Transplant and Cancer Immunology

Learning objectives

By the end of the session students should be able to know

- Classification of transplants
- Histocompatibility antigens
- Types of graft rejection
- Mechanism of graft rejection
- Prevention of graft rejection
- Graft versus host reaction
- Cancer immunology

Transplantation

- Transfer of a graft or transplant (cells, tissues, or organs) from one site to another.
- Individual from whom the transplant is taken is referred to as the donor.
- Individual to whom it is transplanted, is called as the recipient.

CLASSIFICATION OF TRANSPLANTS

Based on	Explanation/ Examples
Organ or tissue transplanted	Kidney, heart and skin grafts, etc.
Based on the anatomical site of the graft	
Orthotopic grafts-	When the tissue or organ grafts are transplanted to their anatomically 'normal' sites in the recipient, then such grafts are known as orthotopic grafts. E.g., as in skin grafts.
Heterotopic grafts	are placed in anatomically 'abnormal' sites, as when thyroid tissue is transplanted in a subcutaneous pocket.

CLASSIFICATION OF TRANSPLANTS

Based on	Explanation/ Examples
Vital and static transplants	
Vital grafts	Live grafts, such as the kidney or heart, are expected to survive and function physiologically in the recipient.
Static grafts	Nonliving structures, like bone or artery which merely provide a scaffolding on which new tissue is laid by the recipient

CLASSIFICATION OF TRANSPLANTS

Based on	Explanation/ Examples
Based on the genetic relationship between the donor and the recipient	
Autograft	Self-tissue transferred from one part of the body site to another in the same individual. Example: • Transferring healthy skin to a burned area in burn patients. • Use of healthy blood vessels of the same person to replace blocked coronary arteries.
Isograft or syngeneic graft	Tissue transferred between genetically identical individuals (e.g. monozygotic twins)

CLASSIFICATION OF TRANSPLANTS

Based on	Explanation/ Examples
Based on the genetic relationship between the donor and the recipient	
Allograft	Tissue transferred between genetically non-identical members of the same species (e.g. kidney or heart transplant).
Xenograft	Tissue transferred between different species. (e.g., the graft of a baboon heart into a man). o Most commonly used graft in transplant centres.

Histocompatibility

- Histocompatibility between the graft and recipient - decide whether the graft is going to be accepted or rejected.
- Graft and recipient tissues histocompatible - graft is accepted.
  - Autografts and isografts are histocompatible.
- Histoincompatible (i.e. antigenically dissimilar) grafts are generally rejected by the recipient.
  - Allografts and xenografts are usually histoincompatible.

Transplantation antigens

- Antigens of allografts against which the recipient would mount an immune response.
  - MHC molecules
  - ABO and Rh blood group systems

Transplantation antigens

- **MHA (minor histocompatibility antigens):**
  - Peptides derived from normal cellular proteins of donated organs.
  - Immune response against MHA molecules is weaker; hence they pose problems of rejection less frequently than MHC molecules.
  - One exception is when a graft is transferred from a male donor to a female recipient.

Eichwald-Silmser effect

- The graft tissues of a male donor (XY) would have some male-specific minor histocompatibility (H-Y) antigens determined by the Y chromosome which will be absent in the female (XX) recipient.
- It is observed that the rejection of the grafts when transferred from a male donor to female recepient is more as compared to female to male transplantation.

TYPES OF GRAFT REJECTION

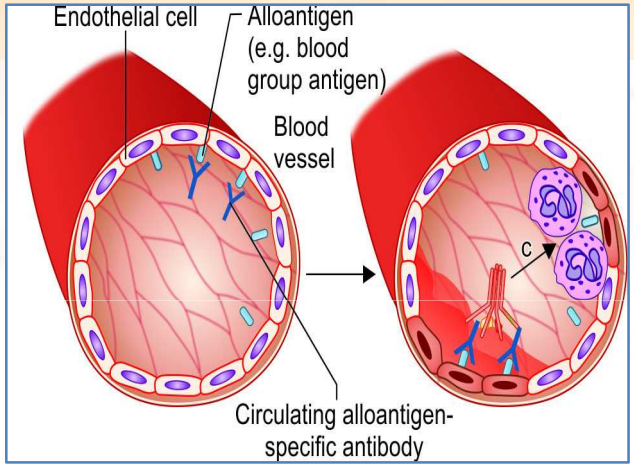
- Based of time taken for the rejection, types of immune response mounted against the graft and clinical & pathologic features:
  - Hyperacute
  - Acute
  - Chronic

Hyperacute rejection

- Occurs within minutes to hours of transplantation.
- Characterized by thrombosis of graft vessels and ischemic necrosis of the graft.
- Mediated by *circulating antibodies* that are specific for antigens on the graft endothelial cells and that are present before transplantation.

