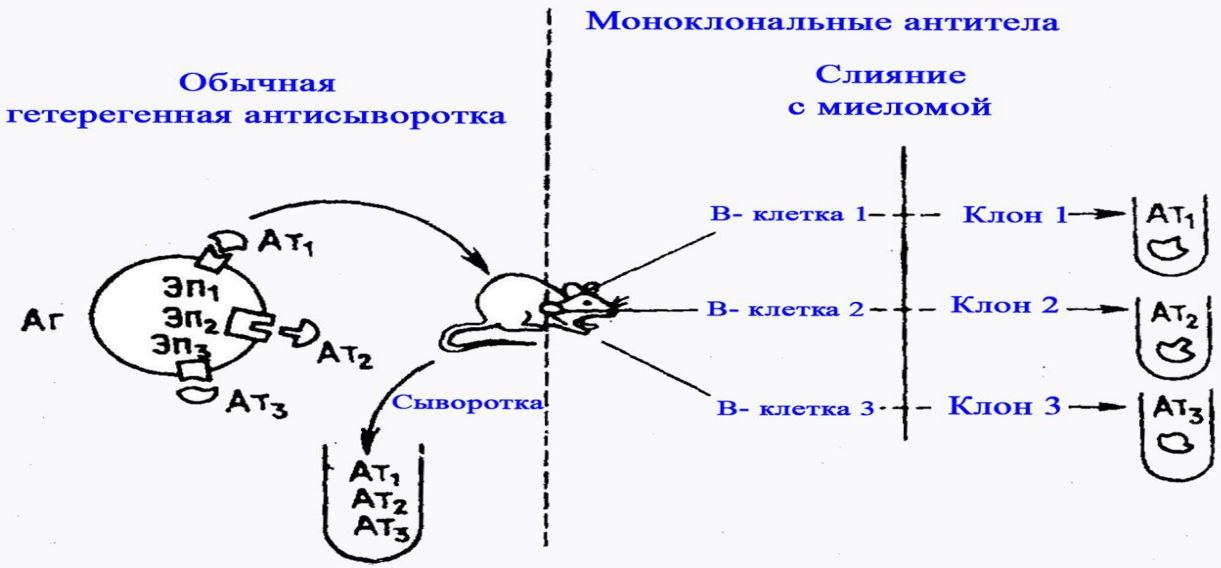
WHAT'S THE NEED TO DEVELOP MONOCLONAL ANTIBODIES?

- In general naturally produced antibodies are non-specific and heterogenous in nature. Hence there are several limitations in the use of polyclonal antibodies for therapeutic and diagnostic purposes.
- Thus there is a need for producing monoclonal antibodies for different antigens.
- George Kohler and Cesar Milstein got noble prize in 1984 for the production of MAbs in large scale.

WHAT ARE MONOCLONAL ANTIBODIES?

- MAb is a single type of antibody that is directed against a specific antigenic determinant(epitope).
- Monoclonal antibodies are specific to antigen and are homogenous.

СРАВНЕНИЕ ПОЛИКЛОНАЛЬНЫХ И МОНОКЛОНАЛЬНЫХ АНТИТЕЛ

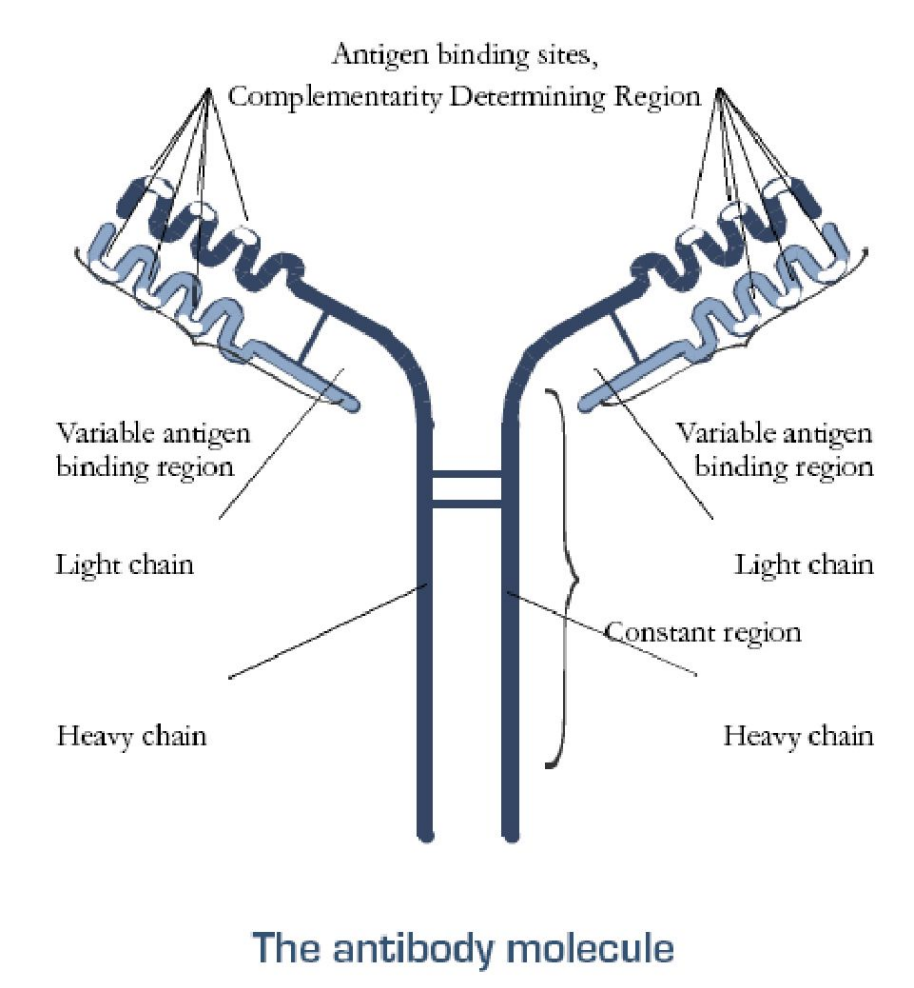


Сравнение обычной антисыворотки, полученной, с моноклональными антителами, полученными in vitro (Джон Ф.Кирней, 1989)

History of Mab development

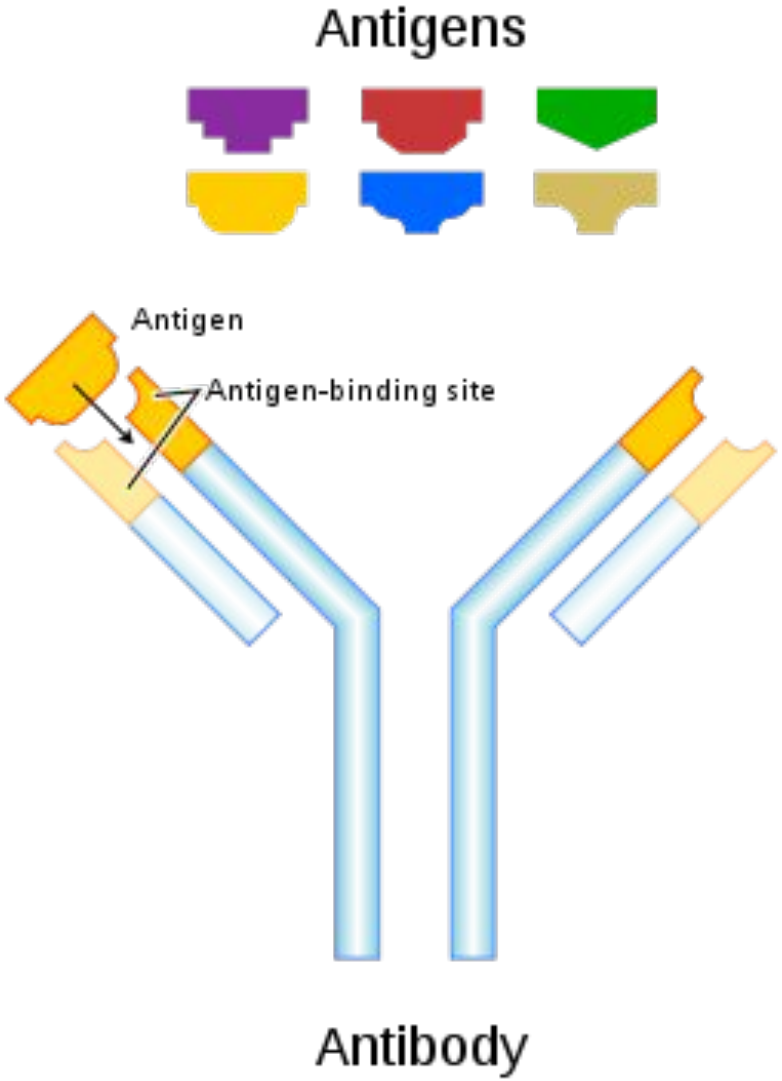
- 1890 Von Behring and kitasato discovered the serum of vaccinated persons contained certain substances, termed antibodies
- 1900 Ehrlich proposed the " side-chain theory"
- 1955 Jerne postulated natural selection theory. Frank Macfarlane Burnet expended.
- Almost the same time, Porter isolated fragment of antigen binding (Fab) and fragment crystalline (Fc) from rabbit y-globulin.
- 1964 Littlefield developed a way to isolate hybrid cells from 2 parent cell lines using the hypoxanthine-aminopterin-thymidine (HAT) selection media.
- 1975 Kohler and Milstein provided the most outstanding proof of the clonal selection theory by fusion of normal and malignant cells
- 1990 Milstein produced the first monoclonal antibodies.

Structure of MAb



The antibody molecule

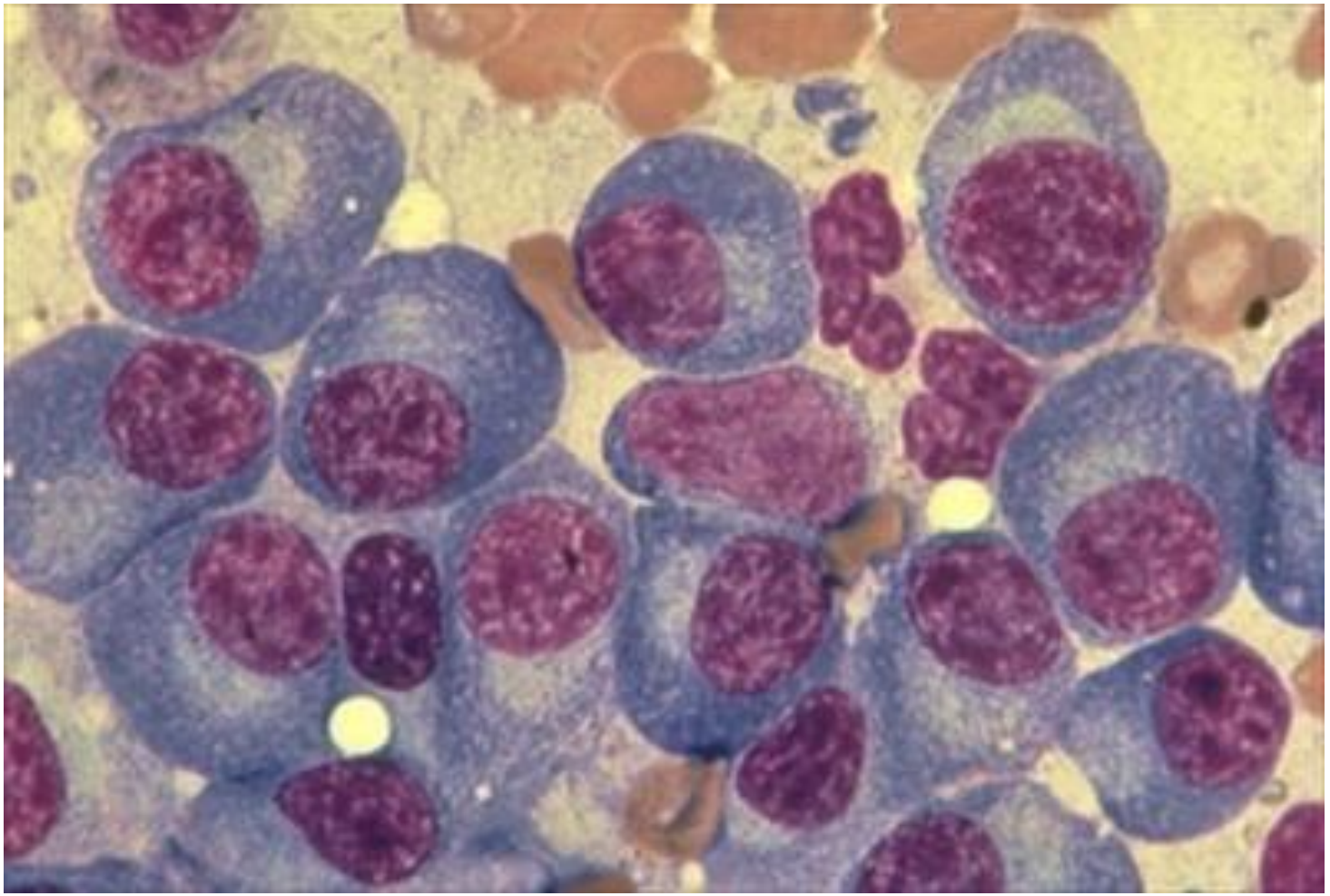
Antigen- antibody binding



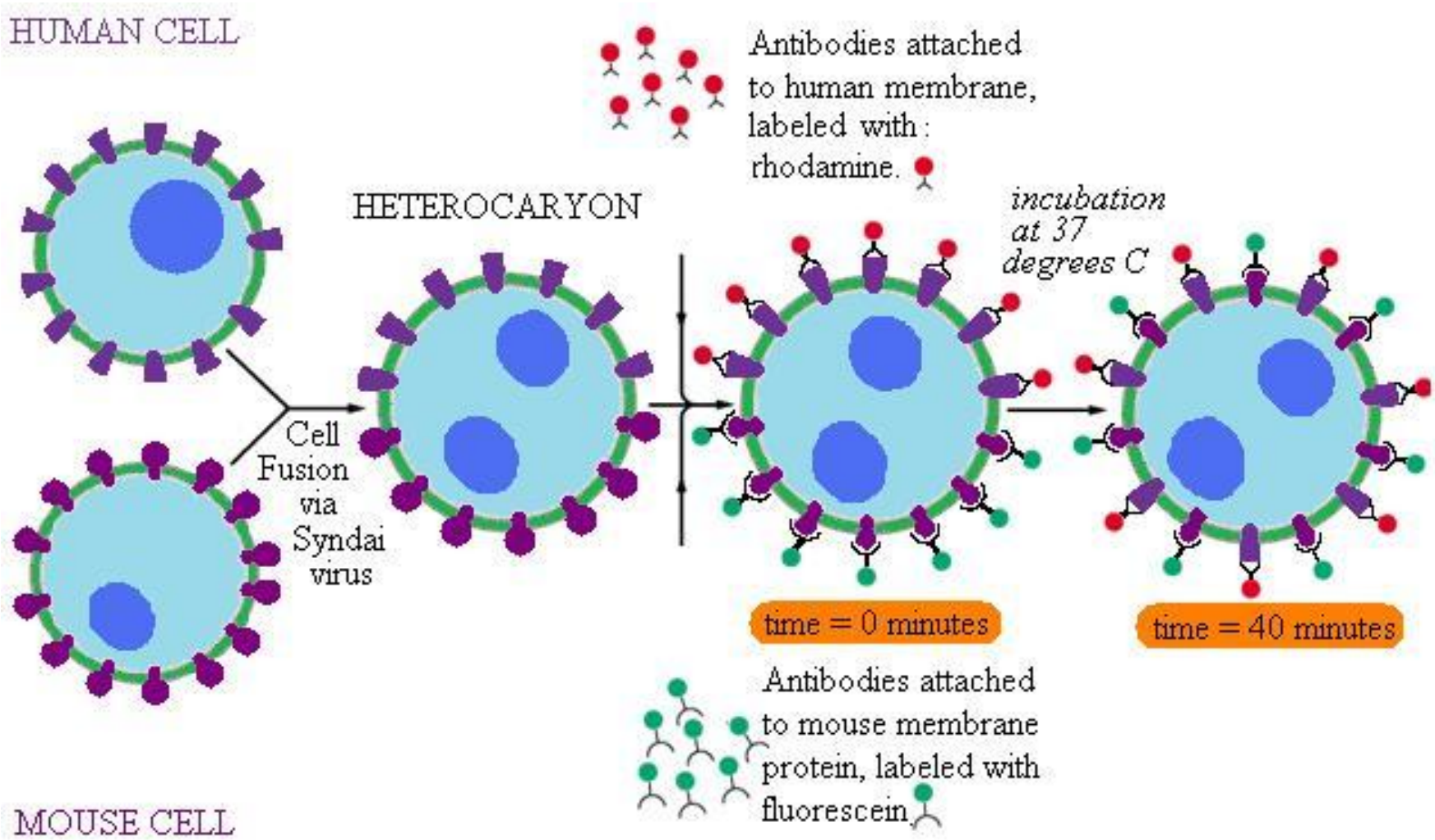
PRINCIPLE INVOLVED IN MONOCLONAL ANTIBODIES PRODUCTION

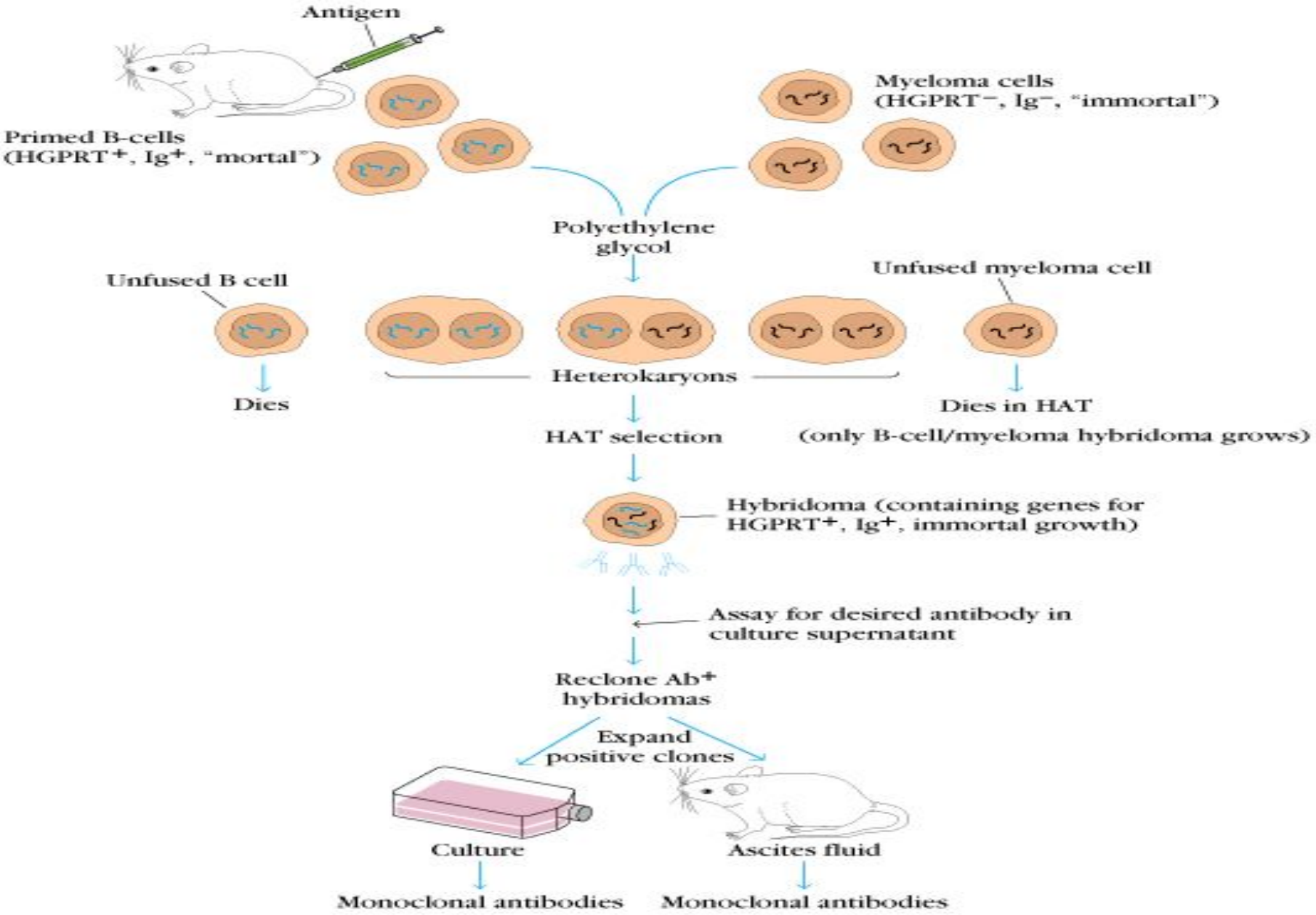
- Hybridoma technology: In this B-Lymphocytes and myeloma cells are mixed together and exposed to PEG for a short period.
- The mixture contains hybridoma cells, myeloma cells and lymphocytes.
- This mixture is washed and cultured in HAT(hypoxanthine aminopterin and thymidine) medium for 7-10 days.
- only hybridoma cells remain in the mixture.

