BCM 317 LECTURE

BY

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OUTLINE

- Immunological tolerance
- Autoimmune diseases
- Immuno – suppresive drugs
- Monoclonal antibodies

Immunological tolerance

- immunological tolerance is a state of unresponsiveness to an antigen induced by the exposure of specific lymphocytes to that antigen, but respond to other antigens normally.

- Like immunological memory, immunological tolerance is antigen specific. It can exist in B cells, T cells or both

- Self-tolerance: Normal individuals are tolerant of their own antigens (self antigen).

Classification of Immunological tolerance

- Immunological tolerance is classified into central tolerance or peripheral tolerance depending on where the state is originally induced- either in the thymus and bone marrow (central) or in other tissues and lymph nodes (peripheral).

Central tolerance

- Central tolerance refers to the tolerance established by deleting autoreactive lymphocyte clones before they develop into fully immunocompetent cells. It occurs during lymphocyte development in the thymus and bone marrow for T and B lymphocytes, respectively.

- In these tissues, maturing lymphocytes are exposed to self-antigens presented by medullary thymic epithelial cells and thymic dendritic cells, or bone marrow cells. Those lymphocytes that have receptors that bind strongly to self-antigens are removed by induction of apoptosis (programmed cell death) of the autoreactive cells, or by induction of anergy, a state of non-activity

Peripheral tolerance

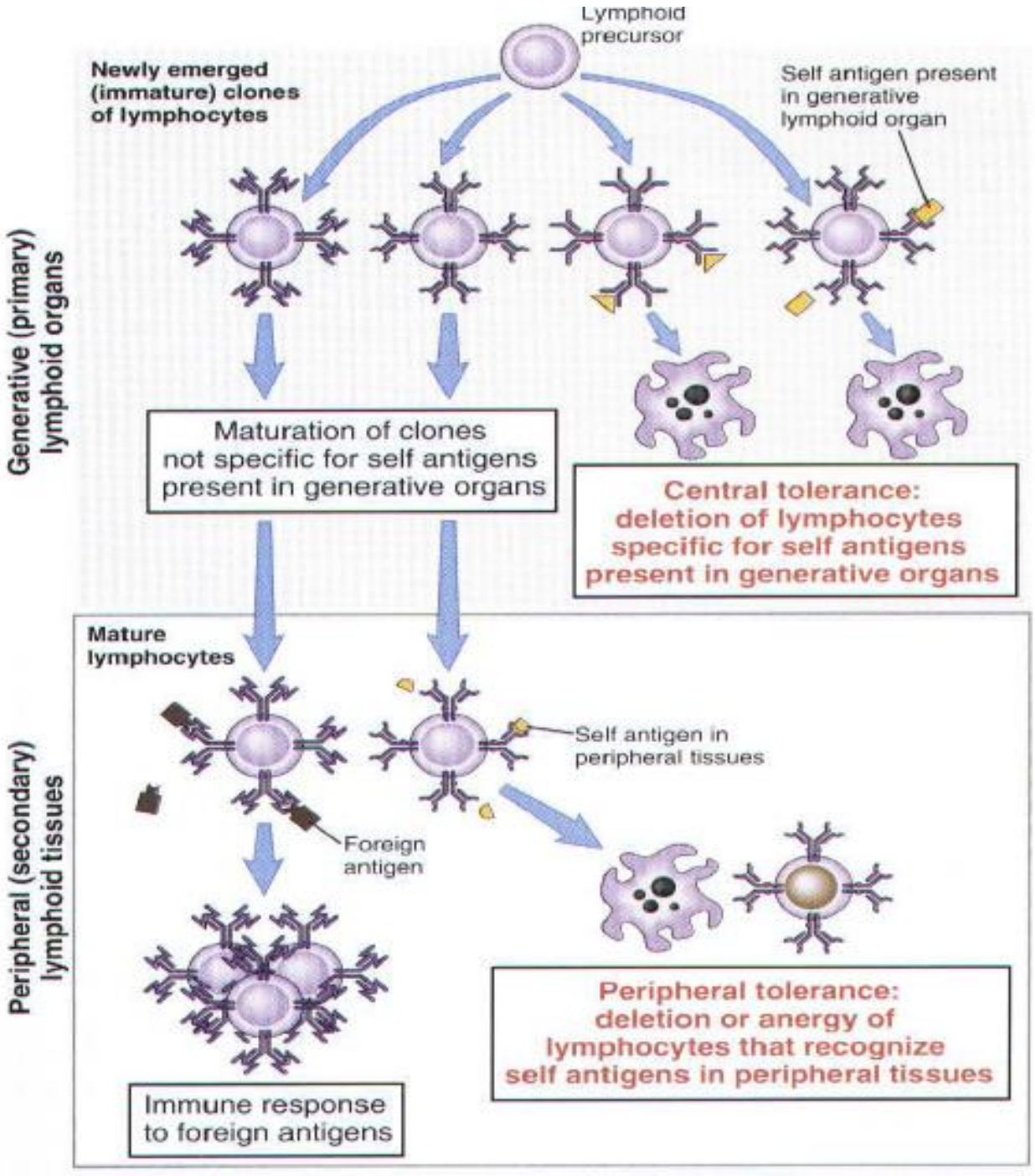
- **Peripheral tolerance** develops after T and B cells mature and enter the peripheral tissues and lymph nodes.

