**Tumor immunotherapy**

Conventional therapy for most tumors is chemotherapy. The hypothesis of fractional cytotoxicity of chemotherapy assumes that a certain concentration of cytostatics, applied in a certain period of time, kills a constant fraction of the population of tumor cells. In this way, each subsequent therapeutic cycle kills the same fraction of remaining cells. For example, if the cytotoxic effect is 99% per cycle, the tumor cell population of 1011 will be reduced to less than one cell in six cycles: [1011 cells] Ã— [0.01]6 <1.

It is generally known that the emergence of drug resistance (cytostatic) is a consequence of random mutations in the population of tumor cells. The possibility of de novo emergence of resistance in any population of tumor cells increases with the number of cells and the number of cell divisions.

Additional problems in the application of chemotherapy are non-selectivity and toxicity. Tumor chemotherapy relies on drugs that kill dividing cells or block cell division, however, with a severe detrimental effect on normal cells, which is accompanied by high morbidity and mortality.

How to create a tumor-specific therapy based on the biological characteristics of the tumor? Biotherapy represents the first attempt to make cancer therapy specific to malignant cells

Biotherapy involves the use of agents of biological origin or agents that modify the biological response. Synonyms: biological therapy, immunotherapy, therapy with biological response modifiers. Immunotherapy is a form of biotherapy that uses and modifies the immune system, the cells and molecules of the immune system involved in the immune response, in the fight against disease.

Three key observations that enabled the emergence of immunotherapy are the spontaneous remission of some tumors, the increased incidence of tumors in immunosuppressed individuals, and the presence of lymphocytic infiltrates in tumors. A common name for agents used in biotherapy is biological response modifiers. Today, large amounts of biological response modifiers can be made in the laboratory and used in the therapy of many diseases, such as malignant tumors, chronic HCV infection, rheumatoid arthritis...

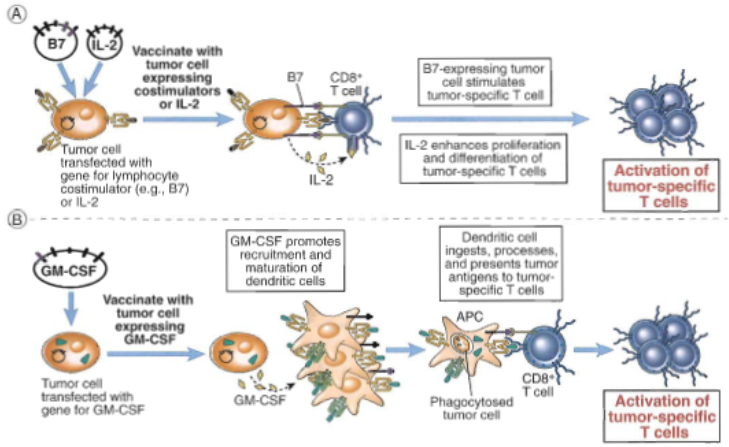
The goal of tumor treatment is the complete removal of malignant cells, and this is rarely achieved. Even in the case of successful application of conventional therapy with clinical remission, a small population of malignant cells is often left behind. Immunotherapy in such circumstances is more likely to work. The immune response to the tumor is specific for tumor antigens, and will not significantly damage normal cells. Tumor immunotherapy is aimed at increasing the host's weak immune response to tumors (active immunity) or administration of tumor-specific antibodies or cells, a form of passive immunity.

**Non-specific immunotherapy**

This type of tumor therapy aims to enhance the response to the tumor by general stimulation of the immune system. As part of non-specific tumor therapy, bacteria that strongly stimulate macrophages, such as Bacillus Calmette-Guerin (BCG) and Corynebacterium parvum, were used. In 1959, Biozzie discovered that when Bacille Calmette-Guérin (BCG), a non-specific activator of the mononuclear phagocytic system, was inoculated into mice, it slowed the growth of some tumors.

The next generation of non-specific immune-stimulators is represented by cytokines, namely IL-2, TNF-α and interferons.