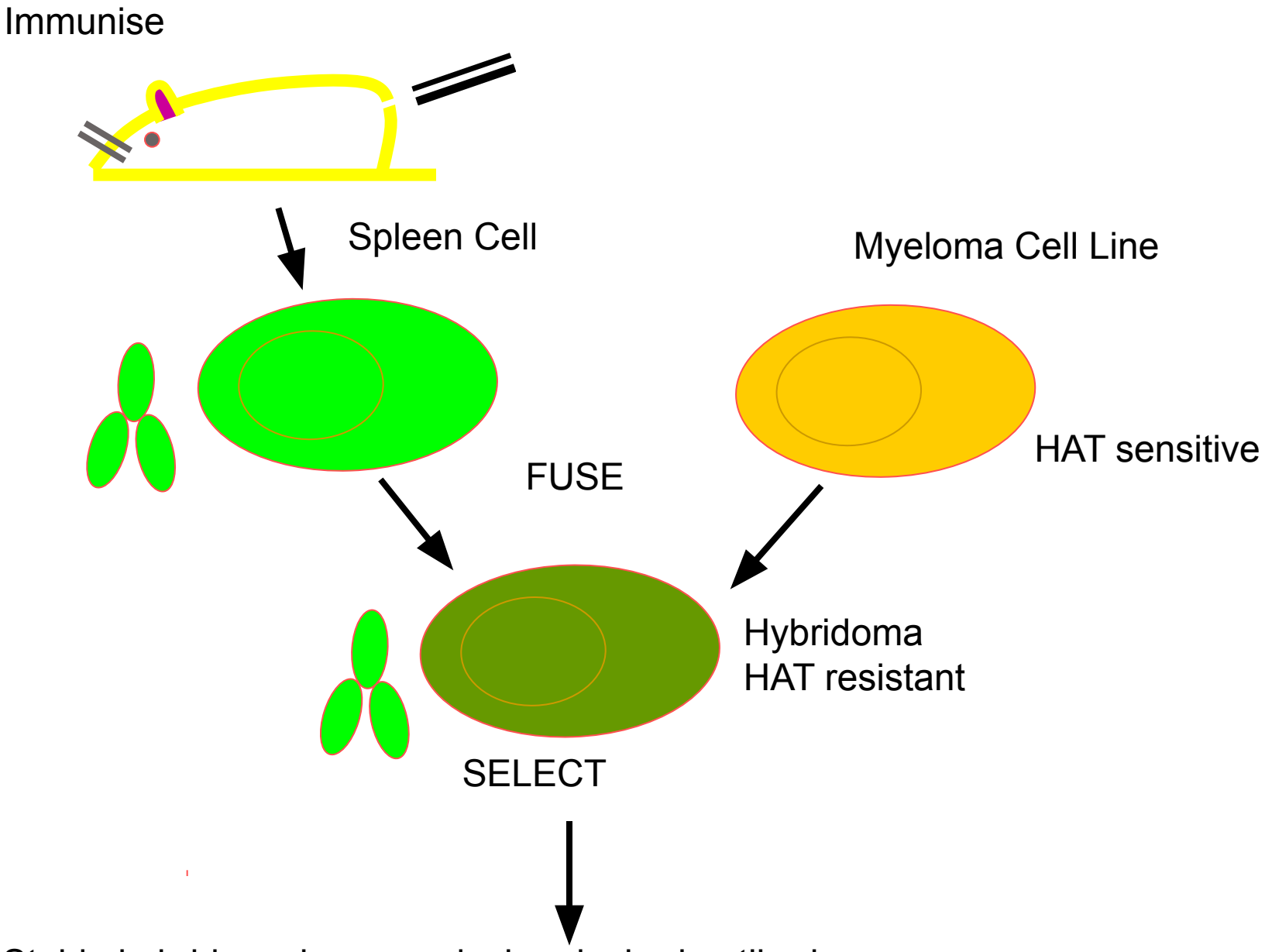
Плазмоцитомы



Слияние клеток человека и мыши




Antigen
Primed B-cells
(HGPRT+, Ig+, "mortal")
Myeloma cells
(HGPRT−, Ig−, "immortal")
Polyethylene
glycol
Unfused B cell
Unfused myeloma cell
Dies
Heterokaryons
Dies in HAT
(only B-cell/myeloma hybridoma grows)
HAT selection
Hybridoma (containing genes for
HGPRT+, Ig+, immortal growth)
Assay for desired antibody in
culture supernatant
Reclone Ab+
hybridomas
Expand
positive clones
Culture
Ascites fluid
Monoclonal antibodies
Monoclonal antibodies


Immunise
Spleen Cell
Myeloma Cell Line
HAT sensitive
FUSE
Hybridoma
HAT resistant
SELECT
Stable hybrid myeloma producing desired antibody

PRODUCTION OF MONOCLONAL ANTIBODIES

- Immunization
- Cell fusion
- Selection of hybridomas
- Screening the products
- Cloning and propagation
- Characterization and storage

Мыши линии *Balb/c*

Immunization

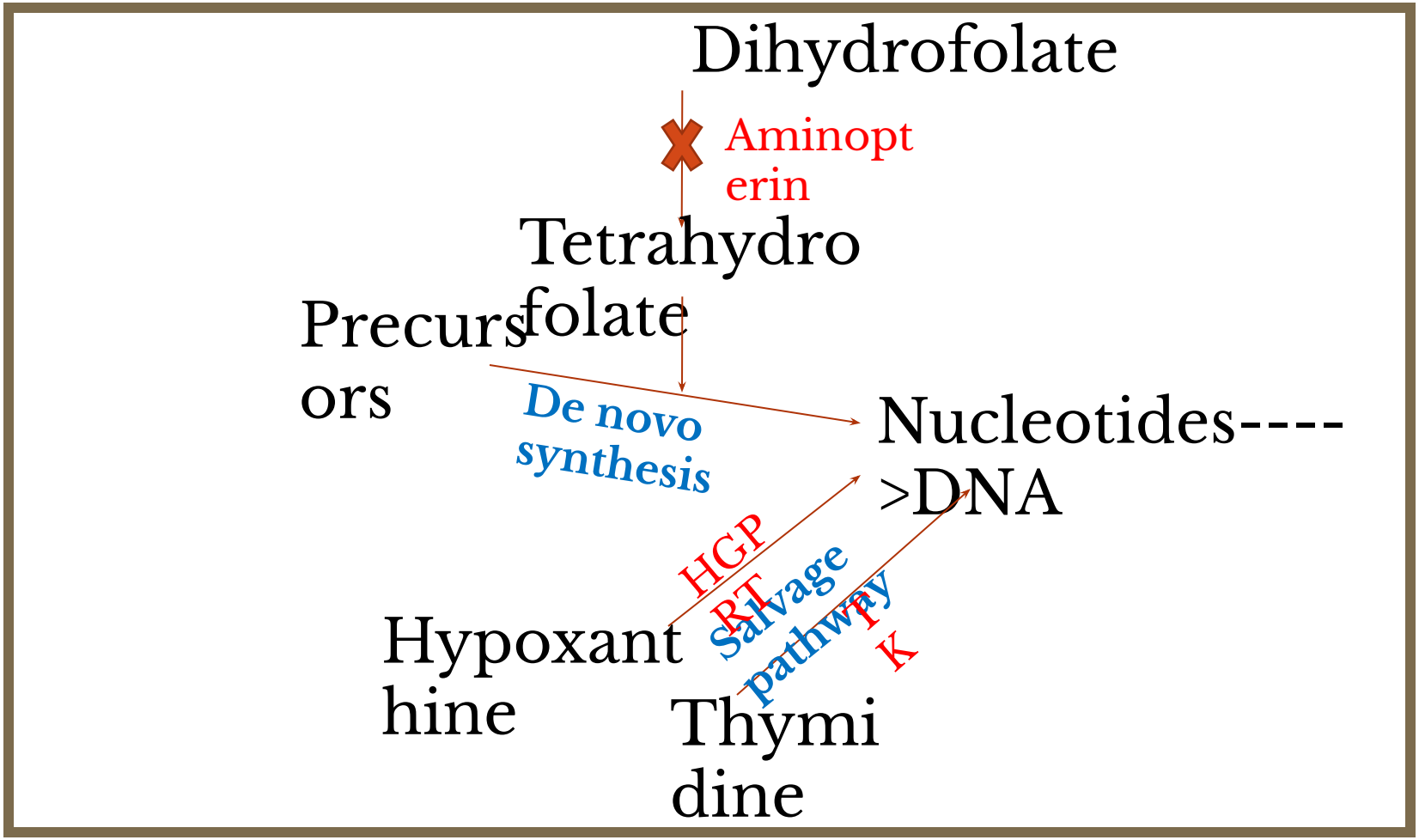
- Immunize an animal usually mouse by injecting with an appropriate antigen along with Freund's complete or incomplete adjuvant.
- Adjuvants are non specific potentiators of specific immune responses.
- Injection of antigens at multiple sites are repeated several times for increased stimulation of antibodies.
- 3 days prior to killing of animal a final dose is given intravenously.
- Spleen is aseptically removed and disrupted by mechanical or enzymatic methods to release the cells.
- By density gradient centrifugation

Иммунизация мыши линии *Balb/c*

Cell fusion

- Lymphocytes are mixed with HGPRT deficient myeloma cells and is exposed to PEG for a short period.
- The mixture is then washed and kept in a fresh medium.
- The mixture contains hybridomas, free myeloma cells, and free lymphocytes.

Synthesis of nucleotides



Selection of hybridomas

- The above mixture is cultured in HAT medium for 7-10 days.
- Due to lack of HGPRT enzyme in myeloma cells, salvage pathway is not operative and aminopterin in HAT medium blocks the de novo synthesis of nucleotides. Hence free myeloma cells are dead.
- As the lymphocytes are short lived they also slowly dissappear.
- Only the hybridomas that receives HGPRT from lymphocytes are survived.
- Thus hybridomas are selected by using HAT medium
- Suspension is diluted so that each aliquot

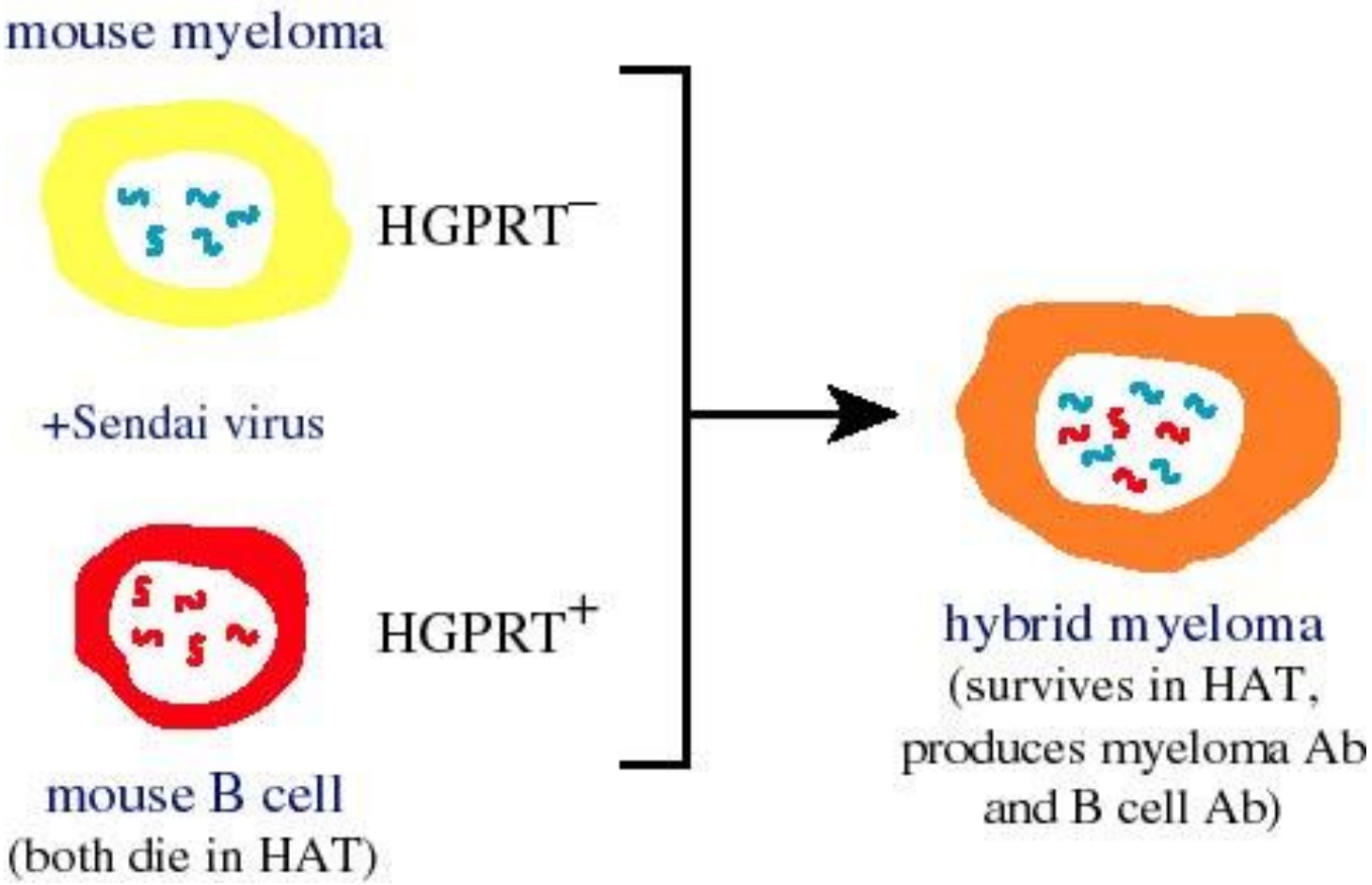
Среда RPMI-1640

Сыворотка плода коровы

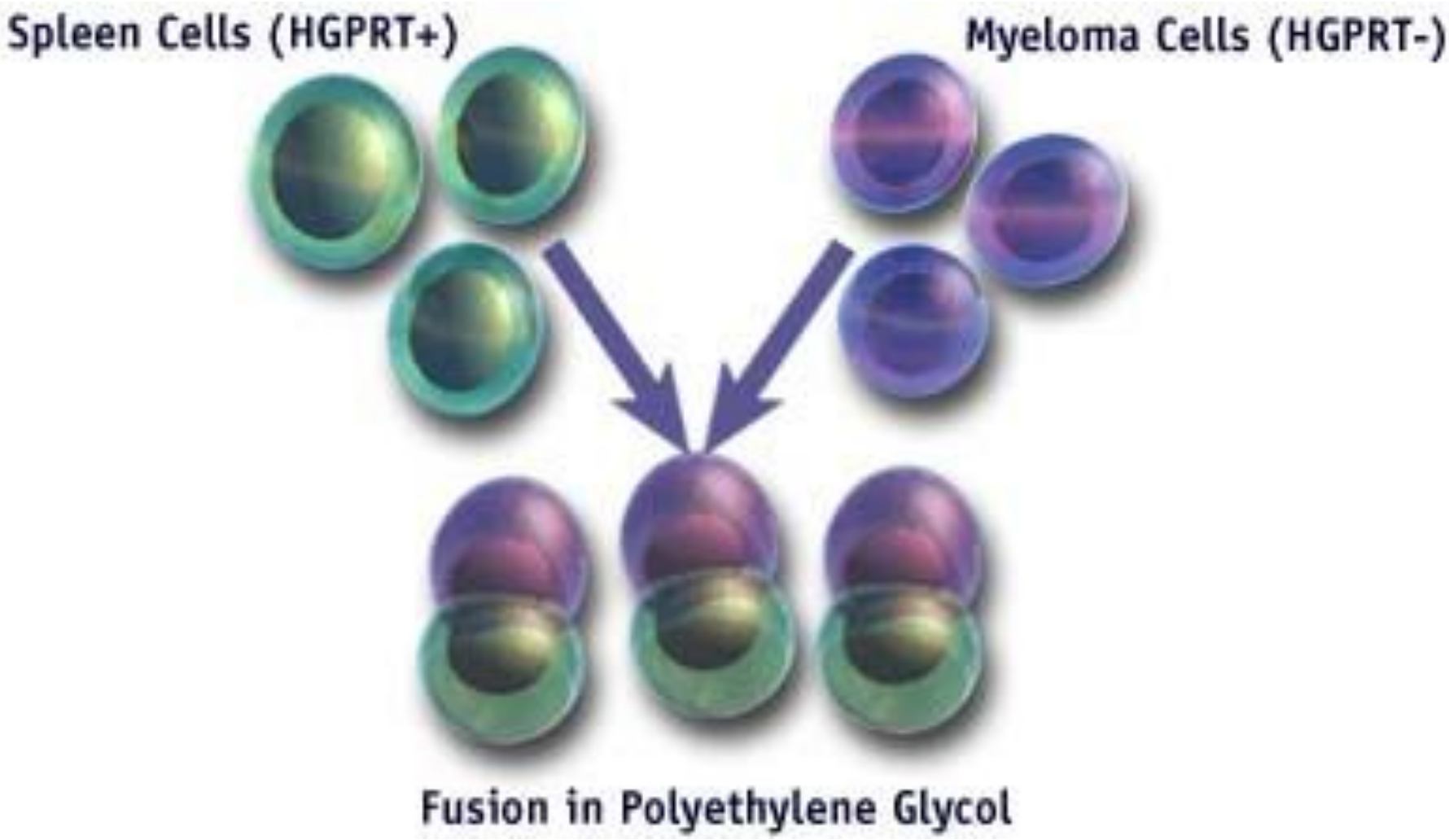
Слияние иммунных лимфоцитов с миеломой

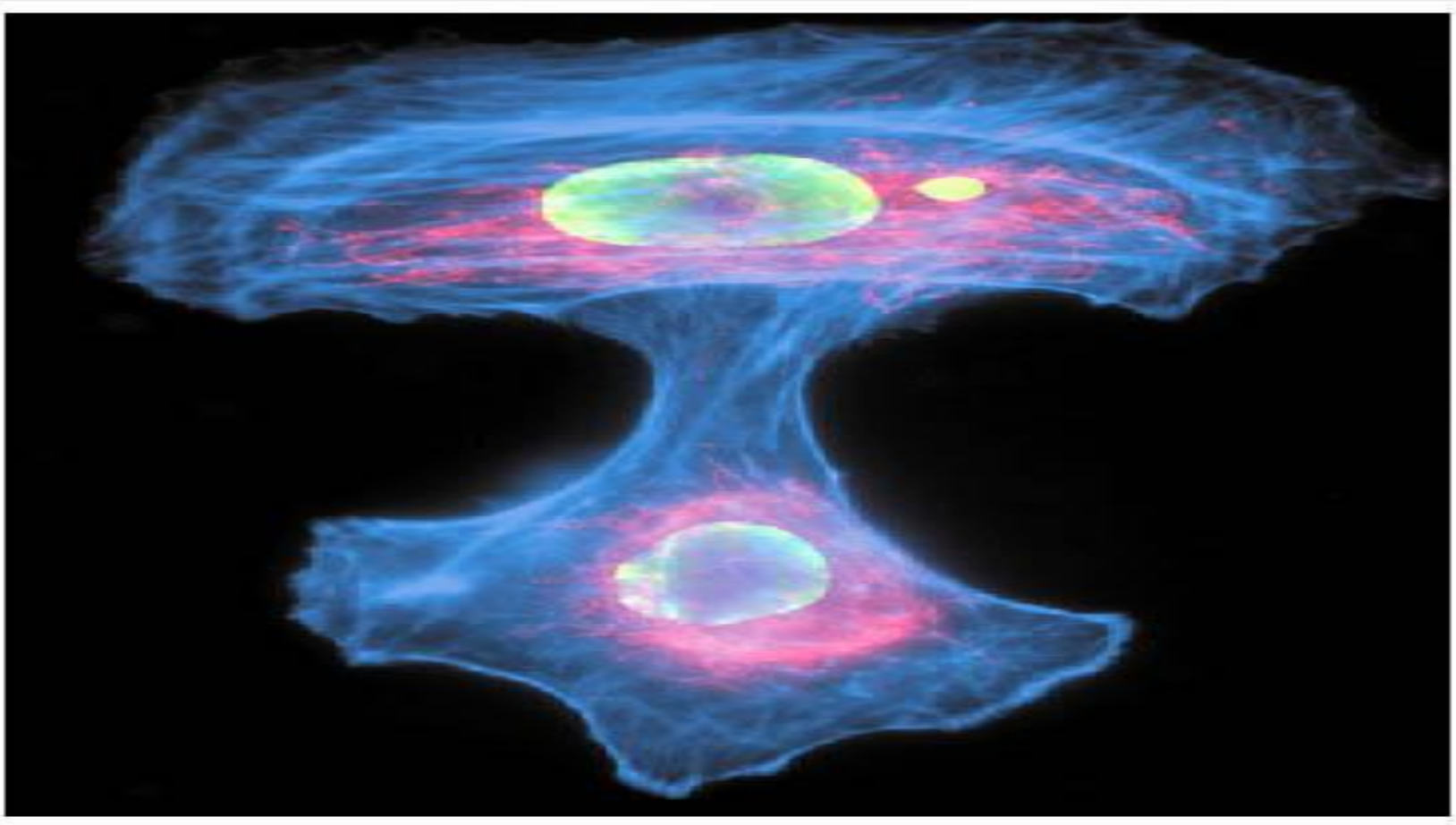
96-луночные планшеты для культуральных работ