- Inappropriate reactivity toward normal, self-antigen that was not eliminated in the thymus can occur, since the T cells that leave the thymus are relatively but not completely safe. Some will have receptors (TCRs) that can respond to tissue specific self-antigens.

Peripheral tolerance

- Those self-reactive T cells that escape intrathymic negative selection in the thymus can inflict cell injury unless they are deleted or effectively inactivated in the peripheral tissue chiefly by natural regulatory T cells (nTreg cells)

Figure 1. Central and peripheral tolerance to self antigens



Physiological importance of immunological tolerance

- Immunological tolerance is the main way the immune system learns to discriminate self from non-self, thus preventing autoimmunity.

- Peripheral tolerance is key to preventing over-reactivity of the immune system to various environmental entities (allergens, gut microbes, etc.)

- immune tolerance in pregnancy is what allows a mother animal to gestate a genetically distinct offspring with an alloimmune response (a response to non-self antigens from members of same species) muted enough to prevent miscarriage.

Applications of immunological tolerance in medicine

Immunological tolerance can be induced artificially that may eventually be exploited clinically to:

- Prevent rejection of transplanted organs
- Treat autoimmune diseases
- Treat allergic diseases

Negative impacts of immunological tolerance on health

- It allows some pathogenic microbes to successfully infect a host and avoid elimination.

- In addition, inducing peripheral tolerance in the local microenvironment is a common survival strategy for a number of tumours.

Autoimmunity

- Autoimmunity is failure of the immune system to distinguish between ‘self’ (body’s own antigens) and ‘non-self’ (foreign antigens). In this case, the immune system goes awry and destroys healthy body cells, leading to autoimmune diseases

- It is a case of mistaken identity by the immune system

- Deficits in central or peripheral tolerance also cause autoimmune disease.

- Prominent examples include celiac disease, diabetes mellitus type 1 (IDDM), sarcoidosis, systemic lupus erythematosus, rheumatoid arthritis.

Autoimmune diseases

- This failure to differentiate between self and non self may result due to some extraneous environmental factors like some viral infections and exposure to some mutagenic agents; can be due to the breakdown and failure of immune regulation and due to some aberration in the genes.

Types of autoimmune diseases

- **Organ specific or localized autoimmune diseases :**As the name indicates in these cases the autoimmunity involves a particular organ.

  Examples are:

_ Type I diabetes

– Hashimoto’ thyroiditis

_ Addison’s disease in which the adrenal glands are affected.