Hyperacute rejection

- Exposure to foreign HLA antigens can occur as a consequence of previous blood transfusions, pregnancy, or organ transplantation → develops antibodies against these antigens.
- If an individual with these pre-existing antibodies to a foreign HLA antigen receives a graft→ graft rejected earlier and more vigorously.



Preformed antibodies react with alloantigens on the vascular endothelium of the graft, activate complement (C) and trigger rapid intravascular thrombosis and necrosis of the vessel wall.

Hyperacute rejection

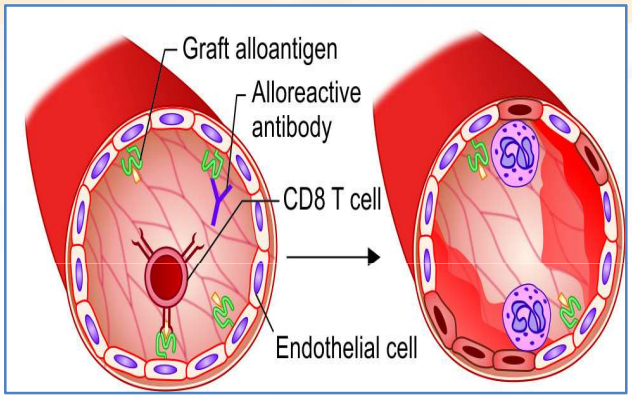
- Not a common problem in clinical transplantation - because it can be avoided by matching the donor and the recipient.
- Potential recipients are tested for antibodies against the prospective donor’s blood group antigens (by cross matching) and HLA antigens (by HLA typing).

Acute graft rejection

- Occurs within days or weeks after transplantation.
- Due to an active immune response of the host stimulated by alloantigens in the graft.
- Mediated by T cells (mainly cytotoxic T cells, occasionally helper T cells) and antibodies specific for alloantigens in the graft.
- Cytotoxic T cells directly destroy the graft cells, or cytokines secreted by the helper T cells induce inflammation; which destroys the graft.

Acute graft rejection

- Antibodies contribute especially to the vascular component of acute rejection.
- Antibody mediated injury to graft vessels by complement activation by the classical pathway.



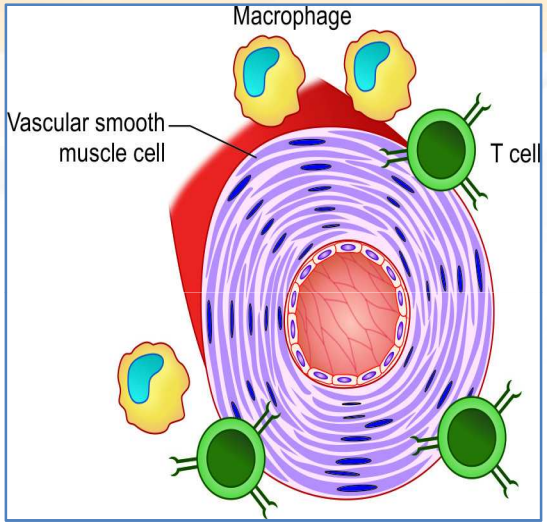
CD8 T cells react with graft alloantigens and destroy the endothelial and parenchymal cells or antibodies react with alloantigens of the graft's endothelium and causes endothelial cell damage via complement activation.

Chronic graft rejection

- Indolent form of graft damage that occurs over months or years, leading to progressive loss of graft function.
- Manifested as fibrosis of the graft and by gradual narrowing of graft blood vessels, called graft arteriosclerosis.

Chronic graft rejection

- T cells that react against graft alloantigens secrete cytokines → stimulate the proliferation and activities of fibroblasts and vascular smooth muscle cells in the graft.