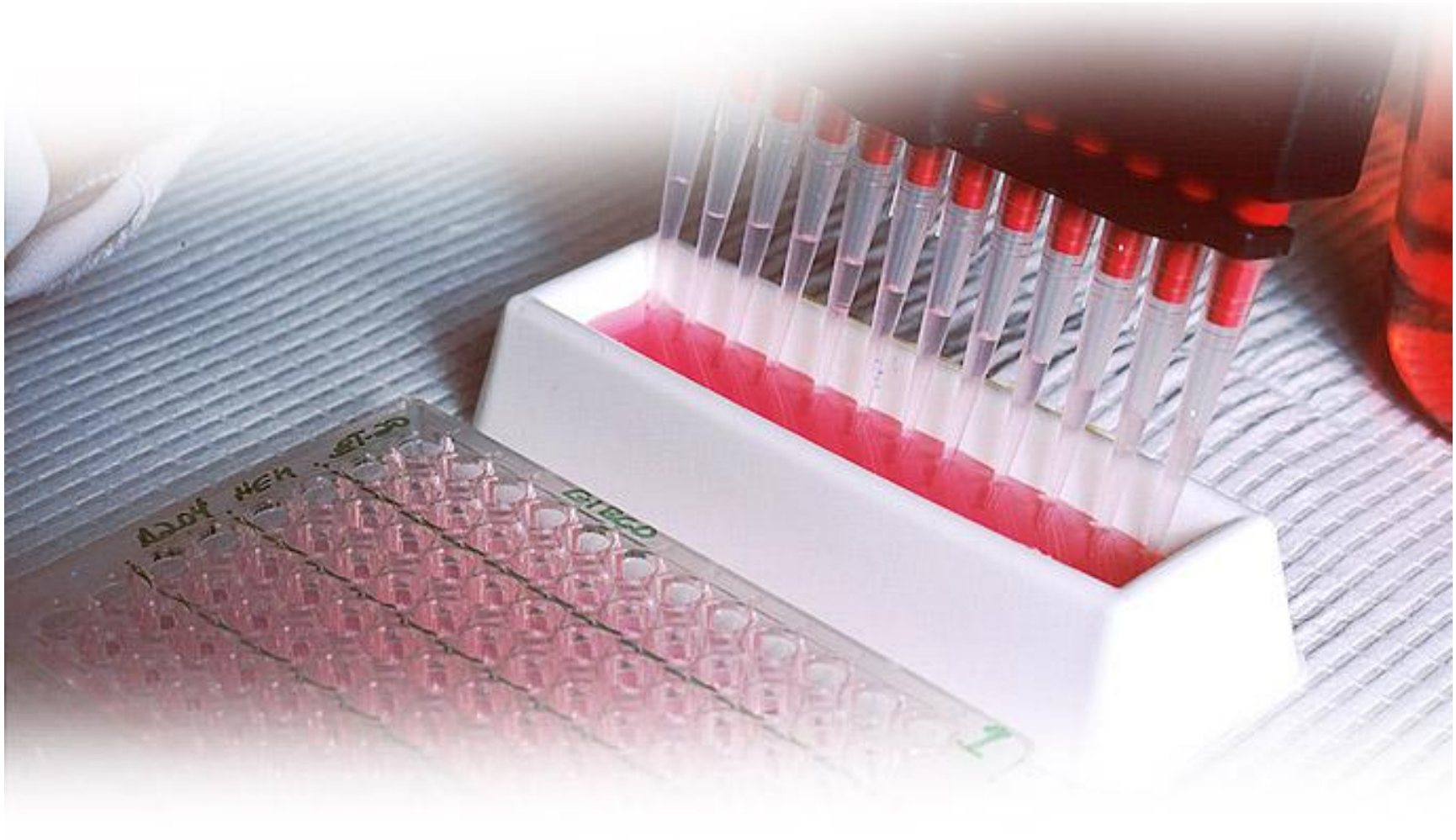
Образование гибридной клетки



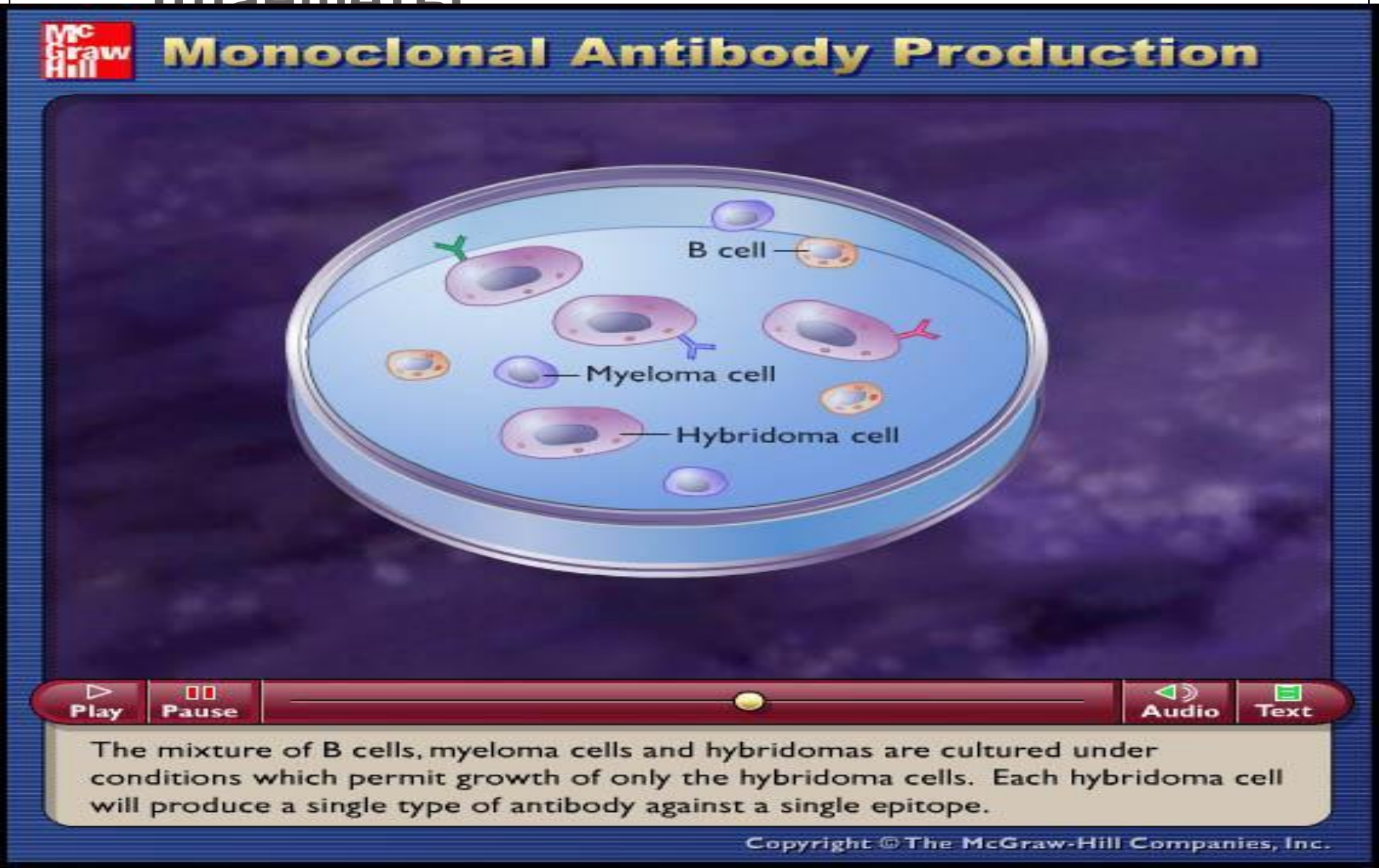
Слияние лифоцитов с миеломой



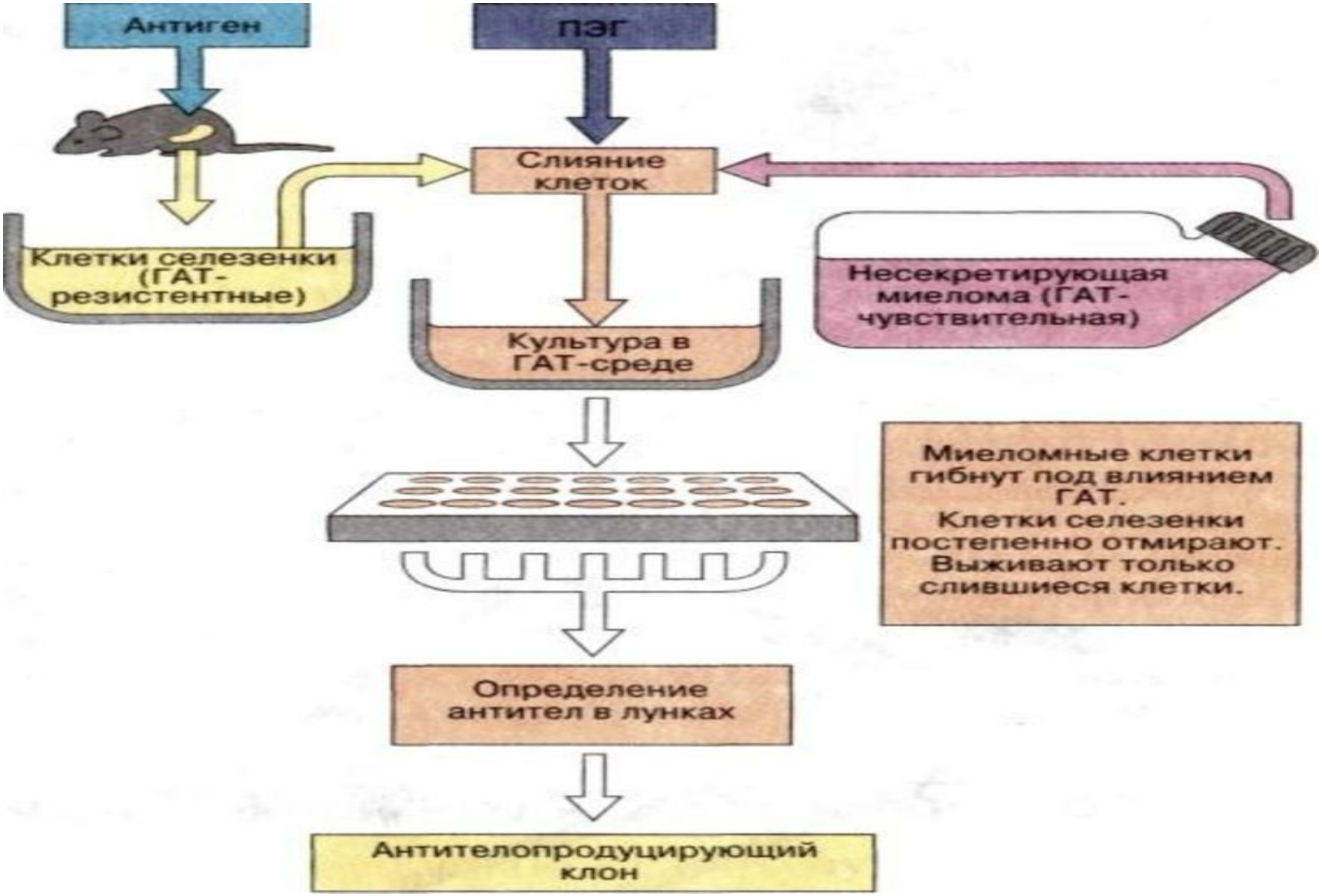




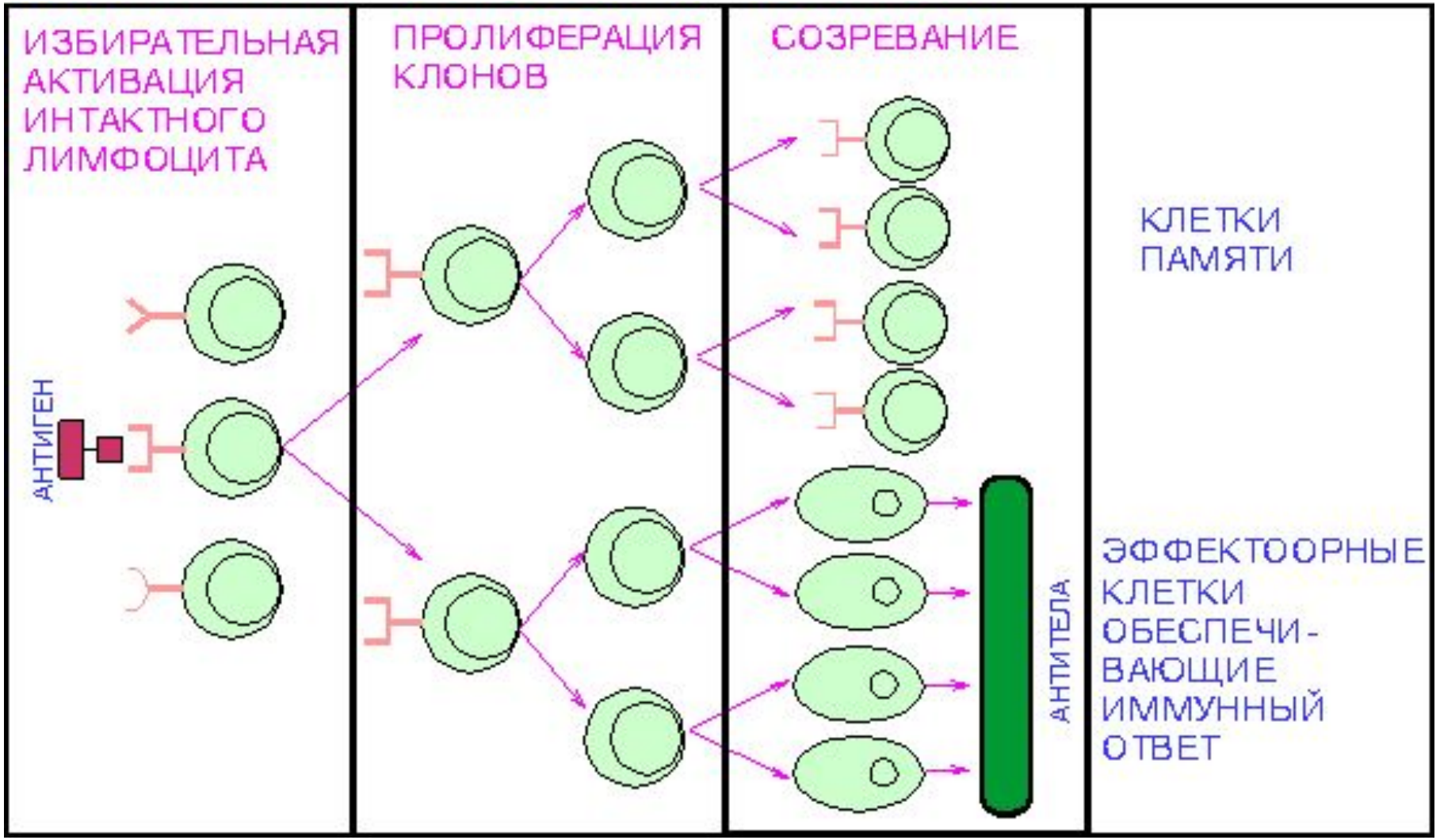
Распределение клеток по лункам планшеты



Культивирование гибридом в CO_2-инкубаторе


Антиген
ПЭГ
Слияние клеток
Клетки селезенки (ГАТ-резистентные)
Несекретирующая миелома (ГАТ-чувствительная)
Культура в ГАТ-среде
Миеломные клетки гибнут под влиянием ГАТ.
Клетки селезенки постепенно отмирают.
Выживают только слившиеся клетки.
Определение антител в лунках
Антителопродуцирующий клон

КЛОНАЛЬНО-СЕЛЕКЦИОННАЯ ТЕОРИЯ БЕРНЕТА



Постулаты теории клональной селекции

- Каждый В лимфоцит имеет рецептор уникальной специфичности.
- Высокоаффинное (прочное) взаимодействие рецептора с антигеном приводит к активации лимфоцита.

Специфичность рецептора сохраняется в процессе пролиферации и дифференцировки лимфоцита.

Что определяет специфичность В-клеточного рецептора?

Антигенраспознающий участок молекулы поверхностносвязанного иммуноглобулина, распознающий только одну антигенную детерминанту.

Виды клеток, образуемые в процессе слияния

1. Неслившиеся клетки лимфоидного органа;
2. Неслившиеся клетки миеломы;
3. Гибриды лимфоцит+лимфоцит и миелома+миелома;
4. **Лимфоцит+миелома**, из которых лишь часть (часто весьма небольшая) стабильно продуцирует антитела нужной специфичности.

Selection of hybridomas

- The above mixture is cultured in HAT medium for 7-10 days.
- Due to lack of HGPRT enzyme in myeloma cells, salvage pathway is not operative and aminopterin in HAT medium blocks the de novo synthesis of nucleotides. Hence free myeloma cells are dead.
- As the lymphocytes are short lived they also slowly dissappear.
- Only the hybridomas that receives HGPRT from lymphocytes are survived.
- Thus hybridomas are selected by using HAT medium
- Suspension is diluted so that each aliquot

Изоляция гибридов лимфоцит+миелома

- от несливших лимфоцитов и гибридов лимфоцит+лимфоцит избавляться не нужно: через несколько дней они умрут сами;

- от несливших опухолевых клеток и гибридов миелома+ миелома избавляются с помощью селективных сред;

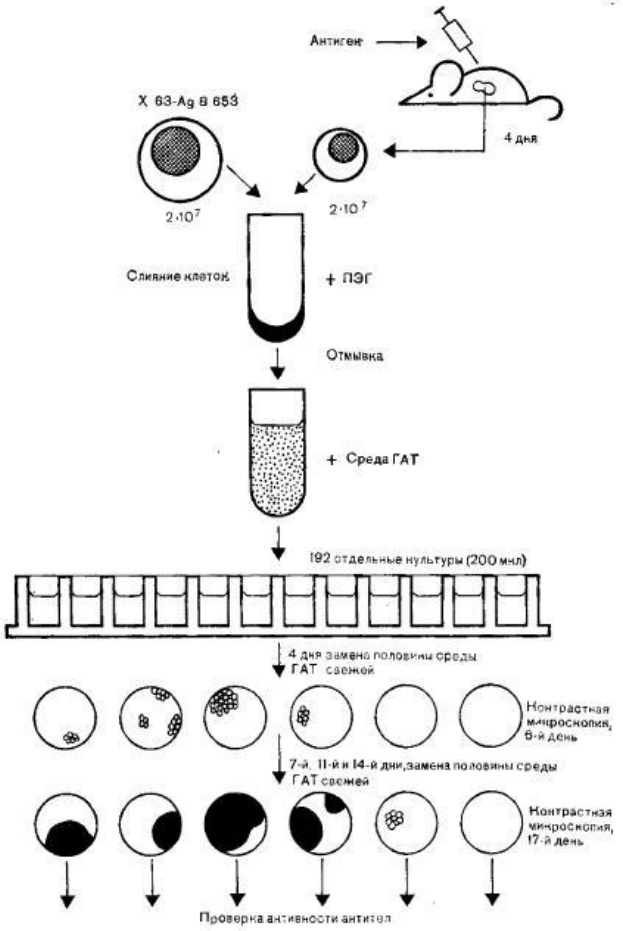
- среди гибридов лимфоцит+миелома отбирают лишь те, которые стабильно продуцируют антитела требуемой специфичности.