_ Multiple sclerosis

– Grave’s disease

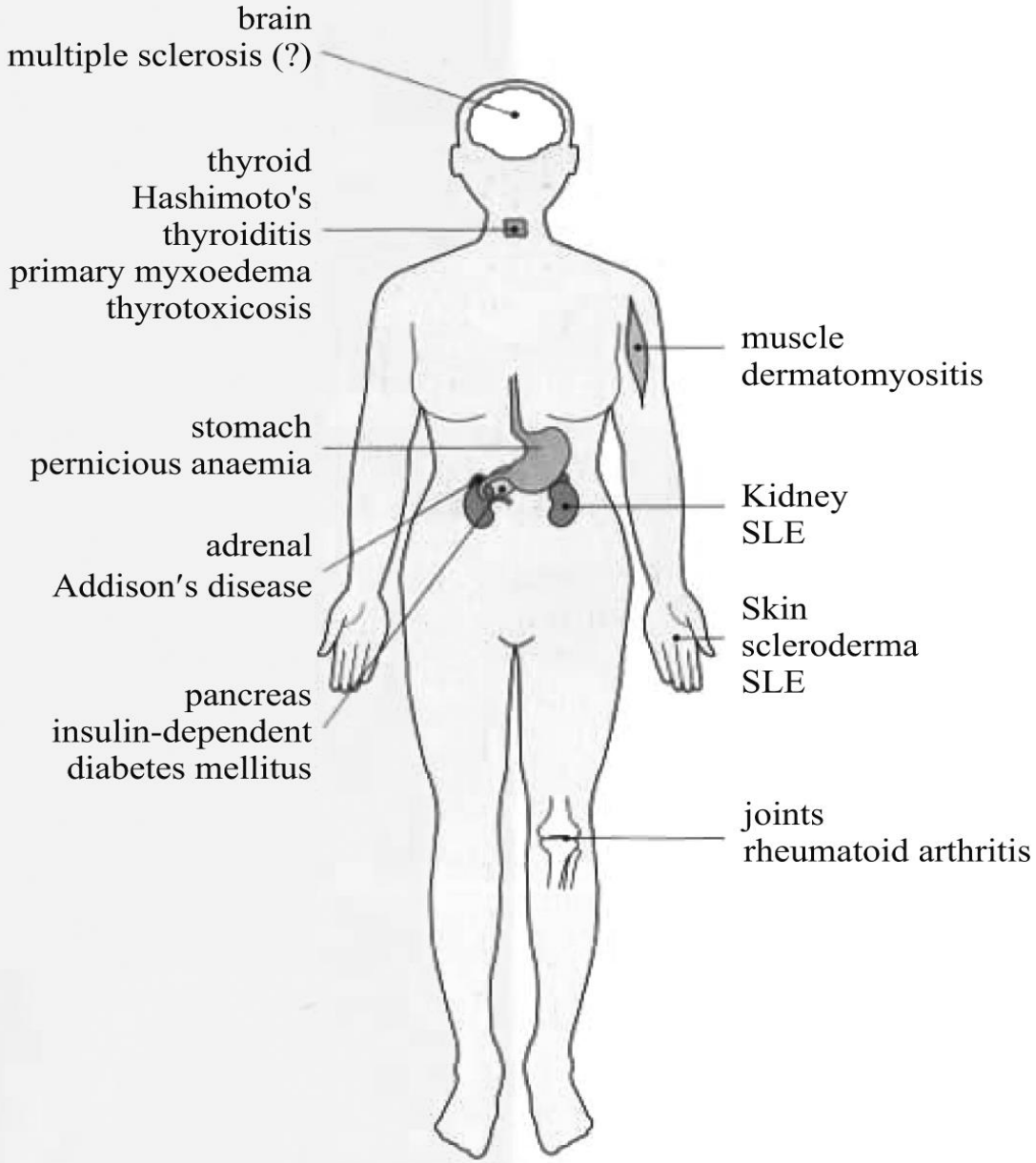
Systemic autoimmune diseases

- These diseases are associated with auto antibodies to antigens which are not tissue specific.

- in case of systemic autoimmune diseases the incriminating antigens and the autoimmunity are distributed in many tissues. Examples of systemic autoimmune diseases are:

– Rheumatoid arthritis
– Systemic lupus erythematosus (SLE)
– Scleroderma
– polymyositis

organ-specific	non-organ-specific



TREATMENT OF AUTOIMMUNITY

The general principle is to somehow stop the immune response to self antigens.

This can be achieved by the following methods:

- Immunosuppression (e.g., prednisone, cyclosporin A);
- Removal of thymus (some Myasthenia Gravis patients);
- Plasmapheresis (to remove Antigen+Antibody complexes);
- T-cell vaccination (to activate suppressing T cells so that immune response
- to self antigens is suppressed);
- Anti-CD4 monoclonal Antibody to inhibit immune response

Immuno-suppresive drugs

- Immunosuppressive drugs are used to reduce the activation or efficacy of the immune system.

- Immunosuppressive drugs/Immunosuppressants are used to control severe manifestations of allergic, autoimmune and organ transplant-related diseases.

- Some of such drugs have a diffuse effect on the immune system while others have specific targets. Such drugs with diffuse effects are more likely to cause damaging adverse effects, but the effectiveness of the more specific drugs may be reduced if their action can be bypassed by alternative metabolic pathways.

Uses of Immuno-suppresive drugs

Clinically they are used to:

- Prevent the rejection of transplanted organs and tissues (e.g. bone marrow, heart, kidney, liver)

- Treatment of autoimmune diseases (e.g. Rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, and ulcerative colitis).

