Module -9

Lecture -4

Tumor immunology

Theory of immune surveillance

Paul Ehrlich - Ehrlich hypothesis
Ehrlich and co-workers in 1909 hypothesized that immune system has the potential to recognize and destroy tumor cells

Lewis Thomas and Macfarlane Burnet in late 1950s also reiterated this idea that preneoplastic cells can be identified before they are visible clinically and finally killed by the immune cells
That is known as the **theory of immune surveillance/Burnet's hypothesis**

Not accepted for quite some time
We know that genes associated with cancer are not alien genes and so their products are also normal proteins. The main idea behind immunological surveillance is that it can distinguish between self and non-self.

How then the immune system would recognize the neoplastic cells?

Burnet's theory was not accepted by many as the tumor incidence in immunocompetent and nude mice was more or less same but now we know why this happened.

Escape from Immune surveillance

Now we know that immune system indeed can recognize preneoplastic cells and several evidences have been provided by experiments in animal models or observations and consequent predictions in humans

Basically the immune system continuously monitors and destroys aberrant cells. However, some cells escape from this monitoring or evade or developed alternate strategies to overcome this surveillance and they grow eventually into cancer cells

It is almost like a war between immunocytes and cancer cells
If immunocytes win we get protection
If cancer cells win we get cancer
Recent studies have identified several ammunitions/weapons/molecular mechanisms/strategies from both the sides

Important Questions in Tumor Immunology

We need to answer three questions to gain a good understanding of tumor immunology

1. How the immune system protects us from getting cancer? or
   What are the molecular mechanisms of this surveillance and protection?

2. Why the immune system fails to protect us in some cases? or
   How do cancer cells escape from these protective mechanisms?

3. Will it help if we answer the above questions in developing antitumor therapies?

Immune system

Before we discuss the details you should recapitulate the details learnt earlier on the following

Innate immunity, Acquired immunity, Cellular immunity, Humoral immunity

NK cells

T_C cells or CTLs

T_H cells

$T_{reg}S$

Macrophages

Dendritic cells

Evidences for immune protection

- Now we know that there are tumor antigens
- First observation was that mice immunized with chemically induced tumors were protected against subsequent rechallenge with the same tumor transplanted into them
- They were called transplantation rejection antigens
- Experimentally induced tumors with different carcinogens, viruses, UV irradiation and spontaneous tumors show the same outcome

Evidences for immune protection

T cell ablation or T-cell deficient mice removed this protection.

Transfer of T cells from an immunized mouse could protect a naïve or unimmunized mouse from tumour challenge

Mice were more susceptible to carcinogen induced or spontaneous primary tumor formation under the following conditions

mice lacking either IFN-gamma receptor or the STAT1 transcription factor required for IFN receptor signaling

or adaptive immunity [RAG2 −/− mice lacking T cells, B cells, and natural killer T (NKT) cells]

Evidences for immune protection

Tumor specific antibodies are detectable in humans with some tumors

Higher TIL presence in tumors correlates with improved survival

- Increased lymphocyte proliferation in draining lymph nodes observed in melanoma and breast cancer

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Evidences for immune protection

Spontaneous regression: melanoma, lymphoma

due to the immune system, it was hypothesized now proven

Regression of metastases after removal of primary tumor: pulmonary metastases from renal carcinoma

Higher incidence of cancer after immunosuppression, immunodeficiency (AIDS, neonates), aging, etc

Immunosuppression leads to increased development of viral-derived tumours (Kaposi / NHL / HPV).

NK cell loss correlates with increased tumour pathogenicity

How Does The Immune System Distinguish Cancer from Normal Cells?

- Cancer cells arise from normal cells – hence many of the proteins expressed by cancer cells are normal proteins and do not trigger an immune response
- But a few proteins are structurally novel – not exposed to the immune system during development and thus are antigenic
- For example mutated Ras oncoproteins or mutant p53 can be recognized by the immune system as “foreign” and the cells that express these proteins can be targeted for destruction

How Does The Immune System Distinguish Cancer from Normal Cells?