Screening the products

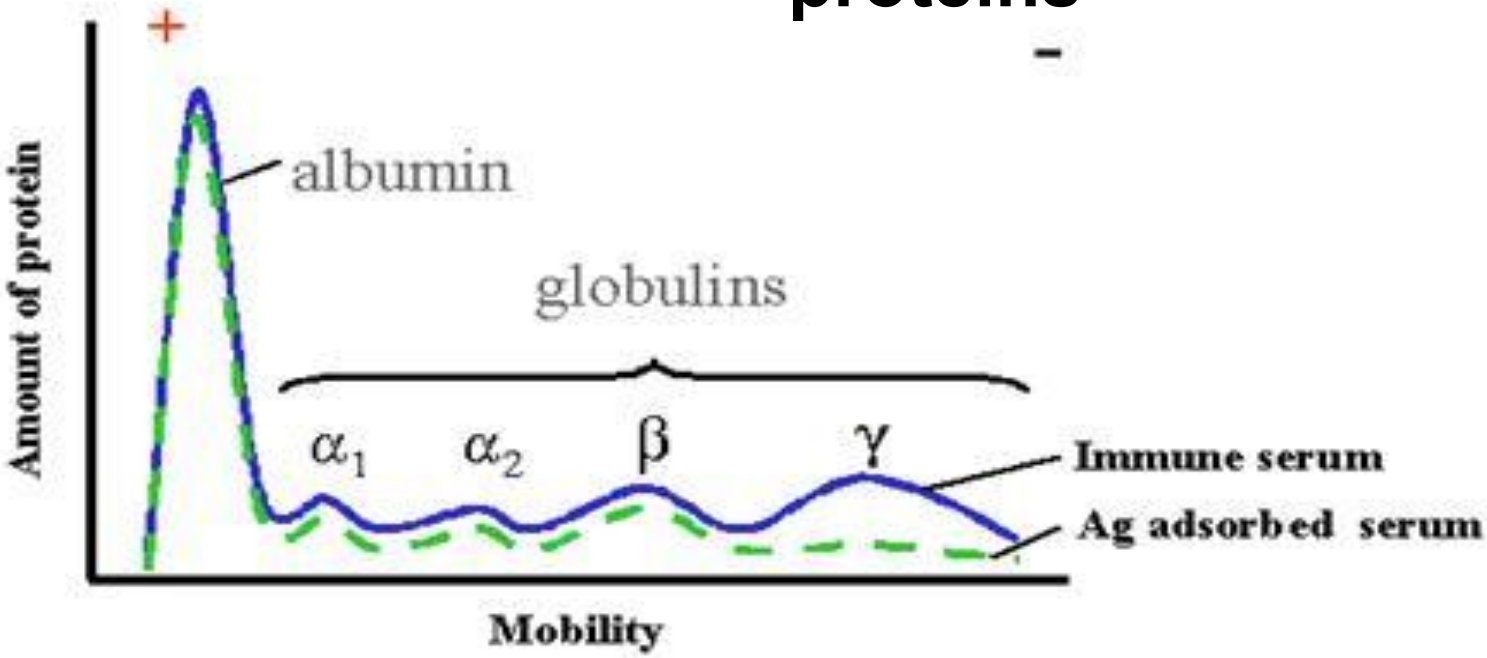
- Screening is done for antibody specificity.
- For this we need to test the culture medium from each hybridoma culture for desired antibody specificity.
- Common tests like ELISA and RIA are used for this.
- In these tests the antigens are coated to plastic plates. The antibodies specific to the antigens bind to the plates. The remaining are washed off.
- Thus the hybridomas producing desired antibodies are identified. The antibodies secreted by them are homogenous and


1980 | 2010
30 years of existence
30 years

Схема получения МКА



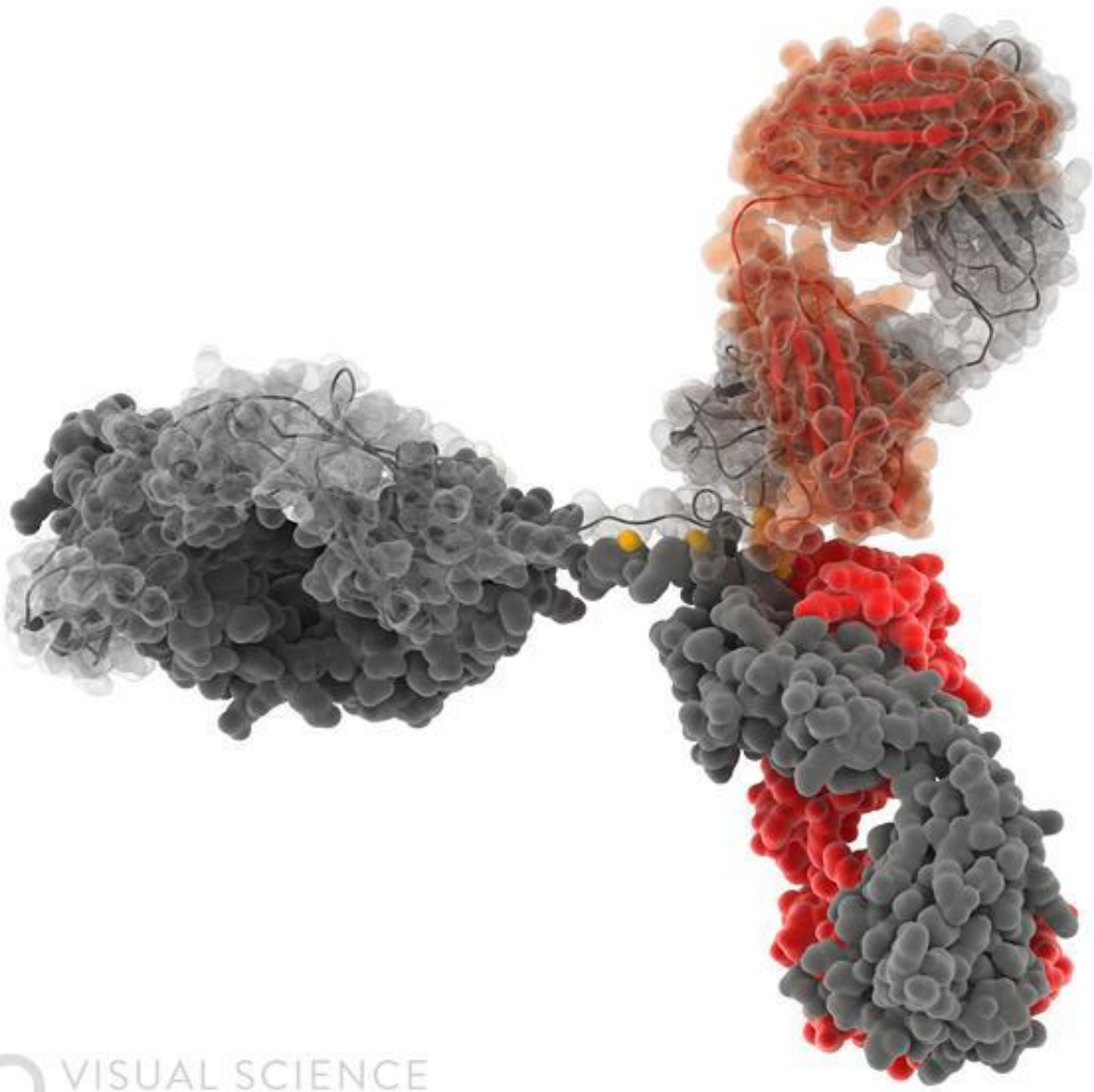
Electrophoretic separation of serum proteins

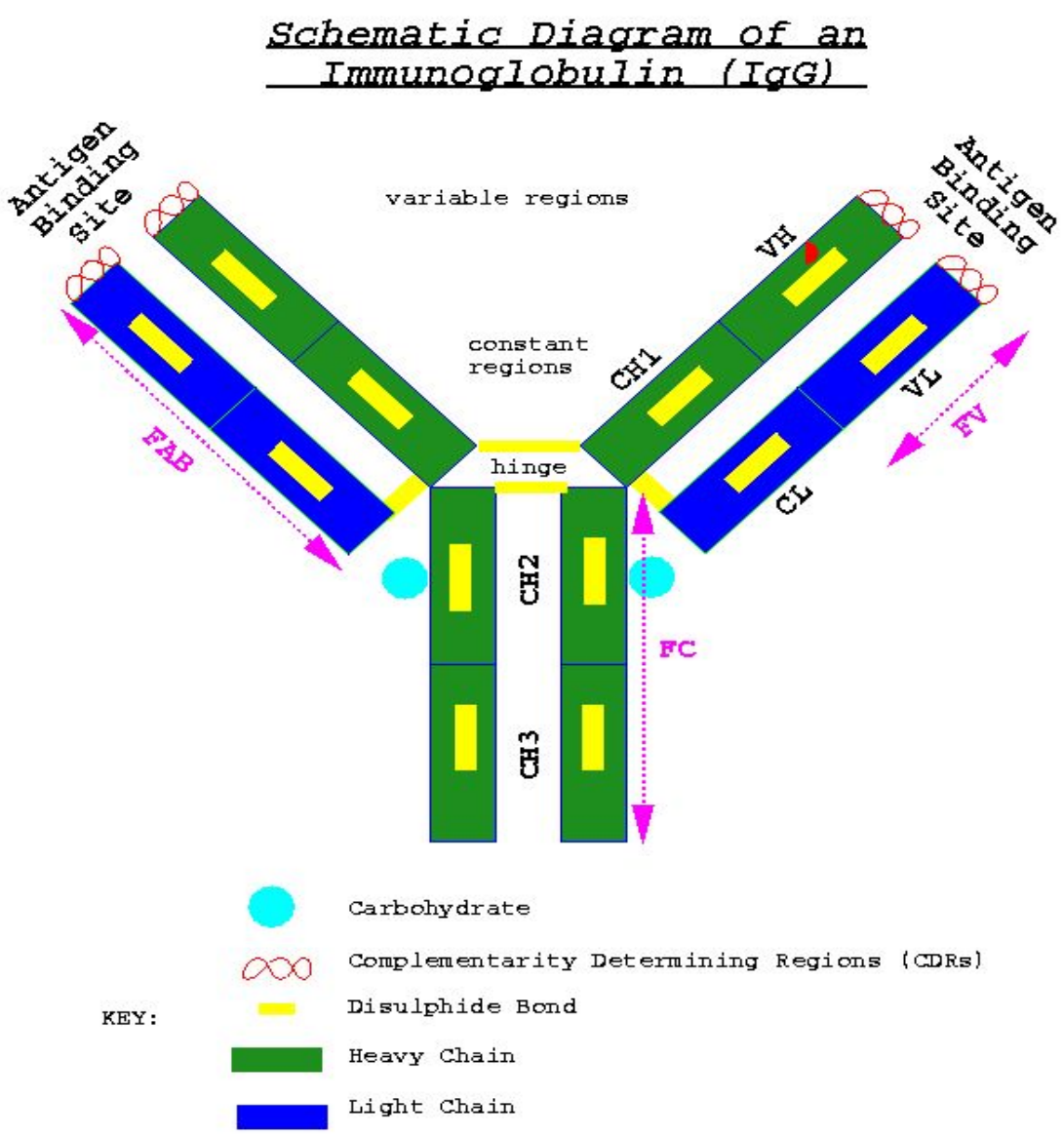


DEFINITION

Immunoglobulin (Ig)

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field





Heavy and Light Chains

All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD)

Disulfide bonds

Inter-chain disulfide bonds

The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.

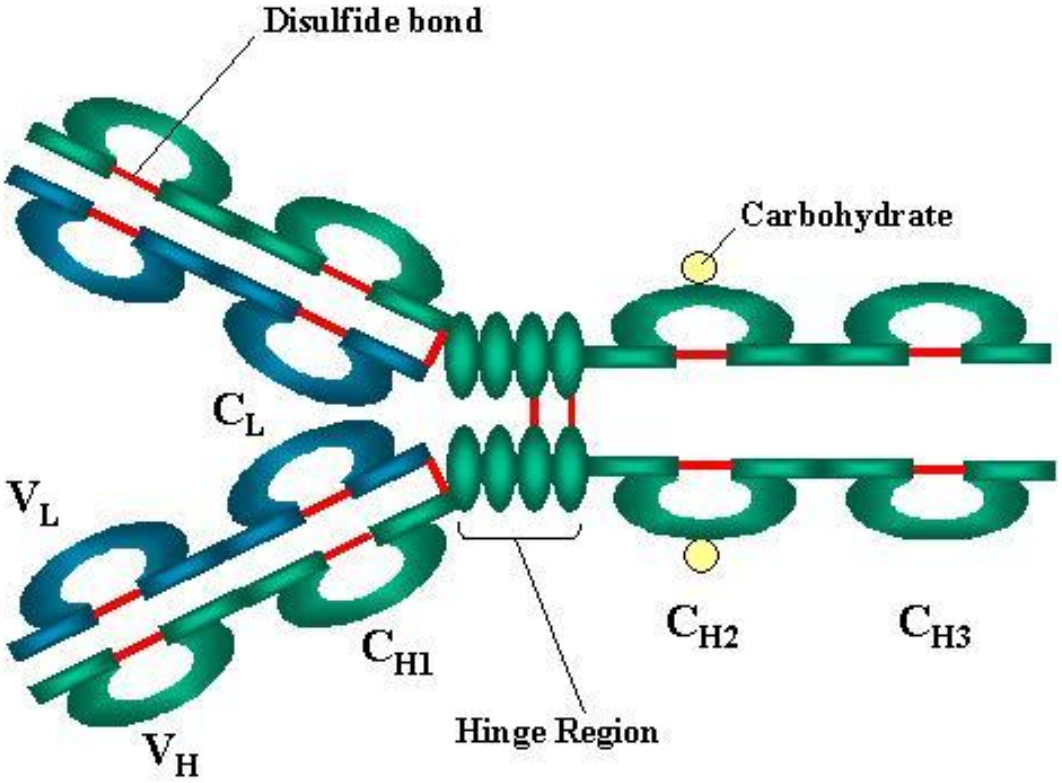
Intra-chain disulfide binds

Within each of the polypeptide chains there are also intra-chain disulfide bonds.

Variable (V) and Constant (C) Regions

When the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain could be divided into two regions based on variability in the amino acid sequences. These are the:
Light Chain - V_L (110 amino acids) and C_L (110 amino acids)
Heavy Chain - V_H (110 amino acids) and C_H (330-440 amino acids)

The basic structure of immunoglobulins



Hinge Region

This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

Domains

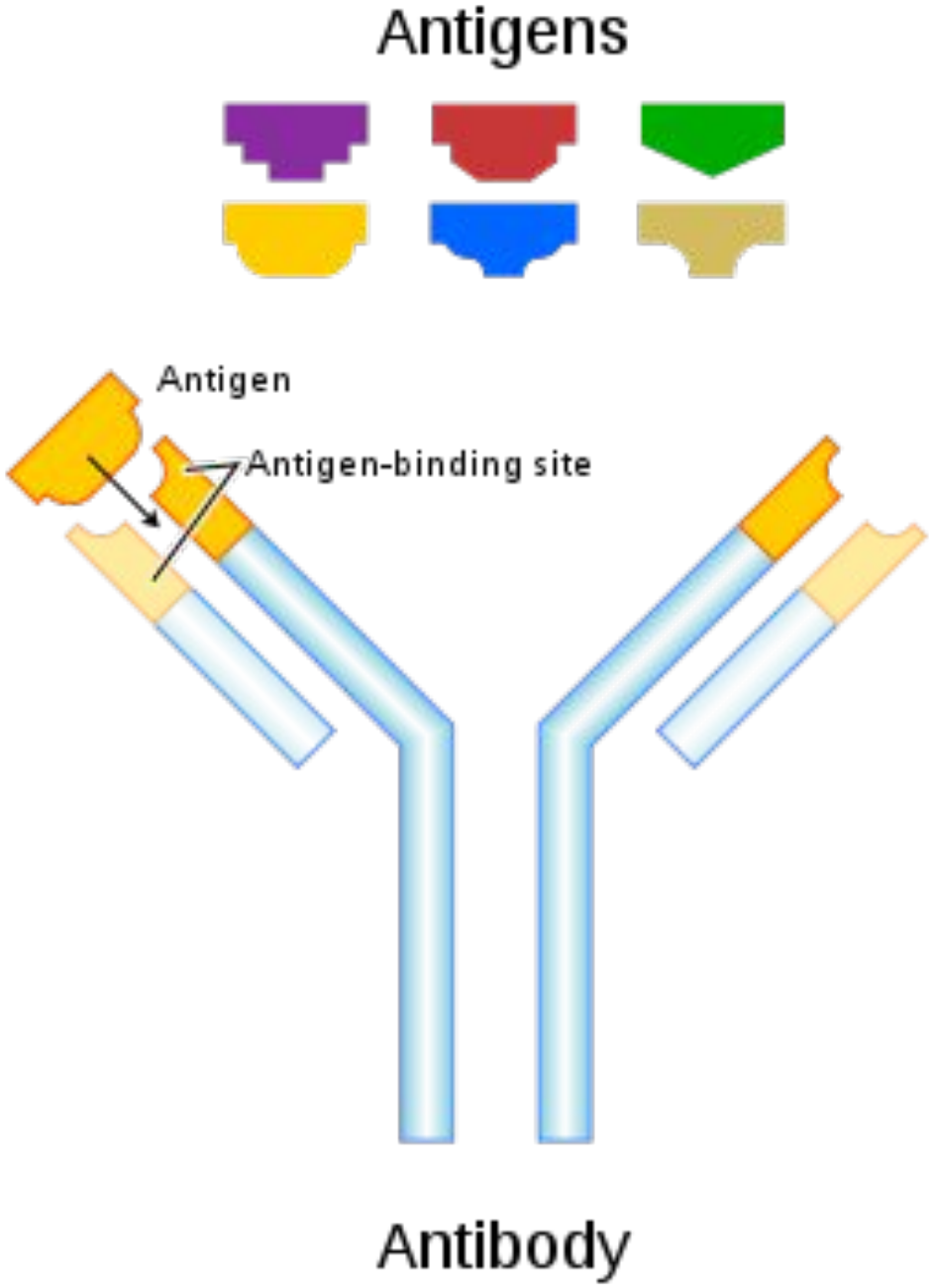
Three dimensional images of the immunoglobulin molecule show that it is not straight . Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond. These regions are called domains.