Tumor cells elicit weak immune responses because they lack co-stimulators and usually do not express type II MHC molecules, so they do not activate helper T lymphocytes. In this sense, the increase in immunity is based on the artificial compensation of co-stimulators and exogenous cytokines that can increase the growth and activation of T lymphocytes, thus replacing the functions of helper T cells (*Figure 1*). An example for increasing co-stimulation of T cells is the transfection of tumor cells with genes encoding B7 costimulatory molecules.



*Figure 1.*

Cytokines constitute a new generation of non-specific immune-stimulating agents and with the use of interferon-α (IFN-α) immunotherapy was officially introduced into clinical practice. IFN has an antiproliferative effect on tumor cells, but also an immunomodulatory effect on various types of immune response. It is used either as monotherapy in low, medium or high doses, in combination with other cytokines (most often IL-2) or as part of chemotherapy. The next most commonly used cytokine, IL-2, was approved in 1992 for the treatment of metastatic renal cell carcinoma, and in 1998 for the treatment of metastatic melanoma. It is used for the production of LAK and TIL cells, but also independently, most often in combination with IFN, and recently in numerous tumors in combination with IL-12, passive mAt immunotherapy and chemotherapy. In high doses, IL-2 has pronounced toxicity in the form of hemodynamic disturbances, and for these reasons, the doses are reduced to ultra-low levels, and the application is reduced to sc administration. Despite the fact that it can lead to tumor necrosis, TNF-α is used in isolated perfusion of limbs affected by sarcomas or melanoma, because it exhibits high toxicity during systemic therapy.

**Specific immunotherapy**

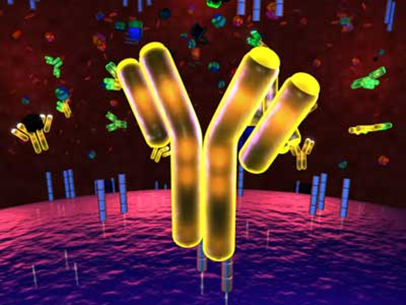
**Passive immunotherapy**

**Antibodies**

Although antibodies can react specifically with tumor cell antigens, their effectiveness in eliminating the target cell depends on other effector mechanisms of the host (phagocytosis and activation of the complement system).

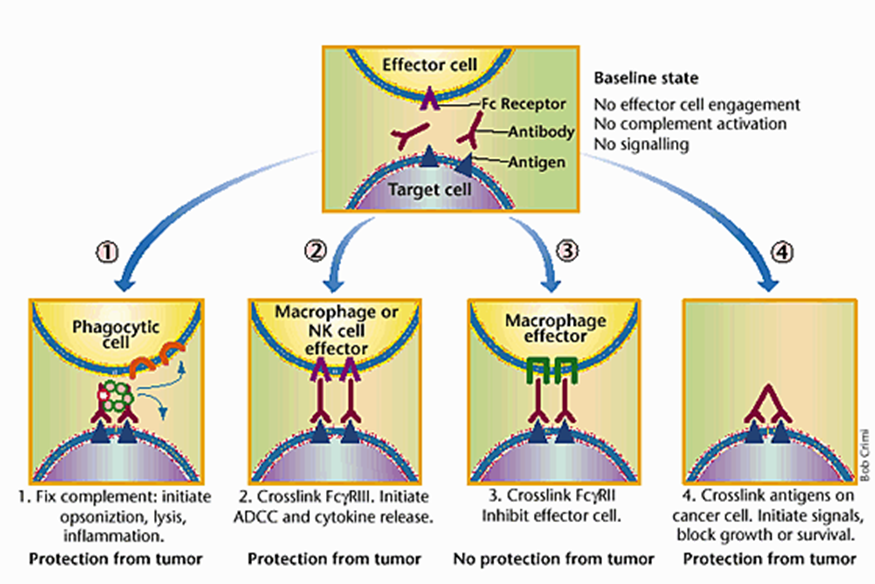
In the immune response to one antigen in the plasma, there is a mixture of different antibodies produced by various clones of B lymphocytes in response to different parts (epitopes) of the antigen, so-called polyclonal antibodies.