Tumor cells accumulate numerous mutations during the course of their rapid proliferation – this may give rise to a number of structurally altered proteins that can be targeted by the immune system

Defects in DNA mismatch repair cause microsatellite instability (MIN) which results in a very high rate of mutations – cancers that exhibit MIN have a higher degree of T cell infiltration and lower rates of metastasis

Tumor Specific Antigens

- They are present only in tumors and not in normal tissues

- The human tumor antigens discovered include
- differentiation antigens (such as melanocyte differentiation antigens),
- mutational antigens (such as p53),
- viral antigens (such as human papilloma virus proteins)
- These evidences clearly disprove the notion that tumor antigens are simply overexpressed normal proteins and therefore cannot ellicit immune response

Tumor Associated Antigens

- They are not limited to malignant tissues and are also present in normal cells

Normal proteins that have not elicited immune tolerance due to various reasons

- Proteins that were expressed only during embryogenesis – the oncofetal antigens (example: Carcino Embryonic Antigen CEA)
- Proteins that are sequestered in areas inaccessible to the immune system or not presented to immune cells due to the lack of MHC class I molecules

- Example: many cell types in the testis/ovary

- cancer/testis (CT) antigens are expressed in germ cells of testis and ovary but silent in normal somatic cells (such as MAGE and NYESO-1).

Molecular mechanisms of immune protection against tumors

Both innate and acquired immunity work together to eradicate tumors as shown by several in vitro experiments. How far this is true in vivo is the challenge.

Natural killer cells (NK cells) play an important role in killing the cancer cells

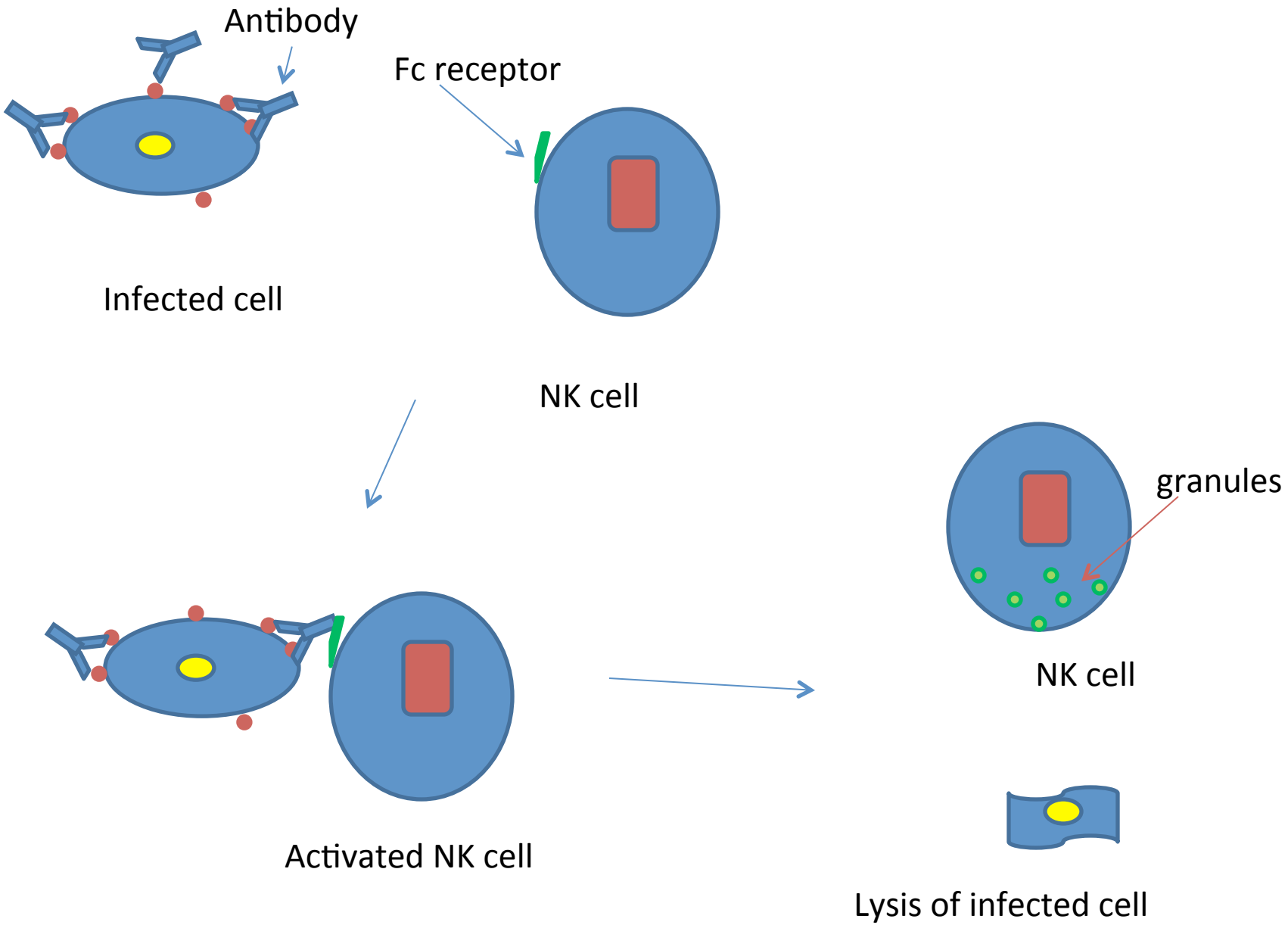
Fc receptors (FcyRIII or CDl6) present in NK cells recognize antibody bound infected cells and attack them