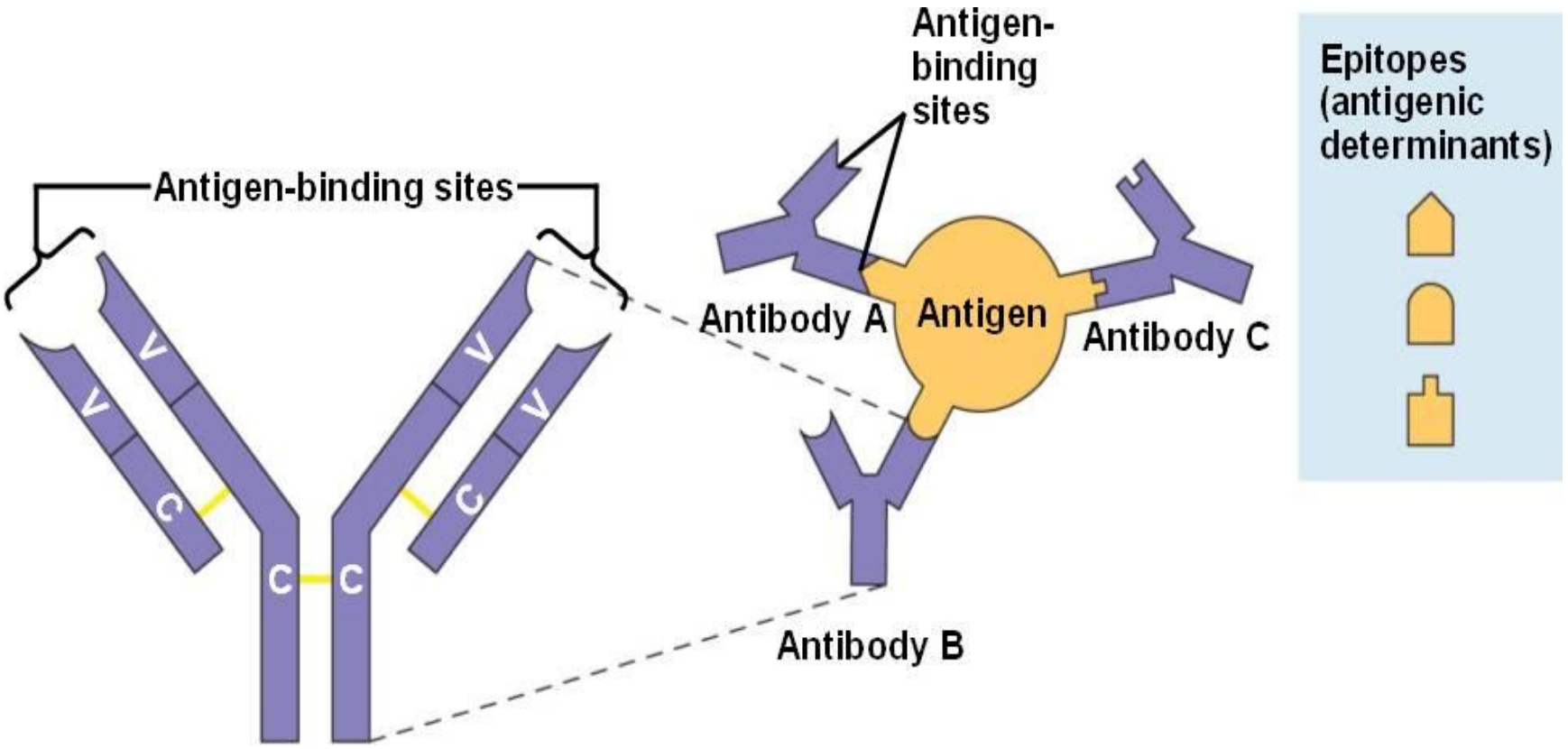
Light Chain Domains - V_L and C_L

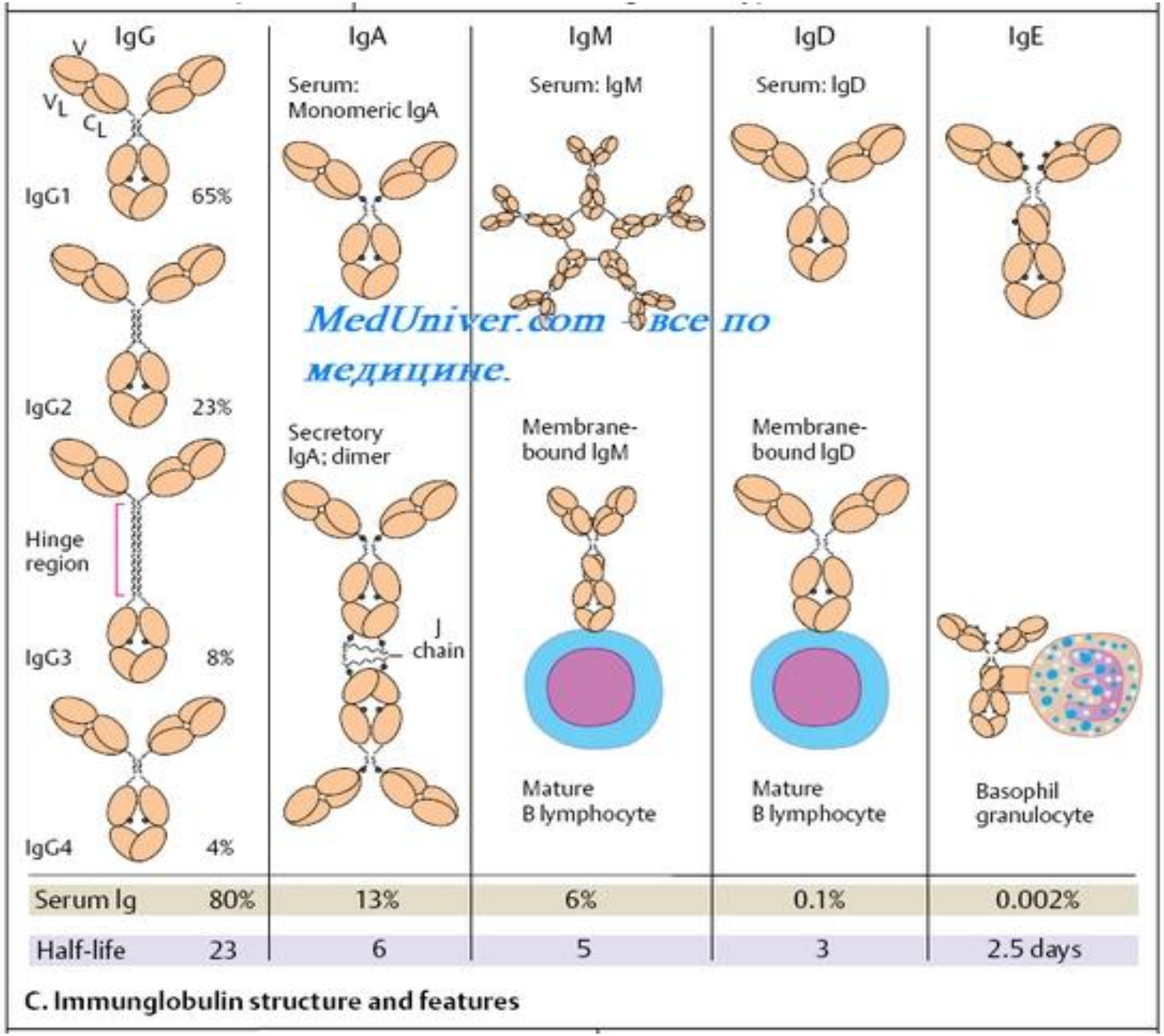
Heavy Chain Domains - V_H, C_{H1} - C_{H3} (or C_{H4})

Oligosaccharides

Carbohydrates are attached to the C_{H2} domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.


Antigens
Antigen
Antigen-binding site
Antibody

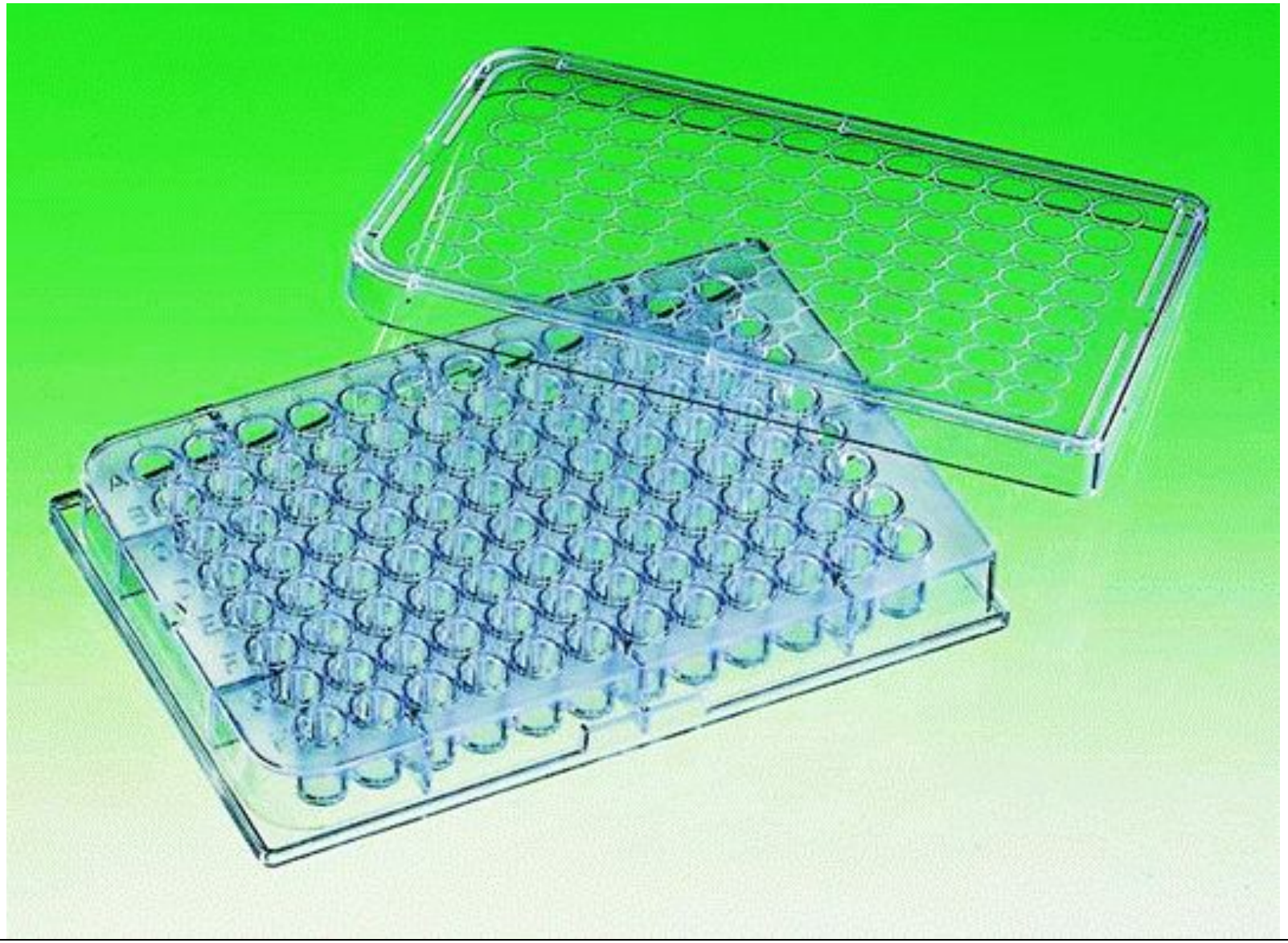

Antigen-binding sites
V
V
C
C
C
V
V
C
Antigen-binding sites
Antibody A
Antigen
Antibody C
Antibody B
Epitopes (antigenic determinants)



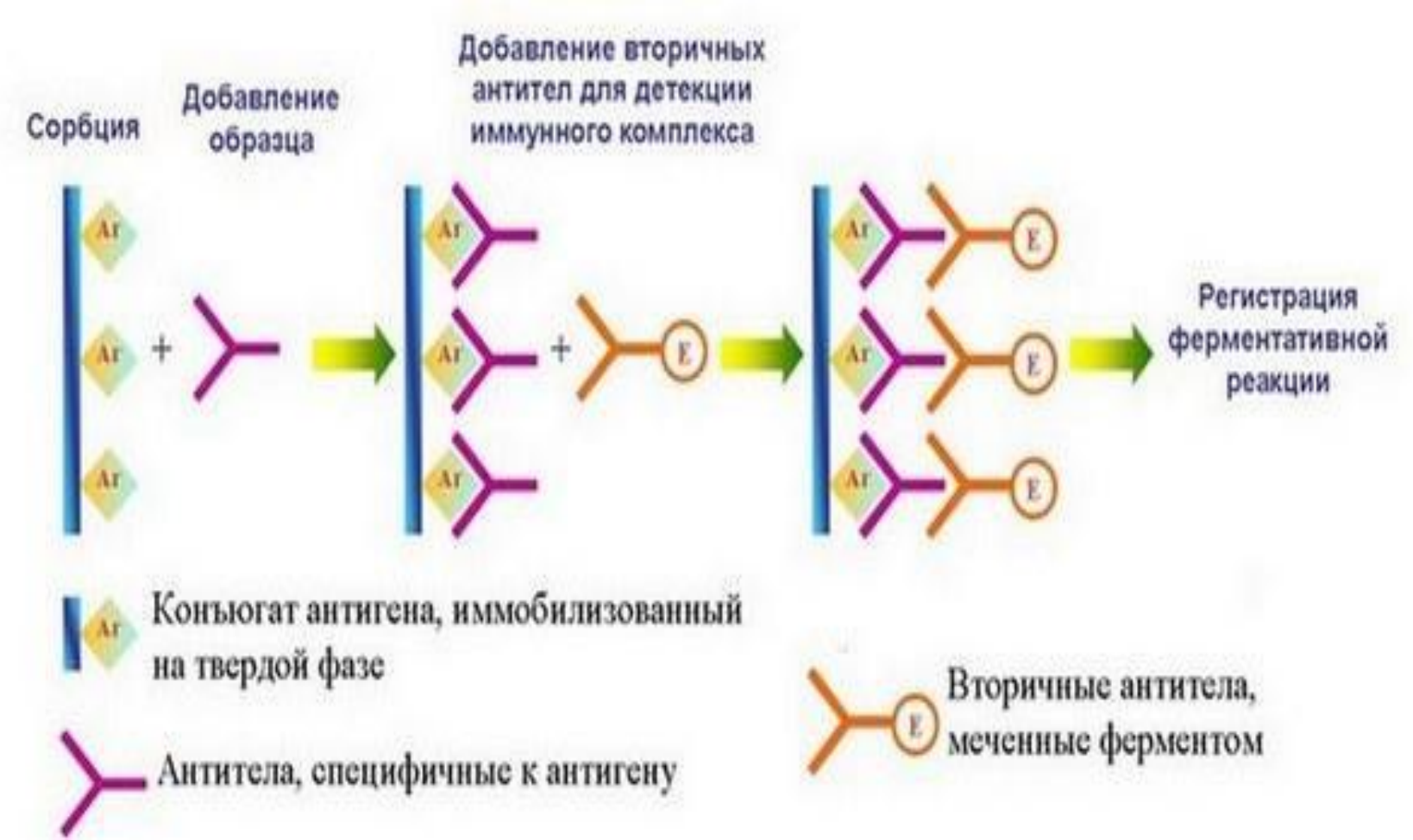
Serum Ig	80%	13%	6%	0.1%	0.002%
Half-life	23	6	5	3	2.5 days

C. Immunglobulin structure and features

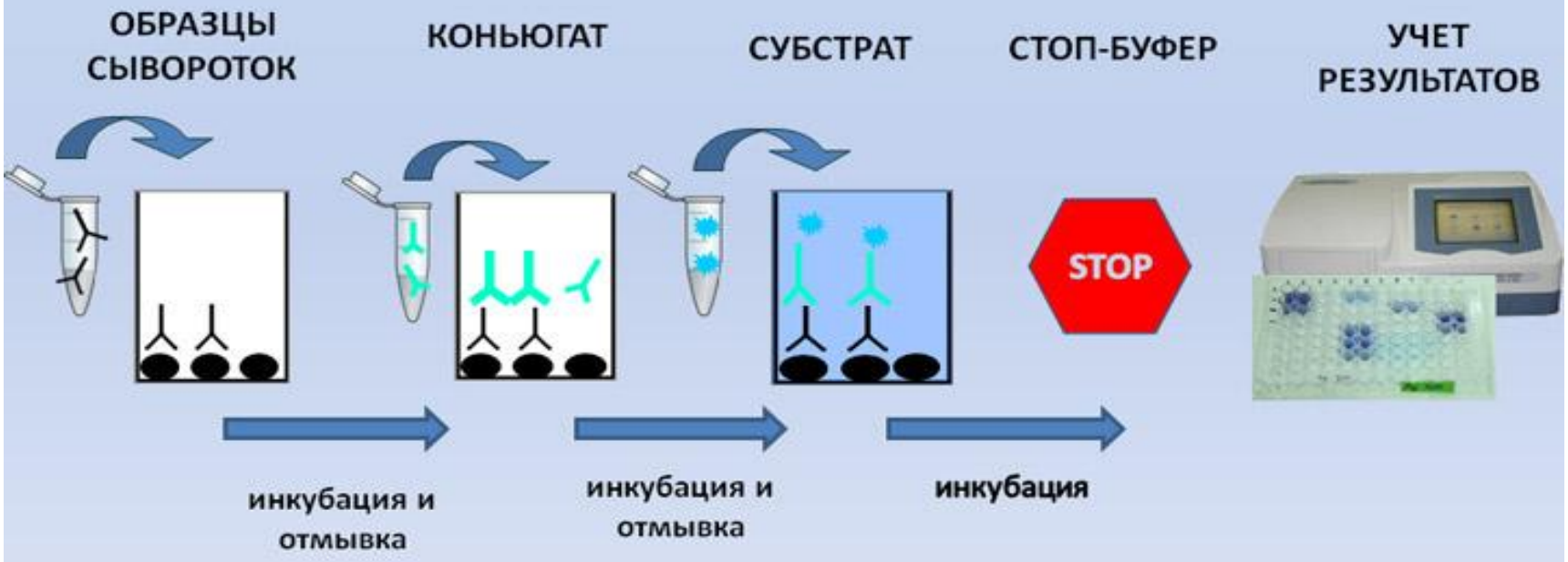
96-луночный планшет для ИФА



ПРИНЦИП НЕПРЯМОГО ИФА

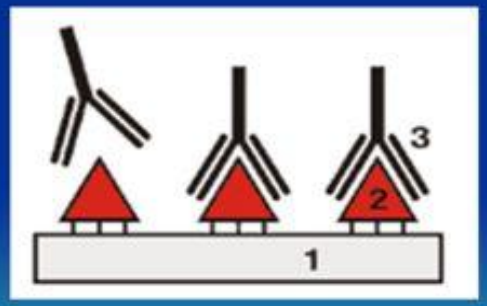


Непрямой вариант ИФА

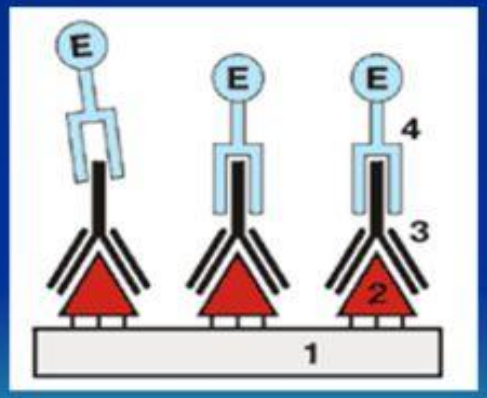


Иммуноферментный анализ

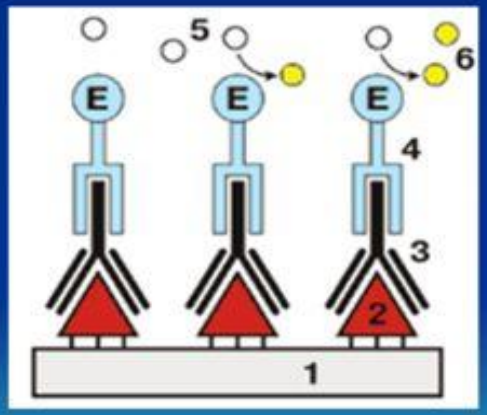
Этапы ИФА:



Взаимодействие
аналита с лигандом

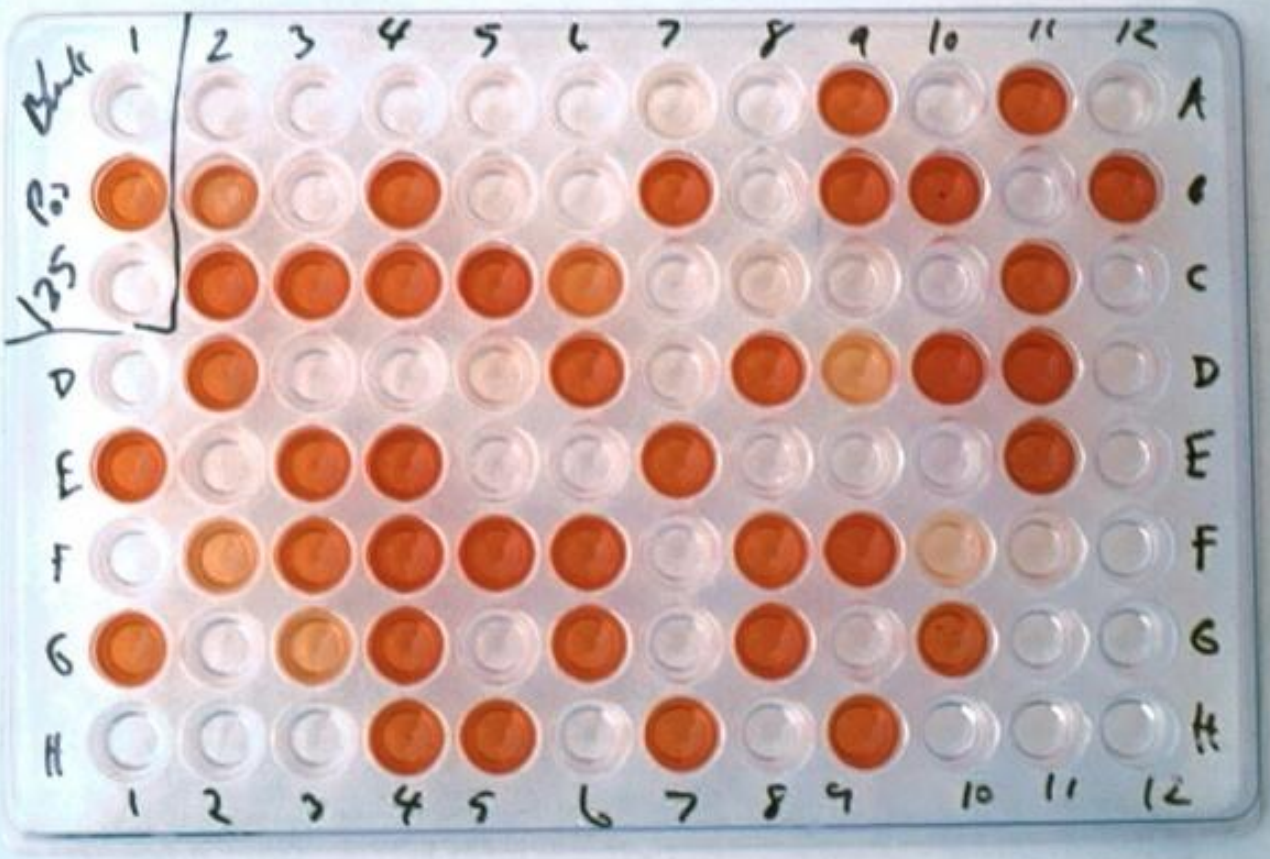


Формирование
меченного комплекса



Измерение сигнала

ELISA testing of western flower thrips for INSV
(red color is a postive result)