Patients with multiple myeloma, a monoclonal plasma cell tumor, produce large amounts of biochemically identical antibodies. Antibodies are produced by neoplastic clones, are present in blood and urine and are called monoclonal antibodies. The discovery that multiple myeloma cells produce monoclonal immunoglobulins served as the basis of the technique for producing monoclonal antibodies. The technique for obtaining monoclonal antibodies, or magic bullets, was first presented by Georges Kohler and Cesar Milstein in 1975. They developed a method to immortalize individual antibody-secreting cells by creating hybridomas that each secrete a separate monoclonal antibody of predetermined specificity. This technique is still the most common approach to obtaining monoclonal antibodies.



The technique is based on the fact that each B lymphocyte secretes a specific antibody. As B lymphocytes have a limited lifespan, it is necessary to immortalize the cells that produce the specific antibody. This was achieved by joining cells - cell fusion - hybridization of a normal antibody-producing B lymphocyte and multiple myeloma cells. Fusion is followed by the selection of newly formed cells that secrete antibodies according to the desired specificity. The cell line of immortal B lymphocytes that produce the desired antibody obtained in this way is called a hybrid, and the antibodies they produce are called monoclonal antibodies.

**Monoclonal antibodies affect tumor growth.**

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Monoclonal antibodies recognize antigens not on target cells, in this case tumor cells.

1. They bind to antigens and activate complement components (small rings between two antibody molecules), which leads to opsonization of tumor cells by phagocytes expressing receptors for complement (orange semicircles), to lysis of tumor cells and an inflammatory reaction followed by the accumulation of inflammatory cells.

2. Monoclonal antibodies bind to activating Fc receptors on effector cells leading to antibody-dependent cellular cytotoxicity (ADCC) or cytokine release.

3. Monoclonal antibodies bind to inhibitory Fc receptors (or both activating and inhibitory ones), thus inhibiting the activation of effector cells.

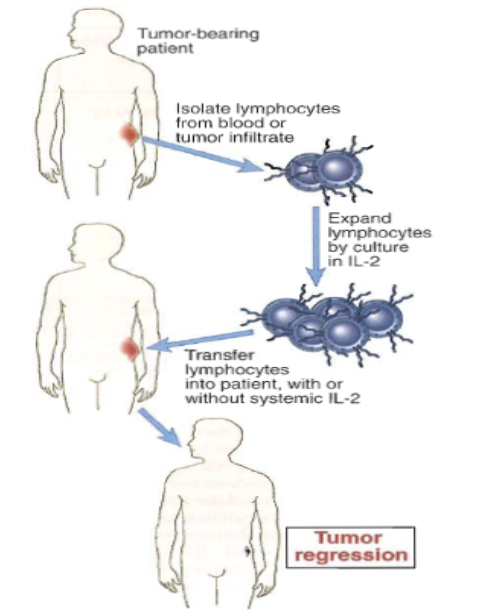
4. Monoclonal antibodies bind directly to growth factor receptors or some other signaling molecules on tumor cells, leading to cell destruction.

The use of monoclonal antibodies, specific for oncogenic products that are expressed at high levels in some tumors, has shown success and has been approved for clinical use. In order to increase the effectiveness of antitumor antibodies (given that mouse monoclonal antibodies are usually used), humanized antibodies are used: hybrid antibodies that have retained their antigenic specificity and have other characteristics of human antibodies. They are obtained by inserting a segment of DNA, which codes for antigen binding sites on murine monoclonal antibodies, into cDNA originating from a human myeloma cell. Therefore, it does not behave as foreign in the human body and does not cause an anti-antibody reaction, which reduces the usefulness of monoclonal antibodies of mouse origin. In order to increase their cytotoxicity, radioactive isotopes, cytotoxic drugs or toxins are attached to the antibodies. As a rule, a large tumor mass with increased interstitial fluid pressure, the existence of a basement membrane and tight junctions between cells make it difficult for antibodies to reach the target cells.