T cells react with graft alloantigens may produce cytokines that induce inflammation and proliferation of intimal smooth muscle cells, leading to luminal occlusion and graft arteriosclerosis

Comparison of various types of graft rejection

Graft rejection	**Time taken for rejection**	**Immune mechanisms involved**
Hyperacute	Minutes to hours	Preformed antibodies (Anti ABO and/or anti-HLA)
Acute	Weeks to months	Cytotoxic T cell mediated
Chronic	Months to years	Chronic DTH mediated Antibody mediated

FACTORS INFLUENCING ALLOGRAFT REJECTION

- The rate of allograft rejection varies according to the-
  - Tissue involved
  - Genetic distance between the donor and recipient
  - Immunological memory

Graft acceptance and graft rejection

- Pathological sequences that take place when a skin graft is placed-

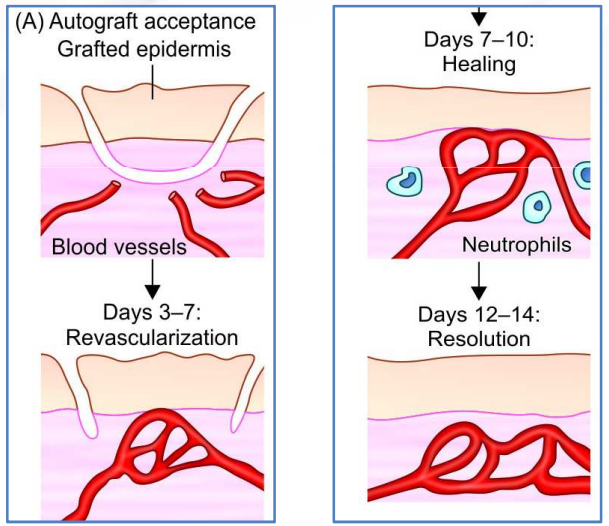
i) as an autograft to the same donor

ii) as an allograft to a recipient for the first time

iii) as an allograft to the same recipient for the second time(leads to first set rejection).

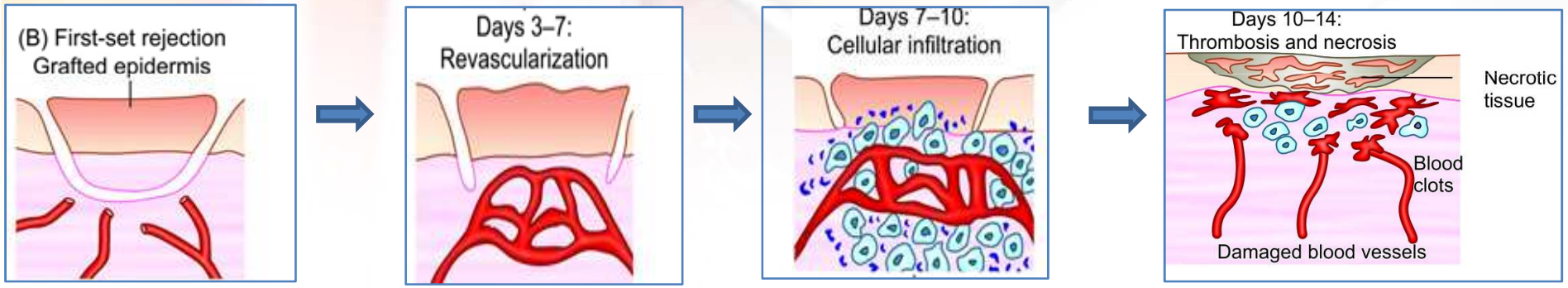
Autograft acceptance-

- When a skin graft is transplanted to the same individual at a different site:
  - Revascularization takes place by day 3-7
  - Healing (within day 7-10)
  - Resolution and acceptance of the graft (by day 12-14).