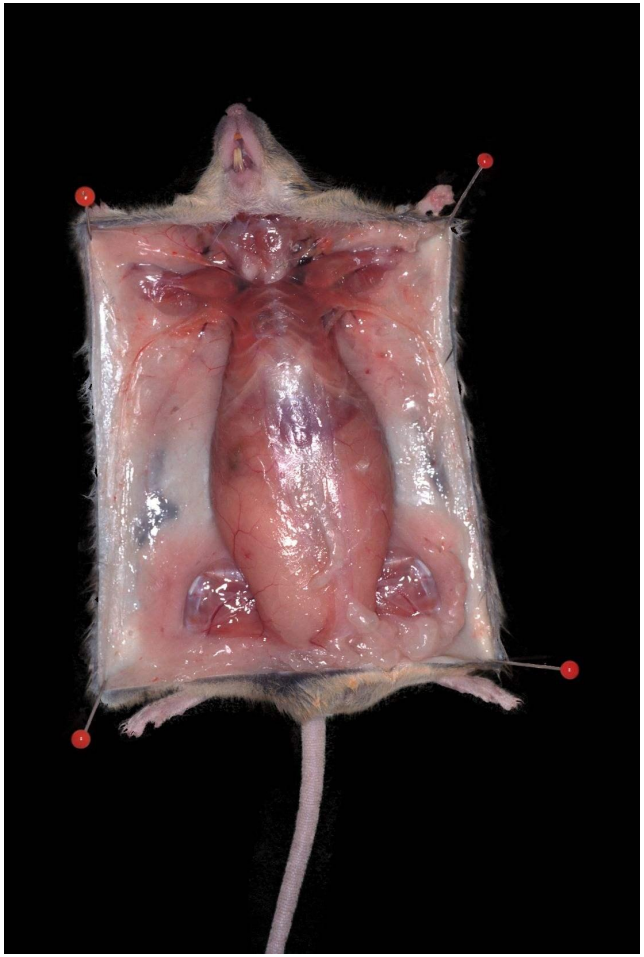


Спектрофотометр для ИФА

Cloning and propagation

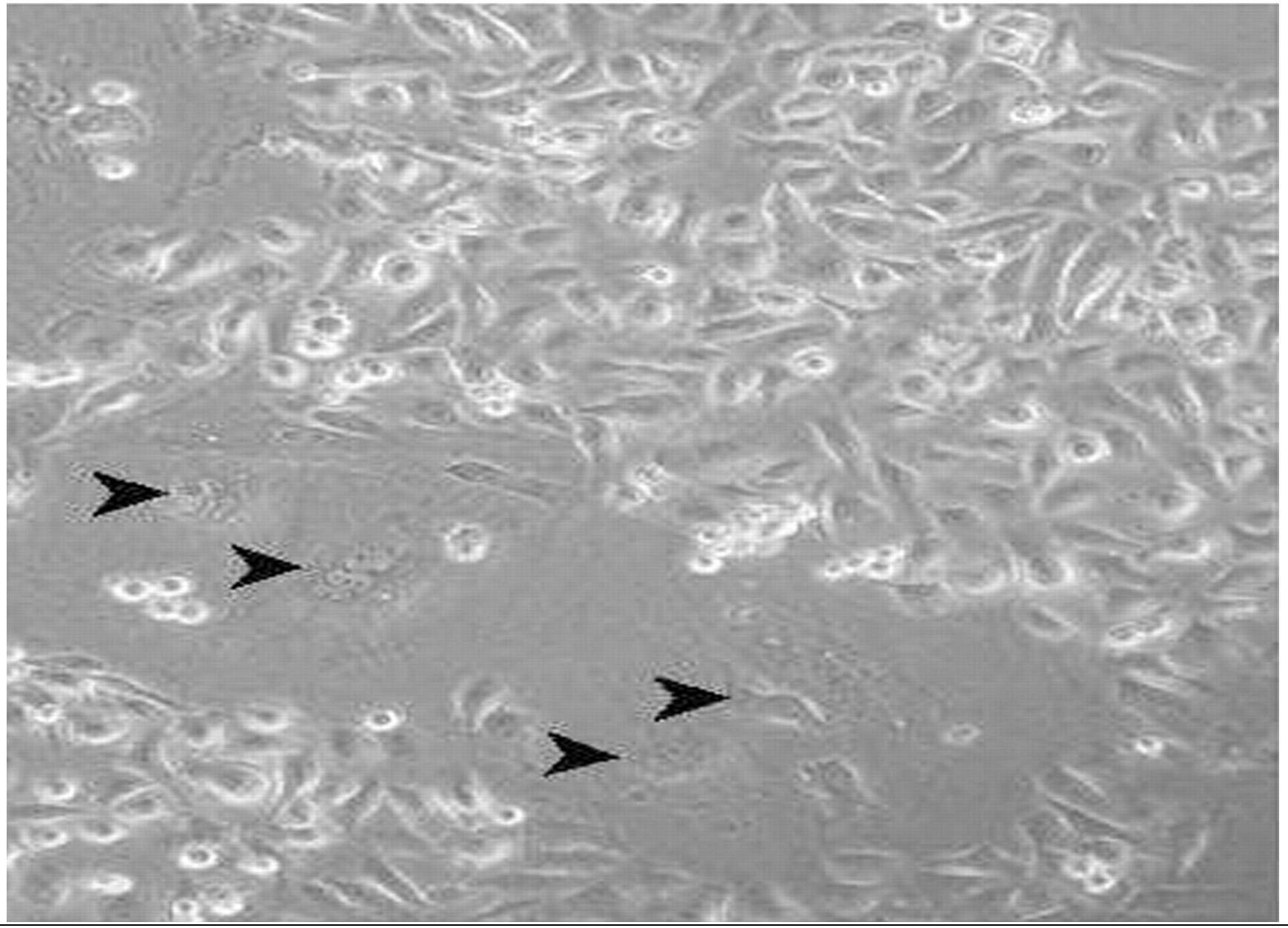
- The single hybrid cell producing the desired antibody are isolated and cloned.
- Usually two techniques are commonly employed for this
- a) Limiting dilution method: Suspension of hybridoma cells is serially diluted so the aliquot of each dilution is having one hybrid cell. This ensures that the antibody produced is monoclonal.
- b) Soft agar method: In this method the hybridoma cells are grown in soft agar. These form colonies and the colonies are monoclonal in nature.

Выделение макрофагов для «питающего слоя»



96-луночные планшеты для культуральных работ

макрофагов



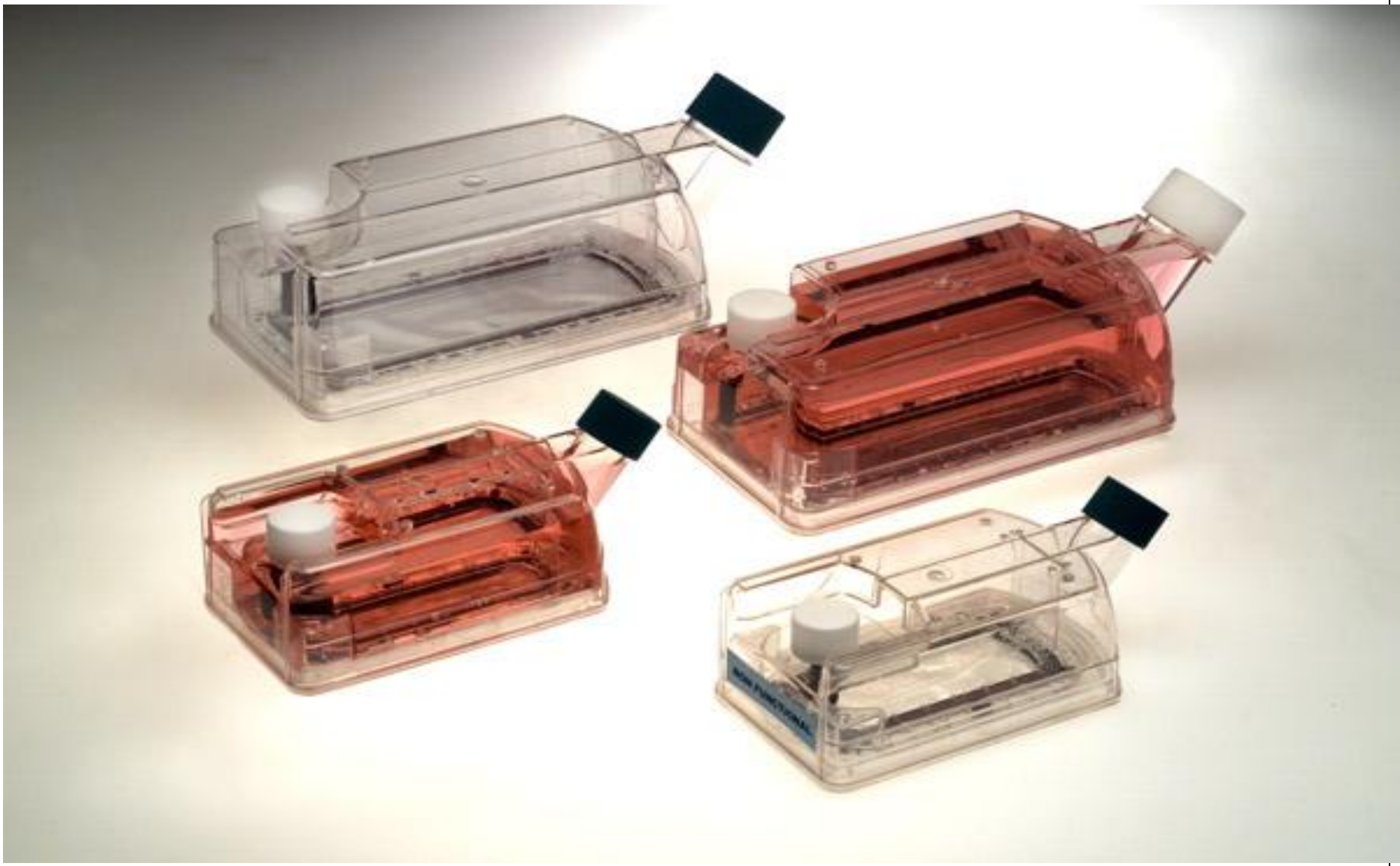
Characterization and storage

- Biochemical and biophysical characterization are made for desired specificity.
- It is important to note the monoclonal antibody is specific for which antigen
- MAbs must be characterized for their ability to withstand freezing and thawing.

Хранение клеток в жидком азоте

Разморозка гибридомных клеток

Накопление МКА в матрасах



Наработка МКА в асцитной жидкости


ÄKTA

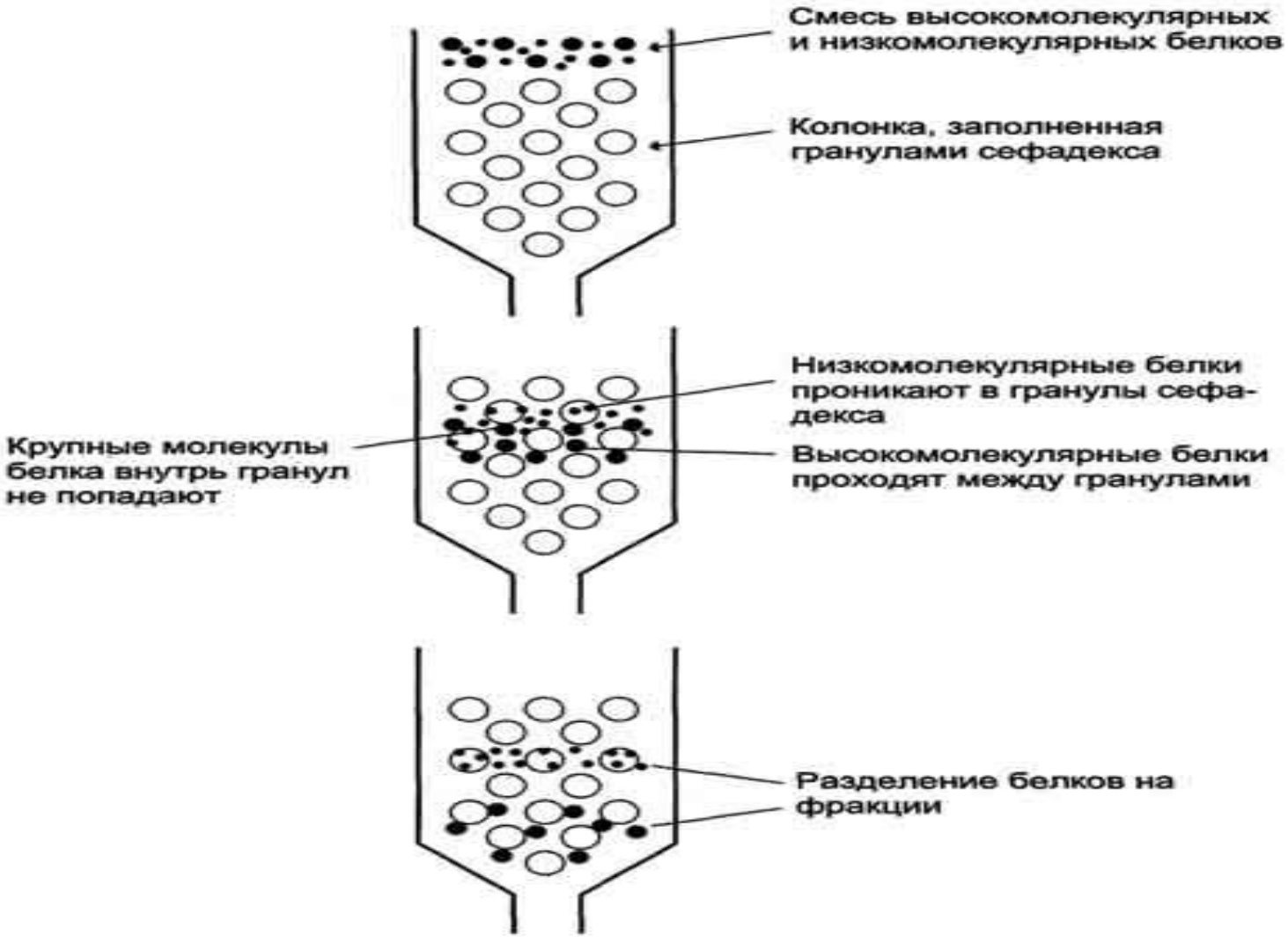
Эксклюзионная хроматография

гель-проникающая

гель-фильтрационная

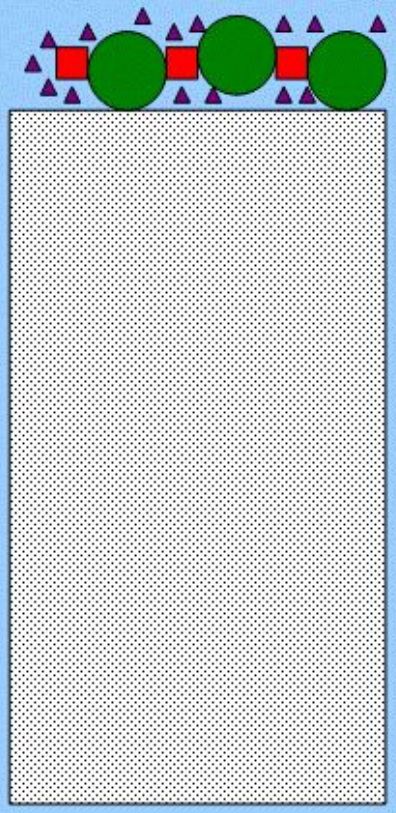
Гель – это сорбент, который готовится на основе природных или синтетических соединений, содержит поры определенного размера

Очистка МКА с помощью гель-фильтрации



Принцип гель-фильтрации

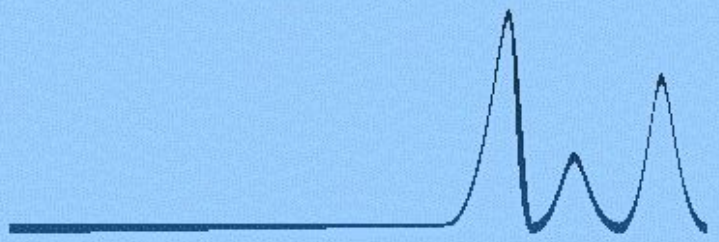
- Шаг 1. Нанесение образца на колонну



Высоко молекулярные соединения (полимеры)

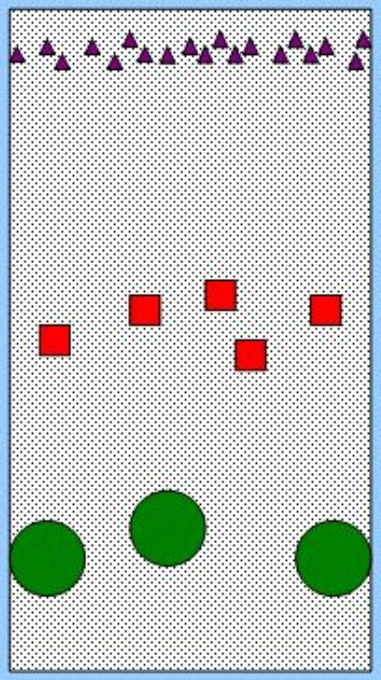
Умеренно молекулярные соединения (мономеры)

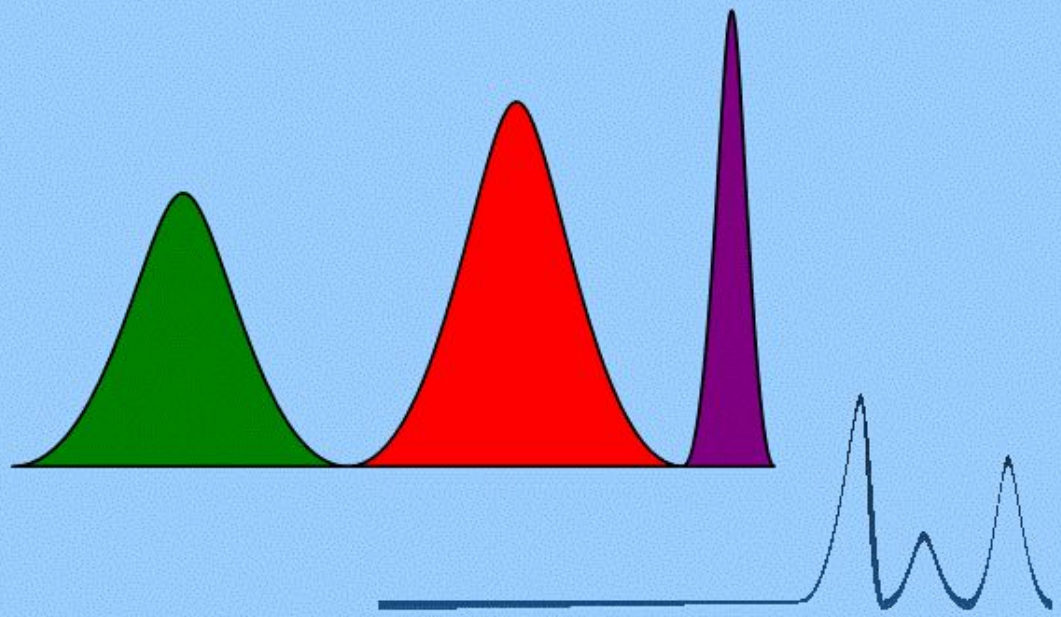
Низко молекулярные соединения (соли)