- Treatment of some other non-autoimmune inflammatory /allergic diseases (e.g long term Allergic Asthma control).

Classification

- Immunosuppressive drugs can be classified into three major groups:

I. Glucocorticoids

II. Cytostatics

III. Antibodies

Glucocorticoids

- Glucocorticoids act by inhibiting genes that code for the cytokines IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8 and TNF-γ.
- Examples are: Prednisolone, azathioprine, mycophenolate or methotrexate.

Side effects of Glucocorticoids

- Patients should be told to expect the common early adverse effects, such as sweatiness, hoarse voice, loss and appetite stimulation.

- Rarely, more serious acute psychiatric disturbances are seen such as agitation, aggression or psychosis.

- Longterm and less reversible, adverse effects include Cushingoid appearance, proximal myopathy, hypertension, hyperlipidaemia, diabetes, cataract formation, peptic ulceration, osteopenia and aseptic necrosis of bone.

CYTOSTATICS

- Cytostatics inhibit cell division. In immunotherapy, they are used in smaller doses than in the treatment of malignant diseases.

- They affect the proliferation of both T cells and B cells.

- They include the following:

➢ **Alkylating agents** (e.g cyclophosphamide)

➢ **Antimetabolites** (interfere with the synthesis of nucleic acids. These include: folic acid analogues, such as methotrexate; purine analogues such as azathioprine and mercaptopurine)

➢ **Cytotoxic antibiotics**: dactinomycin is the most important. It is used in kidney transplantations. Other cytotoxic antibiotics are anthracyclines,mitomycin C, bleomycin, mithramycin).

ANTIBODIES

- Antibodies are used as a quick and potent immunosuppression method to prevent the acute rejection reaction.

- They are of two types: Polyclonal antibodies & Monoclonal antibodies

Polyclonal antibodies

- Polyclonal antibodies inhibit T lymphocytes and cause their lysis.

- Polyclonal antibodies affect all lymphocytes and cause general immunosuppression possibly leading to post-transplant lymphoproliferative disorders (PTLD) or serious infections, especially by cytomegalovirus.

- To reduce these risks,treatment is provided in a hospital where adequate isolation from infection is available.

Polyclonal antibodies

- Because of a high immunogenicity of polyclonal antibodies, almost all patients have an acute reaction to the treatment.

- It is characterized by fever, rigor episodes and even anaphylaxis. Later during the treatment, some patients develop serum sickness or immune complex glomerulonephritis.

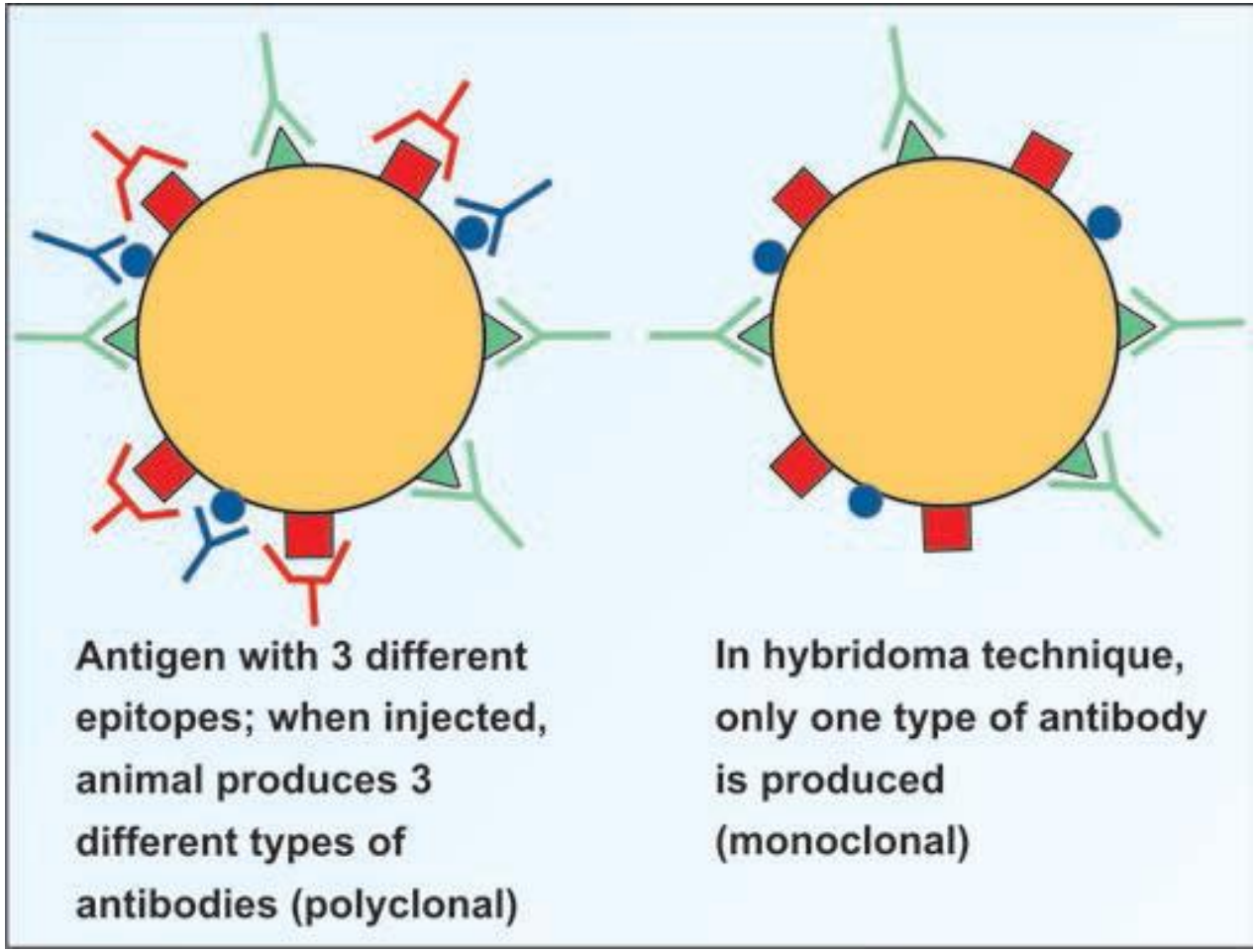
- Serum sickness arises seven to fourteen days after the therapy has begun. The patient suffers from fever, joint pain and erythema that can be soothed with the use of steroids and analgesics. Urticaria (hives) can also be present.