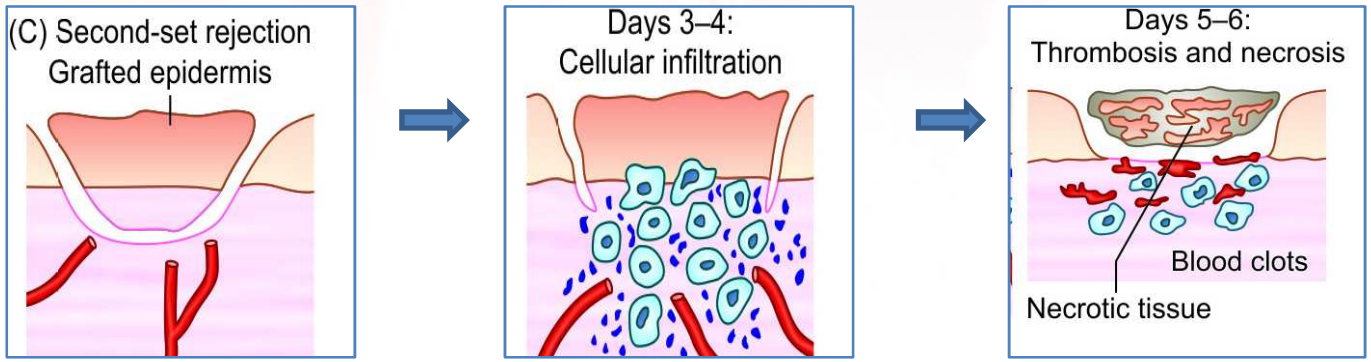
First-set rejection

- Definition – Type of primary rejection when an allograft is placed for the first time from a donor to a recipient.



Second set rejection

- If, in a recipient that has rejected a graft by the first set response, another graft from the same donor is transplanted, it will be rejected in an accelerated fashion.



MECHANISM OF GRAFT REJECTION

- Graft rejection is caused principally by a T cell-mediated immune response to alloantigens expressed on the graft cells, primarily the MHC molecules.
- Peptides present in the groove of allogeneic:
  - Class I MHC molecules - derived from proteins synthesized within the allogeneic cell.
  - Class II MHC molecules – are proteins taken up and processed by the allogeneic APCs.

MECHANISM OF GRAFT REJECTION

- The process of graft rejection can be divided into two stages:
  - Sensitization phase- which involves alloantigen (mainly graft MHC molecules) presentation to recipient's T cells
  - Effector stage - which immune destruction of the graft takes place due to activation of recipient's T cells.

Sensitization phase

- T cells in the recipient may recognize donor alloantigens in the graft in two different ways depending on what cells in the graft are displaying these alloantigens to the recipient T cells.:
  - Direct pathway
  - Indirect pathway

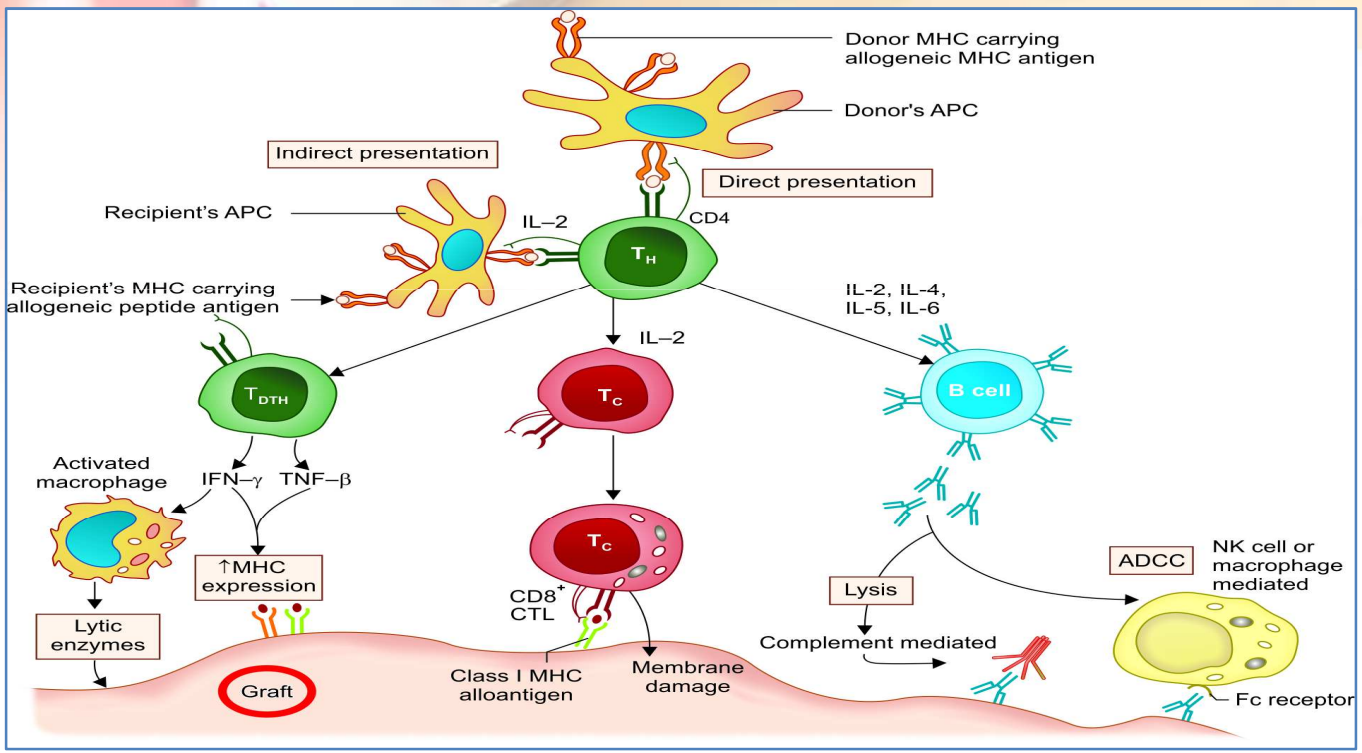
Direct pathway of alloantigen presentation