Принцип гель-фильтрации

- Шаг 2. Элюция образца через гель

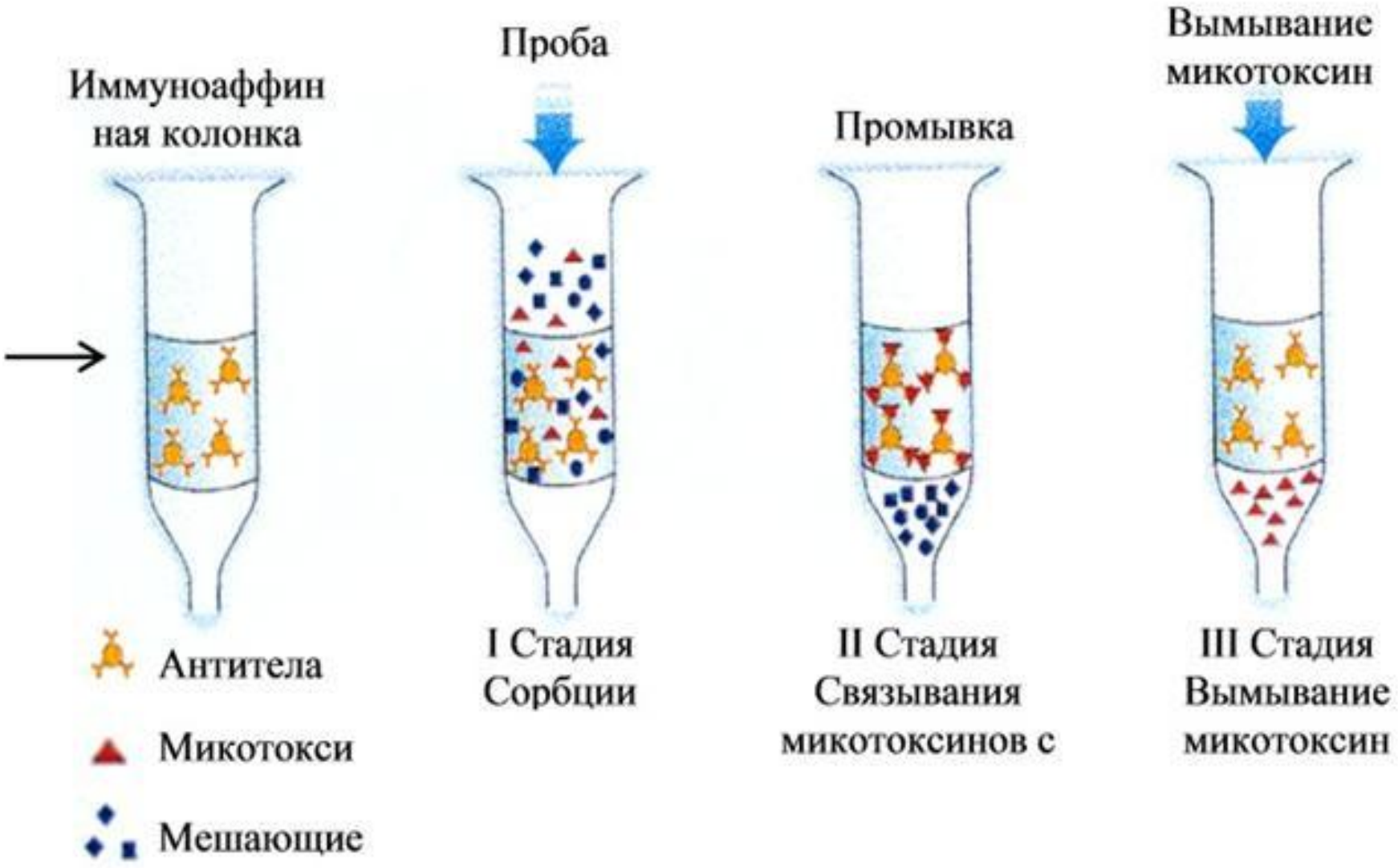


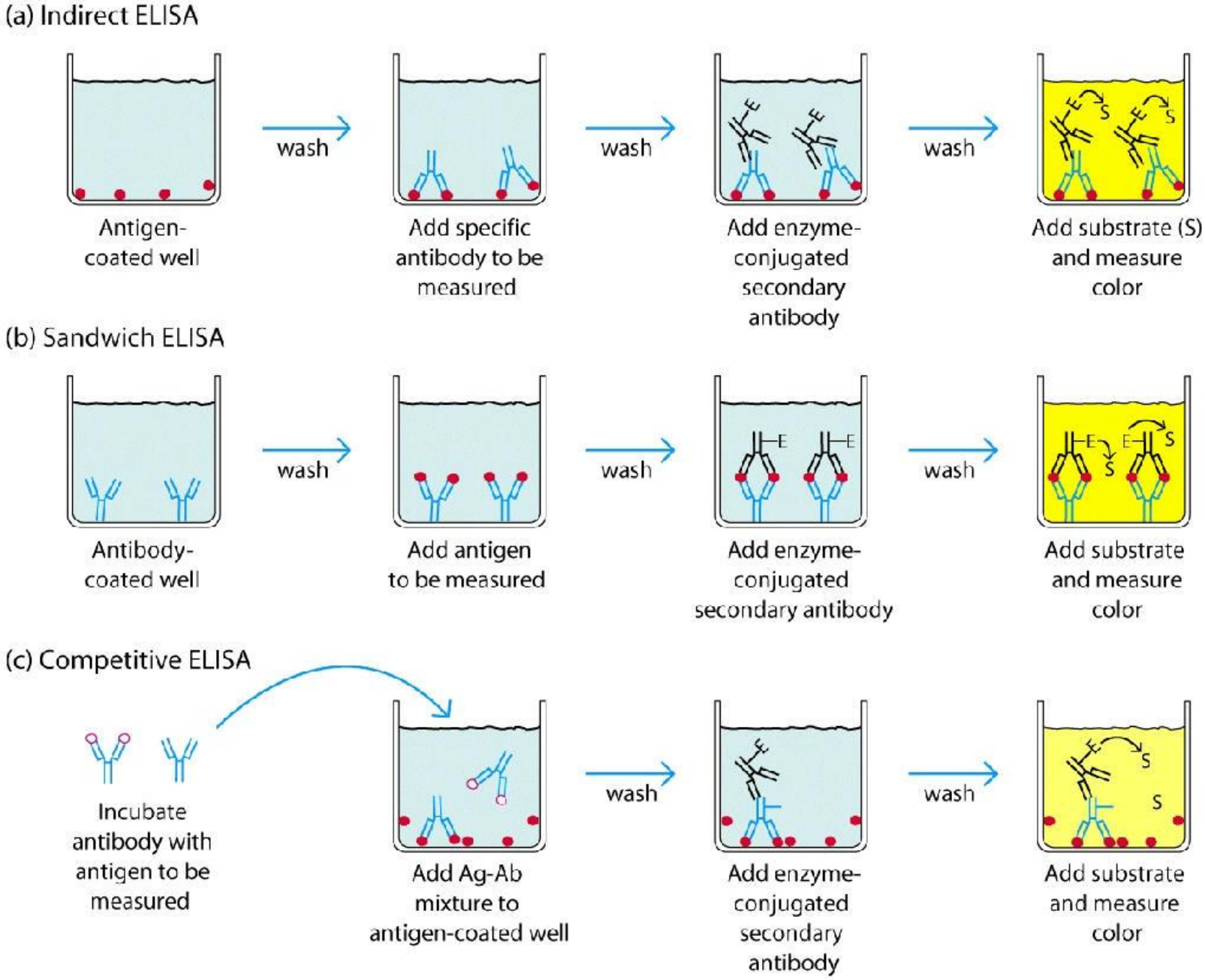


Large scale production

- Encapsulating the hybridoma cells in alginate gels and using a coating solution containing poly-lysine is employed.
- These gels allow the nutrients to enter in and antibodies to come out.
- Damon biotech and cell-tech companies are using this technique for commercial production of MAbs.
- They employ 100-litres fermenters to yield about 100g of MAbs in about 2 weeks period.

АФФИННАЯ ХРОМАТОГРАФИЯ




(a) Indirect ELISA
wash
wash
wash
Antigen-coated well
Add specific antibody to be measured
Add enzyme-conjugated secondary antibody
Add substrate (S) and measure color
(b) Sandwich ELISA
wash
wash
wash
Antibody-coated well
Add antigen to be measured
Add enzyme-conjugated secondary antibody
Add substrate and measure color
(c) Competitive ELISA
Incubate antibody with antigen to be measured
wash
wash
Add Ag-Ab mixture to antigen-coated well
Add enzyme-conjugated secondary antibody
Add substrate and measure color

Engineered antibodies

- MAbs derived from mouse are murine derivatives. As they are not human origin, they show HAMA(human antimouse antibody) response.
- To overcome this we need to cleave the antibody into its respective Fc and Fab fragments.
- Fab fragments are less immunogenic and their smaller molecular size may facilitate penetration into tumor tissue and result in a longer half-life.
- Engineering is needed to reduce the immunogenicity.

Engineered antibodies

Chimeric antibodies:

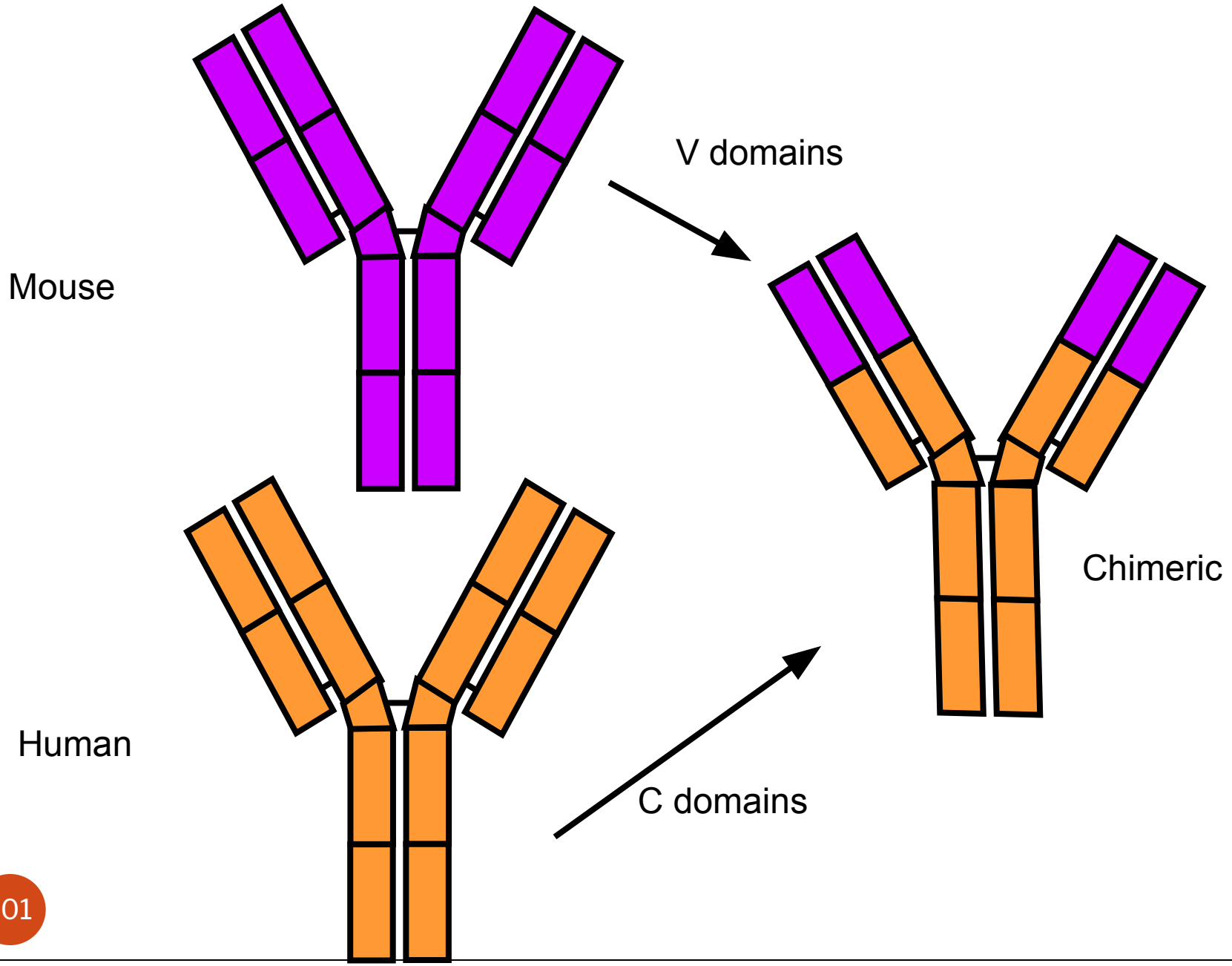
- Hence the murine antibodies are immunogenic to humans, the obvious solution for this is to clone a fully human antibody. But it has many problems like ethical clearance, difficult to culture, impossible to obtain many of the appropriate antibodies.
- To over come HAMA(human antimouse antibody) response, a chimeric antibody is prepared with Fc region of human IgG and Fab regions of murine origin by the use of DNA recombinant technology.

Основные проблемы, возникающие при использовании монАТ в терапии

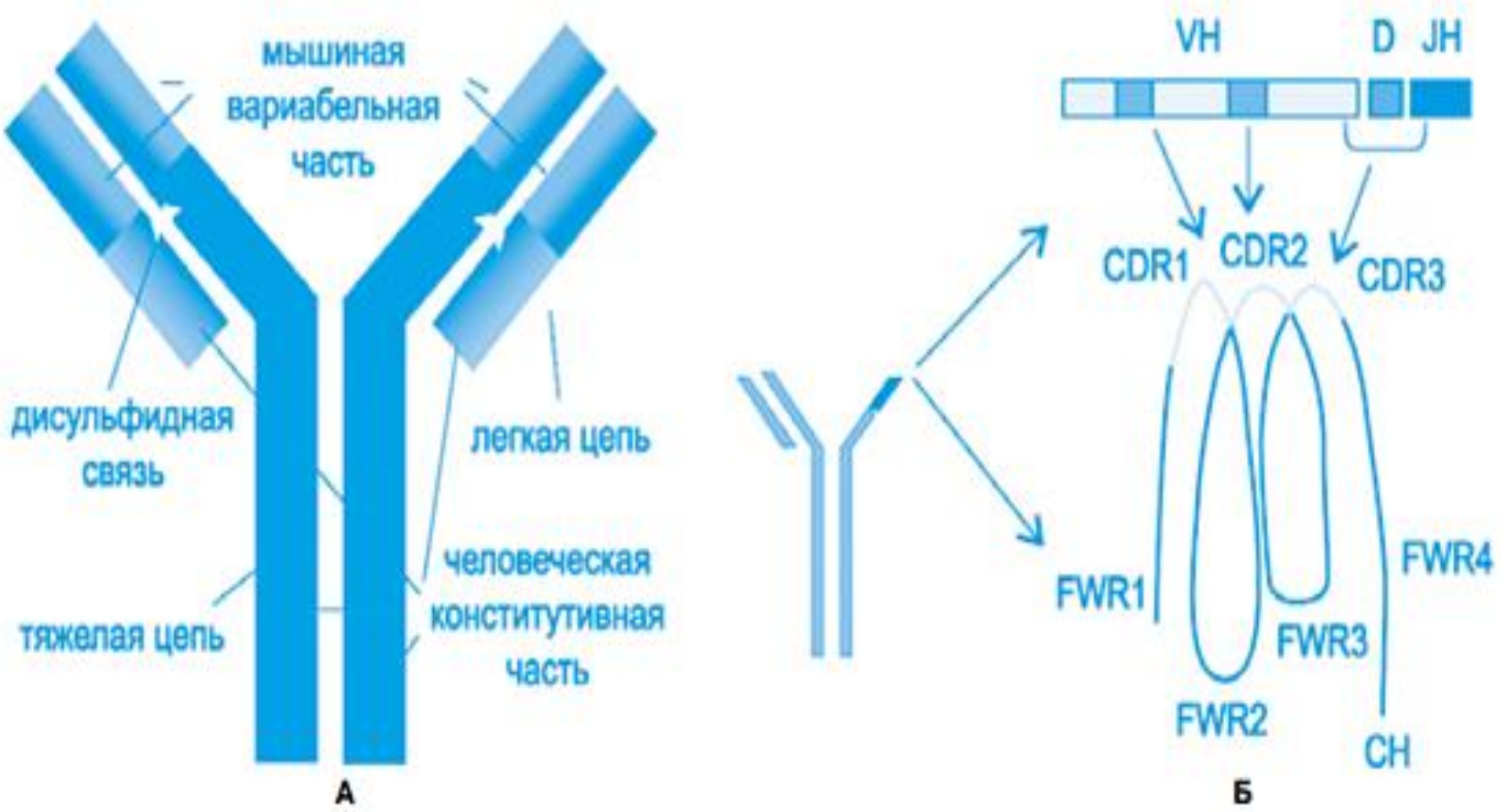
а) Подавляющее большинство получаемых монАТ имеет животное происхождение (мышиные или крысиные), в результате чего иммунная система человека воспринимает их как чужеродный белок и быстро разрушает. МонАТ при этом не успевают проявить свое лекарственное действие;

б) Некоторые монАТ нечеловеческого происхождения могут связывать и выводить из строя жизненно важные молекулы в организме человека, иногда это может привести к летальному исходу;

в) Мышиные и крысиные монАТ являются для человека сильным иммуногеном, и введение их в терапевтических дозах может вызывать аллергические реакции вплоть до анафилактического шока.


Mouse
V domains
Chimeric
Human
C domains

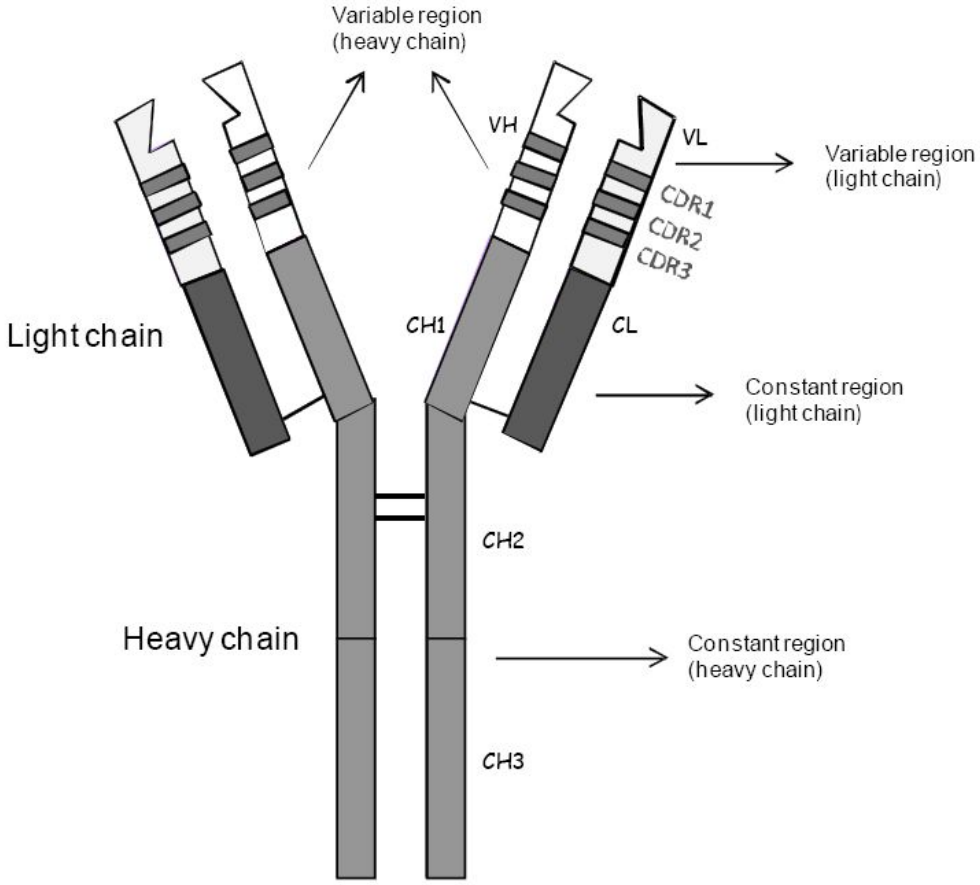
Химерные МКА



Engineered antibodies

Humanized antibodies:

- Though chimeric antibodies elicit less HAMA response than murine antibodies, they are still immunogenic due to their murine regions(30%)
- It is came to know that a small portion(CDR) of an antibody was actually responsible for antigen binding.
- By this humanized antibodies are prepared by recombinant DNA technology with majority of human antibody framework and CDR's of murine antibody.
- Thus humanized antibodies are 95% homology with human antibodies.