Antibodies specific for the CD20 molecule expressed on B lymphocytes are used to treat B lymphocyte tumors, usually in combination with chemotherapy. Since the CD20 molecule is not expressed on hematopoietic stem cells, normal B lymphocytes are restored after treatment with this antibody is completed. Other monoclonal antibodies used in malignancy therapy can block growth factor signaling (eg, anti-Her/Neu for breast tumor) or inhibit angiogenesis (eg, anti-vascular endothelial growth factor antibodies for colon and other tumors).

**Cells**

Cell therapy is based on the in vitro cultivation of mononuclear cells of a person suffering from a tumor in the presence of high concentrations of cytokines, which induces the formation of the so-called lymphokine-activated killer cell (**LAK**). LAK cells arise primarily from **NK cells**, but a number originate from **cytotoxic T lymphocytes** that have lost the property of MHC restricted antigen recognition. These cells with a far greater killing capacity than the original population are returned to the affected individual with further in vivo administration of IL-2 (*Figure 2*). Immunotherapy with autologous LAK cells gave impressive results in the regression of solid tumors in experimental animals. Use in cancer patients is generally limited to advanced cases and varies from patient to patient.



*Figure 2.*

**TIL therapy.** In an attempt to find more effective anti-tumor cytotoxic cells, Rosenberg isolated tumor-infiltrating lymphocytes in 1986. and named them **TIL** (TIL-**tumor infiltrating lymphocytes** - lymphocytes that infiltrate a tumor). In contrast to LAK cells, TIL cells constitute the most tumor-**specific CTL** capable of killing 100–1000 times more efficiently than LAK cells upon in vitro activation with IL-2. TILs are obtained by pulverizing the tumor to a single-cell suspension of mononuclear cells and tumor cells, which is then cultured in vitro for 30 to 45 days with anti-CD3 mAt and IL-2 until the required number of TIL cells is reached, which are then infused with IL- 2 are returned to the patient.

**Active immunotherapy**

Passive immunotherapy of tumors did not give sufficiently satisfactory results, so intensive work is being done on attempts to actively immunize the host against tumors. Nevertheless, one should not lose sight of the fact that the success of these attempts depends on the preservation of the host's immune system, on the one hand, and the ability of the tumor cell used as a vaccine to express a number of products of different genes, on the other hand, while for passive immunotherapy the only condition is that tumor cells cells express the appropriate target antigen. The goal of active tumor immunotherapy is to induce an immune response to the tumor that would prevent further growth and eliminate tumor cells. The identification of tumor antigens that induce an immune response enables a new approach in tumor immunotherapy. There are several categories of alteration of the genetic material of the tumor cell with a consequent increase in immunogenicity. The first includes those procedures that make the presentation more effective, the second includes those that enhance co-stimulation, and the third category aims to, by modifying the microenvironment of the tumor cell, create conditions for better recognition of tumor antigens, more effective activation and expression of the effector function of the cells of the immune system.

**Vaccination**

Vaccination with tumor cells and tumor antigens can result in enhanced immune response against the tumor. Vaccines are made from either killed tumor cells or tumor antigens. Vaccination with tumor cells and tumor antigens can result in enhanced immune response against the tumor. Attempts are underway to immunize cancer patients with purified dendritic cells, either incubated with tumor cells or transfected with genes encoding these antigens and injection of plasmids containing cDNAs encoding tumor antigens. This is the best way to activate CTL.

Cell-based and DNA vaccines are the best ways to induce CTL responses, however the limitation of vaccine treatment is that they should be therapeutic, not just preventive, and it is often very difficult to induce a strong and sufficient immune response that will eradicate all the cells of a growing tumor. The development of virus-induced tumors can be prevented by vaccination with viral antigens or diluted live viruses. This approach has been successful in people in a routine hepatitis B vaccination program, which may reduce the incidence of hepatocellular carcinoma.

Additional reading: Abul Abbas. Basic immunology. Immunotherapy of malignant tumors.