Monoclonal antibodies (mAb)

- Monoclonal antibodies are directed towards exactly defined antigens. Therefore, they cause fewer side effects.
- **Monoclonal antibody** is a particular type of antibody against a specific epitope of the antigen. While polyclonal antibodies are against different epitopes of an antigen

- More recently, hybridoma technology has produced a plethora of monoclonal antibodies against molecules expressed by human immune effector cells.
- Examples are: muromonab-CD3, Basiliximab, daclizumab, rituximab, alemtuzumab

Difference between monoclonal and polyclonal antibodies



Production of mAb

- monoclonal antibodies are produced in multiple myeloma where only one clone secretes a particular type of antibody. But in multiple myeloma, the antibody thus generated is unwanted.

- In the laboratory, **monoclonal antibody** can be generated by **hybridoma tecnology**

Production of mAb by Hybridoma technology

i. The antigen is injected into mice.

ii. Spleen cells from the immunised mice are fused with mice myeloma cells, so as to produce a hybrid cell. Polyethylene glycol (PEG-1500) is used as a fusion agent.

iii. The hybrid cells now contain the gene of normal mice as well as the myeloma cells. (Fig. 2

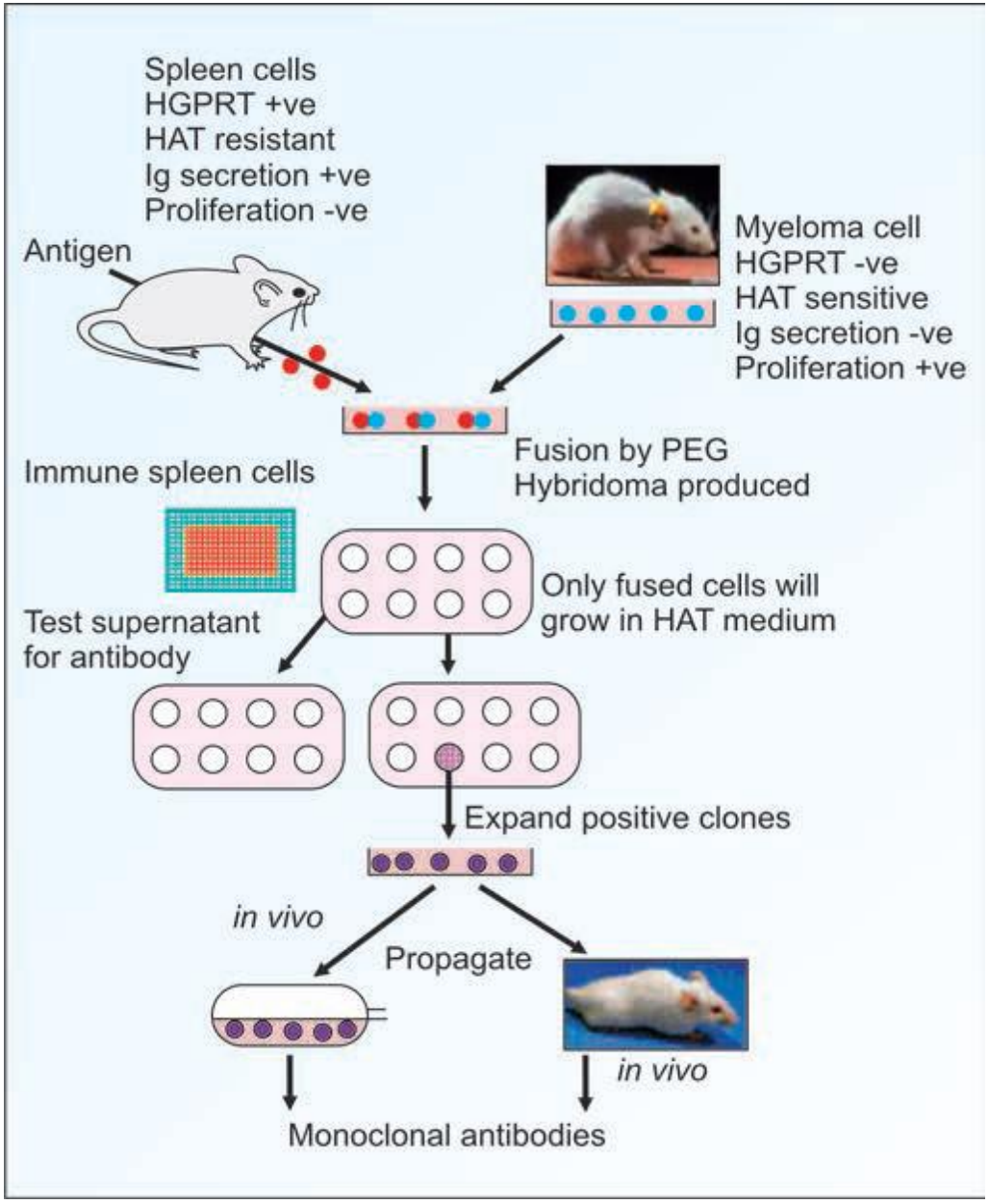
iv. However, hybridization might have occurred between two normal cells. Normal cells lack in the multiplication potential. So all the hybridised normal cells die in the usual culture conditions within 5-6 days.

v. The myeloma cells are defective in the enzyme HGPRTase and so they lack the salvage pathway for DNA synthesis , therefore the non fused myeloma cells also die in the special medium provided.