NK cells kill hematopoietic cancer cell lines and virally infected cells in vitro

NKG2D receptors on NK cells may be activated by their ligands present in cancer cells

IL-2 activated NK cells (lymphokine activated killer cells) are good killers

NK cell deficiency is linked with increased incidence of EBV associated lymphoma


Antibody
Fc receptor
Infected cell
NK cell
granules
NK cell
Activated NK cell
Lysis of infected cell

Molecular mechanisms of immune protection against tumors

Macrophages

Recognizing some surface antigens of tumor cells

Activation of macrophages by interferon-y (IFN-y) produced by tumor-specific T cells

Release of TNF, lysosomal enzymes, reactive oxygen species, and nitric oxide.

T-Lymphocytes

In virally induced tumors if the oncogenic peptides are presented by the MHC I system then CD8+ T cells can kill them
TILs from solid tumors also contain CTLs suggesting their importance

Helper T cells specific for tumor antigens may secrete cytokines, such as TNF and IFN-y
This can increase tumor cell class I MHC expression
Increase the sensitivity to lysis by CTLs.

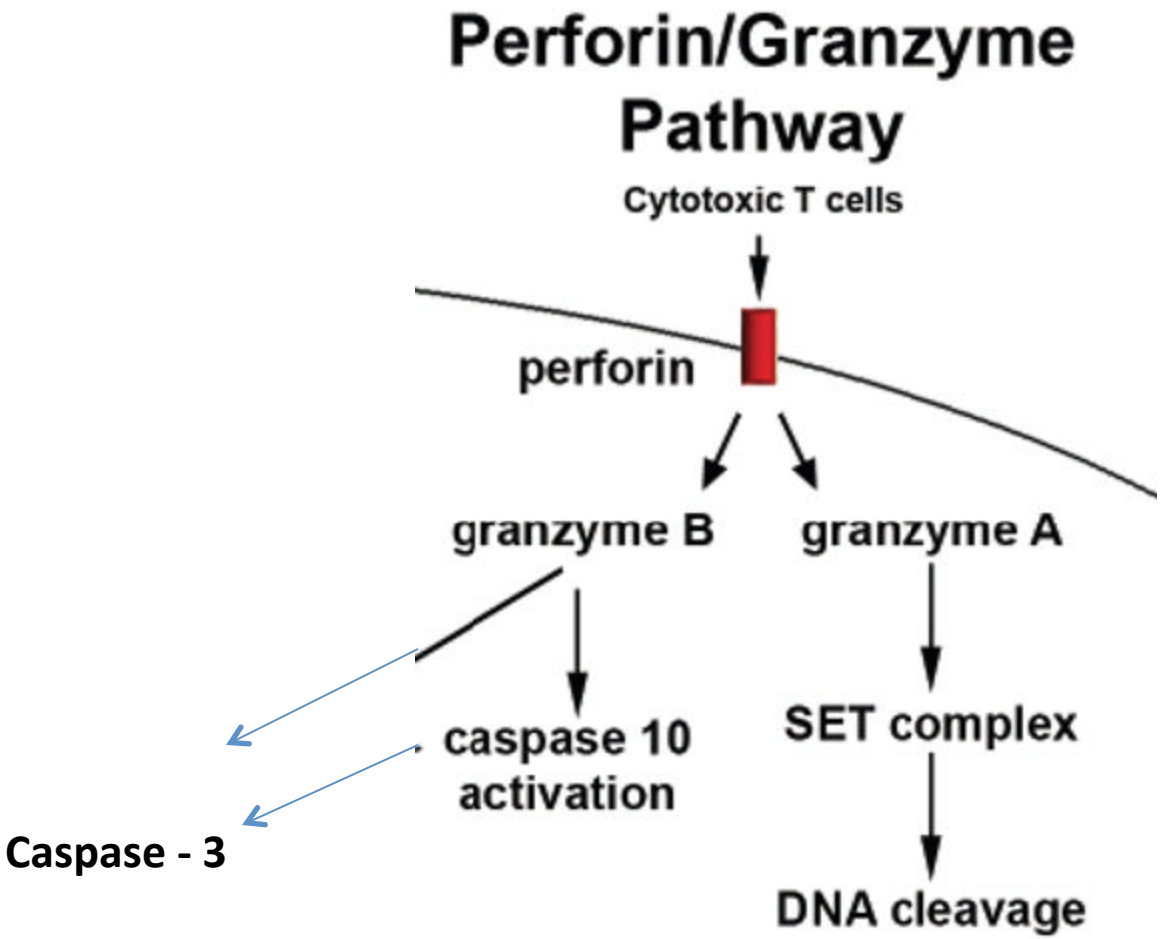
Molecular mechanisms of immune protection against tumors

Antibodies

Antibodies may kill tumor cells by activating complement

or by antibody-dependent cell mediated cytotoxicity in which Fc receptor-bearing macrophages or NK cells carry out the killing of tumor cells.

Molecular mechanisms of immune protection against tumors



Cytotoxic T lymphocytes (CTLs) kill target cells via the extrinsic pathway.
FasL/FasR pathway is the predominant mode of CTL-induced apoptosis