- When the tissues are transplanted, the APCs are also carried along with the graft to the recipients.
- Allogeneic MHC molecules on graft's APCs are directly presented to the recipient's helper T cells.
- Responsible for most of the acute graft rejections mediated by cytotoxic T cells.

Indirect pathway of alloantigen presentation

- Similar to that for recognition of any foreign antigen by the host APCs.
- Graft cells are ingested by recipient APCs, donor alloantigens are processed and presented by the MHC molecules present on recipient APCs to recipient's helper T cells.
- Responsible for most of the chronic rejection mediated by helper T cells via specialized form of chronic DTH reaction.

Mechanisms involved in graft rejection



Effector phase

- **Delayed-type hypersensitivity**-Activated helper T cells differentiate in to T_{DTH} cells → Cytokines are secreted → activate macrophages which destroy the target graft cells by producing lytic enzymes.
- **Cytotoxic T cells**- $CD8^+$ T_C cells kill the graft cells by recognizing the allogeneic MHC-I molecules.
- **Antibody mediated mechanisms**- Cytokines produced by helper T cells activate B cells to produce antibodies → mounts immune response against the graft → mediating hyperacute graft rejections (acute and chronic rejections -minor role). Antibody mediated destruction of the graft by complement mediated lysis or ADCC.

Laboratory tests to determine histocompatibility

- Prior to transplantation, various laboratory tests should be carried out to assess the histocompatibility between the donor and recipient.
- ABO blood group compatibility - blood grouping and cross matching.
- HLA typing

HLA Typing

- Donor's antigens expressed on the surface of leukocytes or their gene to that of recipient are matched.
- HLA compatibility is determined by:
  - **Phenotypic method** (not in use now)**:**
    - Serology: Microcytotoxicity
    - Tissue typing: Mixed lymphocyte reaction
  - **Genotypic methods**

HLA Typing

- **Genotypic methods** (Widely used) **:**
  - PCR detecting HLA genes
  - PCR-RFLP (restriction fragment length polymorphism)
  - PCR-SSOP (PPCR-sequence-specific oligonucleotide probing)
  - PCR-SSP (PCR-sequence-specific primer)
  - PCR- DNA sequencing
  - Conformational analysis

Immunosuppressive therapy

- Hyperacute rejection manifests severely and within minutes, and so the treatment indicated is- immediate removal of the tissue.
- Chronic rejection is generally considered irreversible and poorly amenable to treatment—only re-transplant generally indicated if feasible—though inhaled cyclosporine is being investigated to delay or prevent chronic rejection of lung transplants.
- Acute rejection is treated with therapeutic regimens consisting of one or combination of various immunosuppressive therapies.

Immunosuppressive agents

Corticosteroids	**Prednisolone, hydrocortisone**
Calcineurin inhibitors	Cyclosporine, Tacrolimus
Mitotic inhibitors	Azathioprine Cyclophosphamide Methotrexate
Anti-proliferatives	Mycophenolic acid
mTOR inhibitor (mammalian target of rapamycin)	Sirolimus (rapamycin) Everolimus

Immunosuppressive agents

Monoclonal antibody based	
mAb to CD2 molecule present on T cell surface	OKT2
mAb to CD3 molecule present on T cell surface	OKT3
mAb to CD4 molecule present on T cell surface	OKT4
Monoclonal anti-IL-2Rα receptor antibodies	Basiliximab Daclizumab
Monoclonal anti-CD20 antibodies	Rituximab
mAb to TNFα	Infliximab
Anti-thymocyte globulin (ATG)	
Anti-lymphocyte globulin (ALG)	

GRAFT-VERSUS-HOST REACTION

- Graft mounts an immune response against the host (i.e. recipient) and rejects the host, in contrary to the usual situation of graft rejections, in which the recipient mounts an immune response against the graft antigens.
- The GVH reaction occurs when the following three conditions are present:
  - Graft must contain immunocompetent T cells (e.g. stem cells or bone marrow or thymus transplants)
  - Recipient should possess transplantation antigens that are absent in the graft.
  - Recipient may be immunologically suppressed and therefore cannot mount immune response against the graft.