- **vi. The only cells that survive are the cells where** fusion has taken place between normal spleen cells with myeloma cells. In this case, normal cells provide the HGPRTase enzyme for DNA synthesis.

- **vii. The normal cellular genes also provide the** information for specific antibody synthesis. The

  myeloma cancer genes provide the endless multiplication drive, so that hybrid cells are immortalized (Fig. 2).


Spleen cells
HGPRT +ve
HAT resistant
Ig secretion +ve
Proliferation -ve
Antigen
Myeloma cell
HGPRT -ve
HAT sensitive
Ig secretion -ve
Proliferation +ve
Fusion by PEG
Hybridoma produced
Immune spleen cells
Only fused cells will
grow in HAT medium
Test supernatant
for antibody
Expand positive clones
in vivo
Propagate
in vivo
Monoclonal antibodies

Applications of mAb

- They are used as **Immunosuppressants**
- Monoclonal antibodies are widely used **as diagnostic and research reagents.** They are used in diagnostic kits such as Enzyme linked immunosorbent assays (ELISA), Immunofluorescence to diagnose various diseases.
- **Passive immunization**: High titre antimicrobial human monoclonal antibodies can offer passive protection.
- **Blood grouping**: anti-A monoclonal provides a more reliable standard reagent than conventional antisera.
- **Treatment of cancers**: monoclonal antibody is coupled to a strongly-radioactive atom, such as Iodine-131 to aid in killing the target cancer cells.
- **Purification of antigen**: Isolate antigen from mixtures by monoclonal affinity chromatography.