However, CTLs can also kill tumor cells and virus-infected cells by the secretion of the transmembrane pore-forming molecule perforin
A subsequent exophytic release of cytoplasmic granules occurs through the pore and finally into the target cell.
The serine proteases granzyme A and granzyme B are present in the granules.
Granzyme B cleaves proteins at aspartate residues and activates procaspase-10 and can cleave factors like ICAD (Inhibitor of Caspase Activated DNAse).
Granzyme B also amplifies the death signal by specific cleavage of Bid and induction of cytochrome *c* release.
It can also directly activate caspase-3.
Granzyme B cytotoxicity is critical as a control mechanism for T cell expansion of type 2 helper T (Th2) cells.
Granzyme A activates caspase independent pathways.
Granzyme A activates DNA nicking via DNAse NM23-H1, a tumor suppressor gene product.
Granzyme A protease cleaves the SET complex that usually inhibits the NM23-H1 gene. In addition to inhibiting NM23-H1, the SET complex (SET, Ape1, pp32, and HMG2) is important to protect chromatin and DNA structure.

Immunoediting

Animal models particularly mouse models have shown that immune responses impose a selective pressure on the cells in such a way that some cells die while some clones of reduced immunogenicity survive and get selected for better survival. What this means is that the cells undergo some sort of editing.

This is called **immunoediting**

If a tumor is induced by a chemical carcinogen in immunocompetent mice, then the derived cancer cells are said to be **edited**.

If a tumor is induced by a chemical carcinogen in immunodeficient mice, then the derived cancer cells are said to be **unedited**.