TYPES OF GRAFT-VERSUS-HOST REACTION

- Acute or fulminant GVH disease
  - Occurs within first 100 days of post-transplantation.
  - Major challenge in case of bone marrow transplantation.
- Chronic GVH disease
  - Less severe form.
  - Occurs after 100 days of transplantation.

GRAFT-VERSUS-HOST REACTION

- **Clinical manifestations-**
  - Acute GVH -Damage to the liver (hepatomegaly), skin (rash), mucosa, and the intestine (diarrhea) mediated graft's immunocompetent T cell. Experimentally, GVH can be produced in mice, called as *Runt disease*.
  - Chronic GVH disease also attacks the above organs, but in addition, it causes damage to the connective tissues and exocrine glands.
- **Treatment-** Glucocorticoids (administrated intravenously) are the standard treatment given for both acute and chronic GVH disease.

CANCER IMMUNOLOGY

- **TUMOR ANTIGENS:** Two types of tumor antigens have been identified on tumor cells:
  - Tumor-specific transplantation antigens (TSTA)
  - Tumor-associated transplantation antigens (TATA)

Tumor-specific transplantation antigen (TSTA)

- Tumor-specific antigens are present only on tumor cells and are absent in normal cells of the body.
- May result from mutations in tumor cells that generate altered cellular proteins.
- Cytosolic processing of these proteins would give rise to novel peptides that are presented with class I MHC molecules, inducing a cell-mediated immune response by tumor-specific cytotoxic T lymphocytes.
- TSTA are induced on tumor cells either by chemical or by physical carcinogens, and also by viral carcinogens.

Tumor-specific transplantation antigen (TSTA)

- Tumour specific.
- Methylcholanthrene and ultraviolet light are the examples of chemical and physical carcinogens that are extensively studied.
- In contrast, the TSTA of virus induced tumours is virus specific; all tumours produced by one virus would possess the same antigen.
- Example: Epstein Barr virus which causes nasopharyngeal carcinoma and several types of lymphoma.

transplantation antigens (TATA)

- Not unique to tumor cells and may also be expressed by normal cells but at a very low level.
- Their level gets exponentially high in tumor cells.
- **Oncofetal antigens**- proteins that are expressed on normal cells during fetal life but not expressed in the adult normally.
  - Reactivation of the embryonic genes that encode these proteins in tumor cells results in their expression on the fully differentiated tumor cells.
  - Examples include alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA).
- **Non-oncofetal TATAs:** Examples include Carbohydrate antigens (CA 125, CA 19-9), prostate specific antigen and macroglobulin

TATAs used as tumor markers for diagnosis of cancers

Tumor markers	**Tumor types**
Oncofetal proteins	
Alpha-fetoprotein (AFP)	Hepatoma Testicular cancer
Carcinoembryonic antigen (CEA)	Gastrointestinal cancers Lung, ovarian cancers
Secreted tumor antigens	
CA 125	Ovarian cancers; Other epithelial cancers
CA 19-9	Various carcinomas
Prostate-specific antigen	Prostate cancer
β 2 microglobulin	Multiple myeloma
Hormones	
β subunit of chorionic gonadotropin	Hydatidiform mole Choriocarcinoma; Testicular cancers

IMMUNE RESPONSE AGAINST TUMOR CELLS

- Both humoral and cell-mediated immune responses are induced by tumor antigens that result in the destruction of the tumor cells.
- Cell-mediated response appears to play the major role:
  - Cytotoxic T cell
  - NK cell.

Cytotoxic T cells

- Number of tumors have been shown to induce tumor-specific T_C cells that recognize tumor antigens presented by class I MHC on the tumor cells.
- Expression of class I MHC molecules are decreased in a number of tumors, thereby limiting the role of specific T_C cells in their destruction.