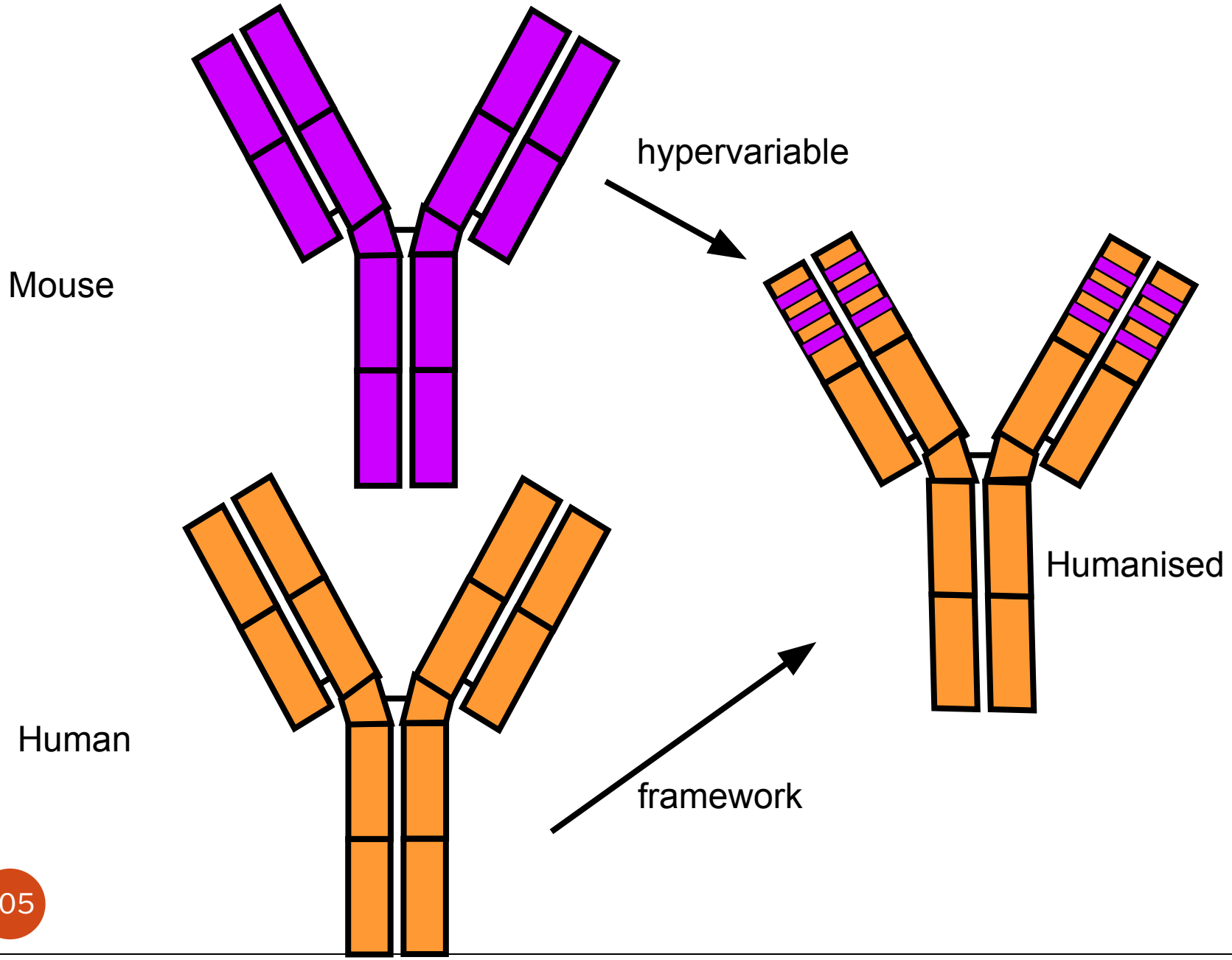


Hypervariable (HVR) or complementarity determining regions (CDR)

Comparisons of the amino acid sequences of the variable regions of immunoglobulins show that most of the variability **resides in three regions** called the hypervariable regions or the **complementarity determining regions** as illustrated in figure. Antibodies with different specificities (i.e. different combining sites) have different complementarity determining regions while antibodies of the exact same specificity have identical complementarity determining regions (*i.e.* CDR is the antibody combining site). Complementarity determining regions are found in both the H and the L chains.

Framework regions

The regions between the complementarity determining regions in the variable region are called the **framework regions**. Based on similarities and differences in the framework regions the immunoglobulin heavy and light chain variable regions can be divided into groups and subgroups. These represent the products of different variable region genes.


Mouse
hypervariable
Humanised
Human
framework

Engineered antibodies

Bispecific antibodies:

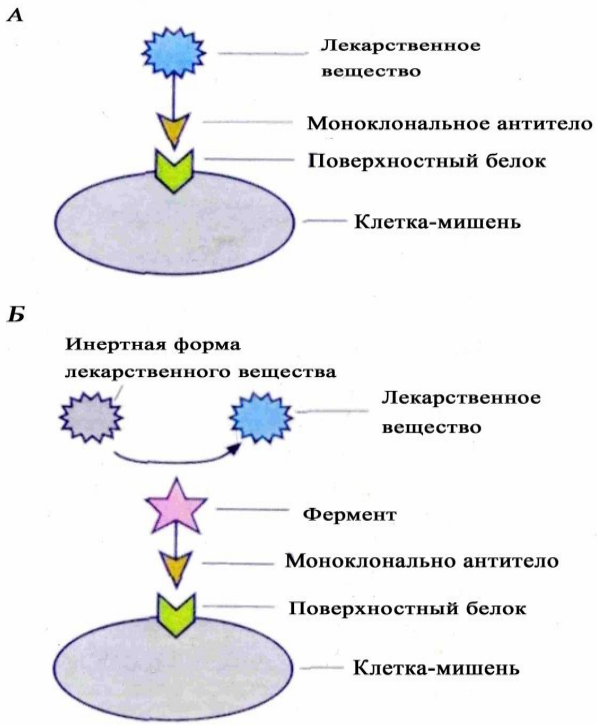
- These are specific to two types of antigens.
- They are constructed by r.DNA technology.
- Each arm is specific to one type of antigen.

Engineered antibodies

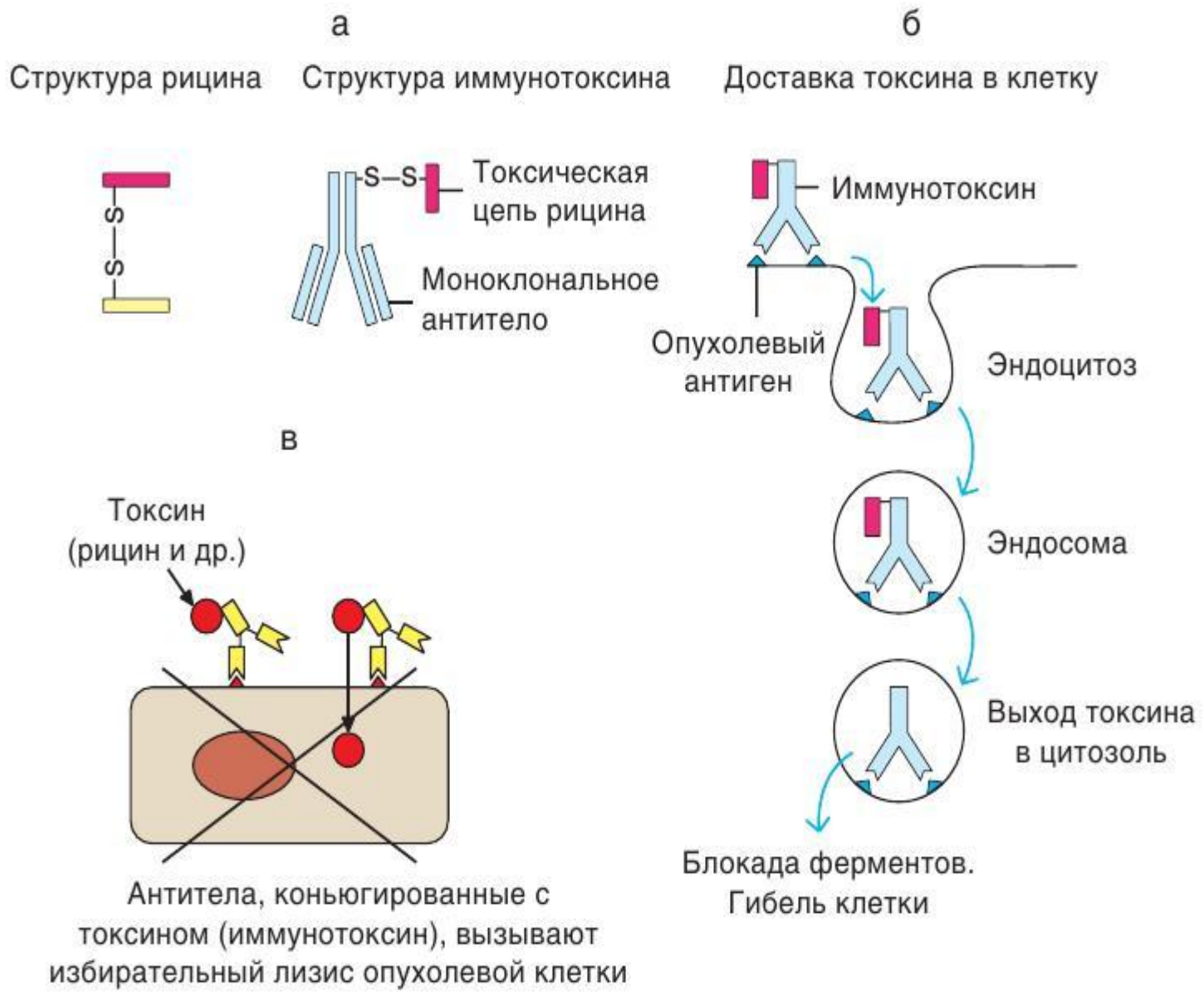
Immunoconjugate:

- For MAb targeted drug delivery, a drug is bound covalently to an antibody that is chosen to target it to the desired site of action.
- Spacer is present between the antibody and the drug.
- Polymer may be present to increase the no. of drug molecules attached to the antibody.
- Drug is non-covalently incorporated into a liposome or microsphere to which the targeting antibody is bound to the

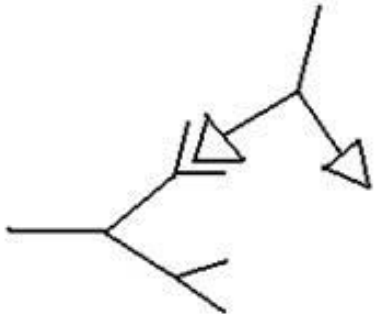
Целевая доставка лекарственных веществ с помощью моноклональных антител



Схематическое изображение системы целевой доставки лекарственного вещества, основанной на использовании моноклональных антител. А. Молекула лекарственного вещества присоединена к моноклональному антителу. Б. К моноклональному антителу присоединен фермент, превращающий инертную форму лекарственного вещества в активную только в непосредственной близости от клетки-мишени. В обоих случаях моноклональное антитело связывается с одним специфическим белком на поверхности клетки-мишени.


а
б
Структура рицина
Структура иммунотоксина
Доставка токсина в клетку
S–S
S–S
Токсическая цепь рицина
Моноклональное антитело
Иммунотоксин
Опухолевый антиген
Эндоцитоз
Эндосома
Выход токсина в цитозоль
Блокада ферментов. Гибель клетки
в
Токсин (рицин и др.)
Антитела, коньюгированные с токсином (иммунотоксин), вызывают избирательный лизис опухолевой клетки

"первые" антитела против антигена

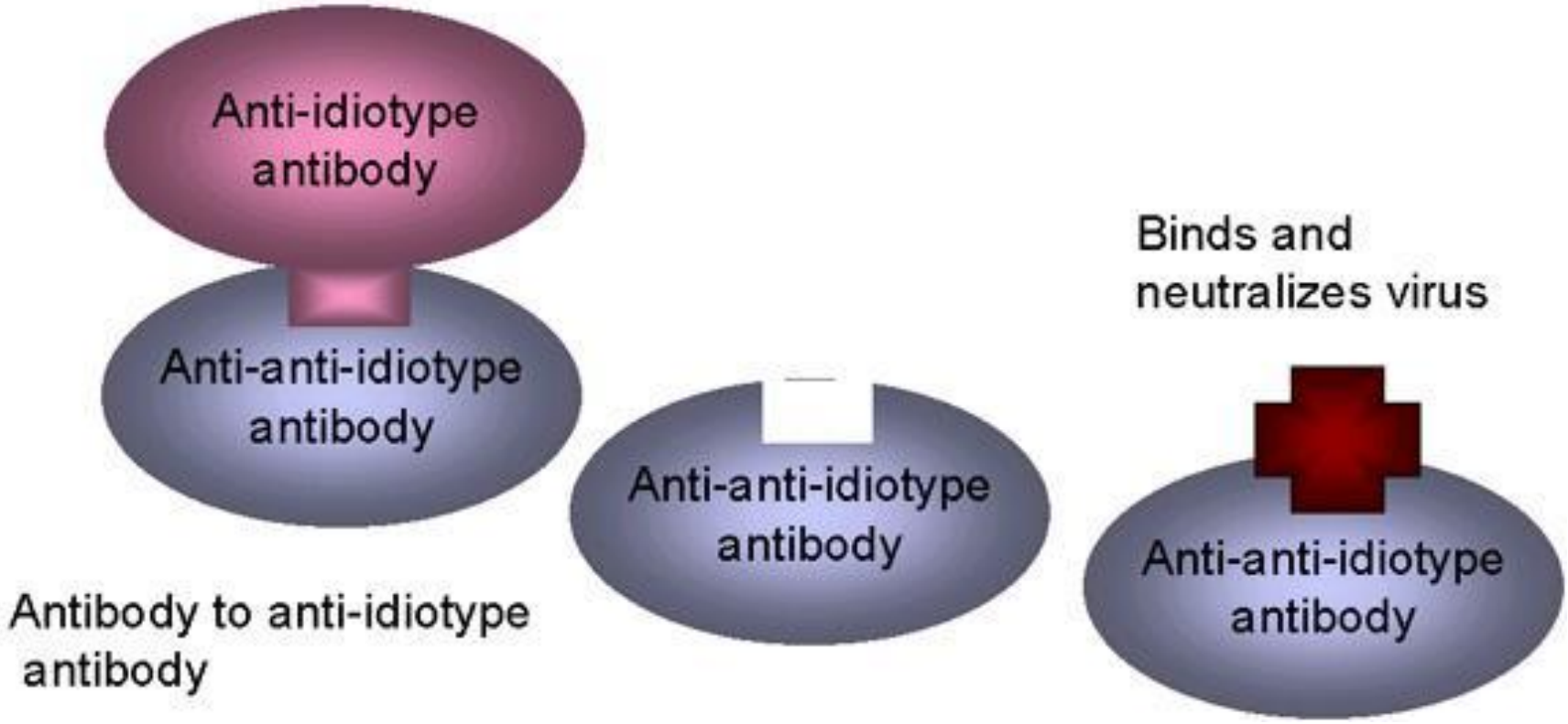


"вторые" антитела против "первых" антител

Рис.3. Схема образования антиидиотипических антител. "Вторые" антитела, направленные против антигенсвязывающего участка "первых" антител, могут копировать антигенную детерминанту и вызывать иммунный ответ, подобный иммунному ответу на антиген.

Anti-idiotype antibody cont 2

Use anti-idiotype antibody as injectable vaccine



Drug targetting

- Principle involved:
- As several classes of the drugs lack specificity for diseased cells, they show their action on other sites of action.

  Ex: cytotoxic action of chemotherapeutic agents is directed against any rapidly proliferating cell population.
- Hence drug targeting is required to overcome this problem.
- Targeting is classified into three categories:

1. Passive targeting
2. Physical targeting
3. Active targeting

Passive targeting

- It is the natural in-vivo distribution pattern of the drug delivery system. It is determined by the inherent properties of the carrier like hydrophobic and hydrophilic surface characteristics, particle size, surface charge, particle number.

  Ex: passive targeting of the lungs is made by modulating the size of the particles to >7µm

  passive targeting of the Reticuloendothelial system is made by modulating the size of the particles to 0.2-7µm

Physical targeting

- In this some characteristics of the environment are utilized for the carrying of the drug to the specific site.

  Ex: thermal sensitive liposomes(local hyperthemia)

  magnetically responsive albumin microspheres

  (localized magnetic field)

Active targeting

- Active targeting is usually done by cell-specific ligands. These are specific to specific cell types. But it is limited to small no. of tumor types.
- Hence MAb targeting is adopted for active targeting. MAb targeting is done by conjugating the drug antibody of the specific targeting type.
- Hence antibody drug conjugates are used as active targeting drug delivery systems.

Drug conjugates

- Toxin conjugates (immunotoxins)

EX: diphtheria toxin, Ricin have been conjugated to the tumor specific antibodies

Ricin has two chains. Amoung these A-chain is cytotoxic and B-chain is non-specific. Hence B-chain is removed and the toxin is conjugated to tumor specific antibody. Thus we increase the specificity of the toxins by using MAbs as active drug targeting systems.

Drug conjugates

- Drug immunoconjugates:

  Agents like chlorambucil, methotrexate and doxorubicin are conjugated with tumor specific antibodies.

  Ex: doxorubicin-BR96 immunoconjugate for Lewis antigen found on the surface of tumor cells.

Advantages of Monoclonal antibodies

- They are homogenous in nature.
- They are specific to a particular antigen with a particular epitope.

  Ex:Rituximab (Rituxan®, anti-CD20) is a good example – this antibody is used for the treatment of lymphoma.

Monoclonals for tumour therapy:

- Cell Depletion
  Rituxan, Campath (naked)
  Myelotarg (drug)
  Zevalin, Bexxar (radioisotope)
- Blocking receptors
  Herceptin
- Attacking vasculature
  Avastin, Erbitux
- Vaccination against idiotype
  Panorex?

Препарат	Активное вещество	Производство	Показания к применению
Герцептин (трастузумаб)	Гуманизированные МкАТ (IgG1) к внеклеточному домену рецептора эпидермального ростового фактора человека 2 типа (HER-2) на опухолевых клетках	«F. Hoffmann-La Roche Ltd» (Швейцария)	Рак молочной железы, яичника, предстательной железы, желудка, легких с гиперэкспрессией HER-2 на опухолевых клетках
Мабтера (ритуксимаб)	Химерные МкАТ к рецептору CD20 на пре-В- и В-лимфоцитах	«F. Hoffmann-La Roche Ltd» (Швейцария), «Genentech Inc» (США)	В-клеточные CD20-положительные неходжкинские лимфомы, хронический лимфолейкоз
Кэмпас (алемтузумаб)	Гуманизированные МкАТ (IgG1k) к рецептору CD52 на нормальных и малигнизированных В- и Т-лимфоцитах	«Schering AG» (Германия)	Хронический лимфолейкоз
Эрбитукс (цетуксимаб)	Химерные МкАТ (IgG1) к рецептору эпидермального фактора роста (РЭФР)	«Merck Serono» (Германия)	Метастатический колоректальный рак, рак головы и шеи
Вектибикс (панитумумаб)	МкАТ к рецептору эпидермального фактора роста (РЭФР) (последовательность идентична IgG2 человека)	Владелец РУ «Amgen Europe., B.V.»	Метастатический колоректальный рак
Авастин (бевацизумаб)	Гуманизированные МкАТ к фактору роста эндотелия сосудов (VEGF)	«F. Hoffmann-La Roche Ltd» (Швейцария), «Genentech Inc» (США)	Метастатический колоректальный рак, рак молочной железы, рак легкого, почечно-клеточный рак

Препараты МкАТ, используемые при лечении аутоиммунных заболеваний

Препарат	Активное вещество	Производство	Показания к применению
Ремикейд (инфликсимаб)	Химерные МкАТ (IgG1) к ФНО человека	«Sentocor» (Нидерланды), «Schering-Plough Central East AG» (Швейцария)	РА, болезнь Крона, анкилозирующий спондилоартрит, псориатический артрит, псориаз
Хумира (адалимумаб)	Рекомбинантные МкАТ к ФНО человека (последовательность идентична IgG1 человека)	«Abbott Laboratories Ltd» (Великобритания), «Vetter Pharma-Fertigung GmbH and Co.KG» (Германия)	РА, псориатический артрит, анкилозирующий спондилит, болезнь Крона
Оренсия (абатасепт)	Димерная молекула из внеклеточного домена CTLA-4 (CD152) и модифицированного Fc фрагмента IgG1 человека (линейный участок тяжелой цепи Ig, соединяющий домены CH2 и CH3)	«Bristol-Myers Squibb Holding PHARMA Ltd» (США)	РА, воспалительные заболевания кишечника, системная красная волчанка, псориатический артрит
Энбрел (этанерцепт)	Димерная молекула из рецептора ФНО и Fc фрагмента IgG1 человека (CH2 и CH3 области)	«Wyeth-Whitehall Export GmbH» (Австрия), «Amgen» (США)	РА, ювенильный полиартрит, анкилозирующий спондилит, псориатический артрит, псориаз

Препараты МкАТ, используемые в трансплантологии

Препарат	Активное вещество	Производство	Показания к применению
Симулект (базиликсимаб)	Химерные МкАТ (IgG1k) к α-цепи рецептора ИЛ-2 (CD25)	«Novartis Pharma AG» (Швейцария)	Профилактика отторжения после трансплантации почки
Зенапакс (дакликсимаб)	Гуманизированные МкАТ (IgG1) к α-цепи рецептора ИЛ-2 (CD25)	«F. Hoffmann-La Roche Ltd» (Швейцария)	Профилактика отторжения после трансплантации почки

Препараты МкАТ, используемые при лечении инфекционных, аллергических и других заболеваний

Препарат	Активное вещество	Производство	Показания к применению
Синагис (паливизумаб)	Гуманизированные МкАТ (IgG1k) к эпитопу А антигена белка взаимодействия (F-белка) респираторного синтициального вируса (РСВ)	«Abbott Laboratories Ltd.» (Великобритания). «Boehringer Ingelheim Pharma GmbH & Co. KG» (Германия)	Профилактика инфекции нижних дыхательных путей, вызванной РСВ, у детей до 2 лет с высоким риском заражения РСВ
Луцентис (ранибизумаб)	Fab-фрагмент МкАТ (IgG1k) человека к фактору роста А эндотелия сосудов (VEGF-A)	«Novartis Pharma Stein AG» (Швейцария)	Неоваскулярная (влажная) форма возрастной макулярной дегенерации
Ксолар (омализумаб)	Гуманизированные МкАТ (IgG1k) к IgE человека, блокирующие связь с рецепторами Fc?-R1	«Novartis Pharma Stein AG» (Швейцария)	Персистирующая атопическая бронхиальная астма, сезонный аллергический ринит