Immunoediting

3 phases of immunoediting have been described

1. Elimination
2. Equlibrium
3. Evasion

Elimination

The elimination phase employs both the innate and adaptive immune systems

They detect the presence of a developing tumor and destroy it before it becomes clinically apparent.

Equilibrium

If some tumor cell variants survive the elimination phase then they enter the equilibrium phase

Here the adaptive immune system prevents tumor cell outgrowth and also shapes the immunogenicity of the tumor cells.

Escape

Those cells which dodged elimination and equilibrium phases enter the escape phase

They emerge as progressively growing, visible tumors.

Escape mechanisms against the immune system

loss of antigens recognized by tumor-specific CTLs correlates with increased growth and metastatic potential

Loss of MHC I APCs is another mechanism so that CTLs do not recognize the cancer cells

Loss of expression of costimulators and MHC II

Evasion From NK-Mediated Attack

- NK cells normally recognize cancer cells in two ways
  - Bound antibodies to the cell surface as described previously
  - Absence of MHC class I molecules
    - MHC class I molecules activate the Killer Inhibitory Receptor (KIR) present in NK cells. These receptors release inhibitory signals and inactivate NK cells
    - Therefore cancer cells suppress antigen display by reducing only some but not all MHC class I molecules

Escape from death

Cancer cells first deactivate the death receptor signaling by mutations at various levels in the Fas receptor / apoptotic pathways
They then acquire the ability to produce soluble FasL

- This FasL does not cause apoptosis in the cancer cells because of the above-mentioned deactivation
- The FasL can bind to Fas receptors on many immune cells causing their death

Resistance to granzyme-mediated apoptosis

- By upregulation Inhibitor of Apoptosis Proteins (IAPs)
- Activating mutations of other anti-apoptotic proteins
- Deactivating mutations of pro-apoptotic proteins

Killing the immunocytes

Another form of counterattack is by secretion of Interleukin 10 (IL-10) or TGF-β

Both are immunosuppressors that kill T lymphocytes by apoptosis

TGF-β also induces apoptosis of dendritic cells and macrophages

For example melanoma cells secrete TGF-β and they bind the lymphocyte progenitor cells expressing TGF-β RII and kill the lymphocytes and the tumor cells escape killing by lymphocytes

Epstein-Barr virus infected cells release IL-10 and thus protect them from the attack by T lymphocytes

Recruiting Treg cells to escape attack from immune cells

Cancer patients often have high no of Treg cells (25-30% of CD4+ lymphocytes as against 5-10% in normal individuals)

- Regulatory T lymphocytes express the **chemokine receptor CCR4** on their surface
- Cancer cells that secrete the **chemokine CCL22** recruit the T_{reg} s to the tumor site

Regulatory T cells (T_{reg}) bind to the same antigens like CTLs and TH cells and thus prevent binding by CTLs and Helper T cells

Treg cells protect cancer cells and so if they are removed, tumor growth is reduced and antitumor immunity increases

Tumor immunotherapy

Specificity of the immune responses is an attractive concept from the point of view of therapy since current therapies affect not only cancer cells but also normal cells

Understanding at the molecular level on the mechanisms of immune responses, evasion by the tumor cells etc has helped to formulate useful therapeutic approaches

In tumor immunotherapy approaches, vaccination is used mainly to treat rather than to prevent cancer

In virally induced cancers however, vaccination could be used for prevention

Vaccination

Several clinical trials are underway to treat melanoma by vaccinating **with killed tumor cells along with adjuvants**. Tumor cell lysates with adjuvants could also be used

Purified tumor antigens or heat shock proteins are also used again in melanoma

Dendritic cells stimulated with antigens are used in melanoma and prostate cancer. Alternatively dendritic cells can be transfected with the **genes encoding** antigens and then used

Tumor cells can be transfected **with genes for cytokines or B7 genes (costimulator)** and this approach has been used to treat melanoma

Other approaches include the use of **DNA vaccines** with plasmids encoding tumor antigens

or **viral vectors** encoding tumor antigens with or without cytokines

Preventive vaccination in virally induced cancers

Tumors with viral etiology can be tackled with preventive vaccination with viral antigens or attenuated live viruses.