NK (natural killer) cells

- Recognition of tumor cells by NK cells is not MHC restricted.
- Activity of NK cells is not compromised; but enhanced by the decreased MHC expression exhibited by some tumor cells.
- Due to withdrawal of inhibitory receptors induced NK cells suppression.
- The inhibitory receptors of NK cells will be no longer functional in the absence of MHC I molecules on the target cells so that the activation receptors become active.

NK (natural killer) cells

- Activation receptors can be Fc receptors on NK cells which can bind to antibody-coated tumor cells, leading to ADCC.
- The importance of NK cells in tumor immunity is suggested by the mutant mouse strain called beige and **Chediak-Higashi syndrome** in humans.
- In each case, a genetic defect causes marked impairment of NK cells and an associated increase in certain types of cancer.

IMMUNE SURVEILLANCE THEORY (Paul Ehrlich)

- Tumor cells may arise frequently in our body but are recognized as foreign and are eliminated by the constant vigilance of our immune system.
- Later, Lewis Thomas revived the theory by suggesting the role of cell-mediated branch of the immune system to patrol the body and eliminate cancer cells.

Immune evasion by tumor cells

- **Anti-tumor antibodies** produced against tumor antigens may have a role in immune evasion.
  - *Blocking factor*-antitumor antibody may act as a blocking factor. The antibody binds to tumor-specific antigen and masks the antigen from cytotoxic T cells and NK cells.
  - *Antigenic modulation*-Certain tumor-specific antigens have been observed to disappear from the surface of tumor cells in the presence of serum antibodies and then to reappear after disappearance of serum antibodies.
- **Masking the immune cells**- Circulating tumour antigens may act as a 'smokescreen', coating the lymphoid cells and preventing them from acting on the tumour cells.

Immune evasion by tumor cells

- **Expressing low levels of MHC I**- Many tumor cells down regulate the expression of MHC I molecules; hence preventing their recognition by cytotoxic T cells.
- **Poor co-stimulatory signals-**The co-stimulatory signal of T cell activation is provided by interaction between the CD28 molecules on T cell surface with the B7 molecules on the APCs. The poor immunogenicity of many tumor cells may be due to lack of the co-stimulatory molecules on APCs.

Immune evasion by tumor cells

- **Secrete soluble factors-** Certain tumor cells secrete soluble factors such as IL-10 and TGF-β that may suppress the immune responses against the tumor cells.
- **Expressing Fas ligand-**Some tumor cells express Fas ligand on their surface, which when interact with Fas (the death receptor) on T cells, causes apoptosis of T cells.
- **↑opportunistic infections-**Patients with advanced cancers have an increased susceptibility to various opportunistic infections which in turn depresses the T cell responses.

CANCER IMMUNOTHERAPY – Cell-based therapies (cancer vaccines)

- Involve the removal of immune cells from patients with cancer, either from the blood or from a tumor →immune cells specific for the tumor will be activated, grown and returned to the person with cancer →where the immune cells provoke an immune response against the cancer.
- Cell types that can be used in cancer vaccines include NK cells, cytotoxic T cells and dendritic cells.
- Only cell-based therapy currently approved for use is dendritic cells (Provenge) for the treatment of prostate cancer.

Monoclonal antibodies

Monoclonal antibodies	Target	Approved for treatment of cancers
Alemtuzumab	CD52	Chronic lymphocytic leukemia (CLL)
Bevacizumab	Vascular endothelia growth factor	Colorectal, lung and renal cancer
Cetuximab	Epidermal growth factor receptor	Colorectal, the head and neck cancer
Ipilimumab	CTLA4	Metastatic melanoma
Rituximab	CD20	CLL Non-Hodgkin lymphoma
Tositumomab	CD20	
Trastuzumab	ErbB2	Breast cancer

Cytokine therapies

- Regulate and coordinate the behavior of the immune system. Examples include:
  - Interferon-α is used in the treatment of hairy-cell leukaemia, AIDS-related Kaposi's sarcoma, follicular lymphoma, chronic myeloid leukemia and malignant melanoma.
  - Interleukin-2 is used in the treatment of malignant melanoma and renal cell carcinoma.

Cancer vaccine

- **Preventive cancer vaccines**:
  - Example - HPV and hepatitis B vaccine
  - Prevent the emergence cervical and liver cancers respectively.
- **Therapeutic cancer vaccines:**
  - Used to treat existing cancers.
  - Research is ongoing for preparation of such vaccines.
  - Vaccines against some oncogenic viruses have proven extremely effective.