This approach has been used successfully in human cervical cancer with antigens from the viral coat proteins of human papilloma viruses

Similar approach is being used for hepatocellular carcinoma against hepatitis B virus

Adoptive cellular therapy

removing peripheral blood leukocytes from cancer patients
Culturing the cells in high concentrations of lL-2
Injecting the LAK cells back into the patients

Another similar approach is to isolate TILs obtained from surgical resection specimens

expand the TILs in culture with IL-2.

Human trials with TIL therapy are ongoing.

In leukemia patients, giving alloreactive T cells along with hematopoietic stem cell transplants can help to destroy the tumors

Allogeneic MHC molecules present on the recipient's hematopoietic cells, including the leukemia cells interact with the graft. However, such graft-versus-host disease may be dangerous since that may be mediated by the same donor T cells

Immunotherapy with Antobodies

A few antibodies are already in the clinic and over 100 of them are under various stages of development for their suitability to cancer therapy

opsonization and phagocytosis and activation of the complement system may be the mechanisms by which these tumor cells are cleared by NK cells and macrophages

Anti-CD30 antibodies to treat lymphomas, currently in clinical trials

Herceptin, a monoclonal antibody to HER-2/n2u/ErbB2 is already in clinical use in combination with standard drugs to treat human breast cancer

Antibodies that block the epidermal growth factor receptor (EGFR) are approved for the treatment of colorectal tumors.

Anti-VEGF antibodies are now approved for clinical use, in combination with chemotherapeutic agents, to treat metastatic tumors.

immunotoxins

Tumor specific antibodies can be combined with toxins – **immunotoxins**
Toxins are internalized along with the antibody by endocytosis
Difficulties are there with this approach. Specificity should be good.
Antibody should reach appropriate target without getting much affected by phagocytic cells
Toxins, drugs or isotopes used may also have systemic effects on normal tissues

Antiidiotypic antibodies

In B cell lymphomas clones of B cells expressing a particular antibody (**idiotype**) proliferate – **antibodies raised against these idiotypes** can target the lymphoma cells selectively
Not cost effective – requires specific antibodies for *each individual*

Graft versus Tumor

- In hematopoietic malignancies the immune cells themselves are the culprits
- Treatment involves ablation of the bone marrow to clean the system of neoplastic stem cells and transplantation of another person's bone marrow cells

- An unexpected side-effect of this treatment was the Graft versus Tumor (GvT) response that prevents the neoplastic immune cells of the host from proliferating – the only effective method for cure of Chronic Myelogenous Leukemia (CML)

The bone marrow cells removed from the patient are treated with antibodies or immunotoxins specific for tumor antigens to kill any tumor cells.

The treated marrow is transplanted back into the patient to reconstitute the hematopoietic system destroyed by irradiation and chemotherapy.

Tumor promotion and the immune system

Many cancers are associated with inflammation
Viral and bacterial associted cancers are known
Even other cancers without infectious cause also show chronic inflammation

Chronic activation of innate immune cells, notably macrophages, is characterized by angiogenesis and tissue remodeling, both of which favor tumor formation.

Innate immune cells can also contribute to malignant transformation of cells by generating free radicals that cause DNA damage and lead to mutations in tumor suppressor genes and oncogenes

mast cells, neutrophils, and macrophages, secrete soluble factors that promote cellcycle progression and survival of tumor cells

The adaptive immune system can promote chronic activation of innate immune cells in several ways, including T cell mediated activation of macrophages in the setting of persistent intracellular microbial infections. Thus, the adaptive immune system may indirectly enhance the tumor-promoting activities of the innate immune system

Summary

We discussed the origin of the concept of immune surveillance

Explained how people were not accepting that concept for a long time

How the modern experiments have come out with convincing evidences

Existence of tumor antigens and tumor associated antigens

We also discussed the concept of immunoediting

Mechanisms by which immune cells work against tumor development

Mechanisms by which tumor cells suppress the immune system

Strategies used to treat cancer using cytokines, tumor antigens and antibodies

The effects of chronic inflammation etc

END