

Ambiguous Role of Immunity in Malignant Neoplasms

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ABSTRACT

Cancers have been one of the most serious illicit diseases threatening people around the world since ancient times. Their origin, growth and spread thorough the body is accompanied by specific immune manifestations, which of mechanism is in principle identical, but differ in a number of essential aspects. The participation of immunity in the cancerogenesis was not entirely clear until the beginning of the twentieth century, when not much was known about the complexity of the immune system or its functional manifestations. All that was known was that immunity was directed primarily against infectious agents. It was only later that it became clear that there is also an anti-tumor immunity, which not only destroys malignant cells and suppresses their growth, but also selects more viable and more resistant tumor cells, thereby promoting their growth and metastatic spreading of the tumor. It is just the participation of immunity in the cancerogenesis that allows the use of immunotherapy for treatment of cancer.

Key words: Neoplasm; Immunity; Surveillance; Cancer

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INTRODUCTION

The term trained immunity resulted from over 30 years of reports of enhanced immunity after reinfection in animals lacking adaptive immunity. Glucan, natural polysaccharide and a part of a PAMP family, has been used in enhancements of the innate immune response^[1,2] and is one of the key factors in trained immunity^[3]. Glucans can induce trained immunity particularly via the cells of the monocyte lineage where they influence the epigenetic and metabolic reprogramming^[4]. It is interesting to note that whereas glucan-primed monocytes respond to restimulation by developing trained immunity, they respond to lipopolysaccharide priming by tolerance^[5], despite the fact that glucan form app. 65% of lipopolysaccharide, suggesting that different PAMP can induce opposing functions.

INFLAMMATION AND TRAINED IMMUNITY

The main cellular factors participating in inflammation are monocytes and macrophages. In chronic inflammation is most of all involved natural immunity, particularly its “trained” part. Immunologists hypothesized for decades that, opposite to adaptive immunity, the natural immunity have no immunological memory. However, recent research found indisputable proofs that natural immunity can adapt and “remember”. After repeated contact with nonself molecular pattern such as Pathogen Associated Molecular Patterns, PAMP) (for review see^[6]) and Danger Associated Molecular Patterns DAMP)^[7] we can observe epigenetic modifications and changes in programming of monocytes, T lymphocytes and NK cells, resulting in increased production of nonspecific humoral factors (cytokines, interferons etc.) and increased response of natural immunity against PAMP/DAMP molecules occurring either on infectious agents or tumor cells. This developing immune response is slowly improved by involvements of additional immune mechanisms, particularly cooperating

immunocompetent T and B lymphocytes producing various humoral factors including specific antibodies. These vector can be oriented not only towards PAMP/DAMP signals, but under specific circumstances also against own healthy cells, leading to autoimmune diseases.

IMMUNE SURVEILLANCE

The idea that immune system might protect against developing cancer growth originated more than 110 years ago by Paul Ehrlich^[8]. Based on his own experience, Ehrlich assumed that tumors develop mostly in long-living organisms. Present limited knowledge of immunology, however, did not allow any experimental proof. Ehrlich suggested that it is immune system which eliminates most of aberrant cells. Only 50 years later, Sir Burnet^[9] and Thomas^[10] came with a hypothesis of immune surveillance, which was further supported by Klein's group showing the existence of specific tumor antigens (for review see^[11]), as it demonstrated the involvement of immune system in tumor development and progression. The idea of immunological (or immune) surveillance, popular in the 50s and 60s, postulated that T cell mediated immunity evolved as a specific defense against tumor cells.

Following decades, however, did not confirm the existence of immunological surveillance, as tumors were shown to occur spontaneously and in athymic, i.e. immunodeficient mice. That led many scientists to refusal of the whole idea of immune surveillance during oncogenesis. The main arguments were that the DAMP present on tumor cells represent only weak signals which do not result in induction of immune response. Other authors speculated that immune system ignores or at least tolerates tumor cells, because they developed from normal cells by blastic transformation, or that constant activation of pro-inflammatory branch of natural immunity blocks its immunoprotective functions, subsequently allowing transformation of cells and subsequent development of tumors^[12].

At the beginning of 1990s, the hypothesis of immunological surveillance was born again. The main reasons for the renewed interest were improved models of immunodeficient animals and observations of IFN- γ and its functions in rejection of transplanted tumors. Subsequently, numerous authors confirmed that role of immune system in inhibition of cancer growth^[13].

DUAL ROLE OF IMMUNITY

On one hand, immune system destroys viruses responsible for tumorigenesis. On the other hand, it also blocks the generation and development of inflammatory microenvironment, which might eliminate invading pathogenic agents, which helps the development of tumors. It is clear that immune system has dual function^[14]. It kills tumor cells via activation of immunocompetent cells activated by PAMP/DAMP molecules and thus suppresses their multiplication. On the other hand, it can support proliferation of cancer cells because it selects cells better suited to resist immune reactions. This duality results in occurrence of specific microenvironment in the primary tumor, which not only allows survival of primary cancer cells, but even makes their proliferation easier. Tumor starts to grow and metastasize into additional tissues and organs.

IMMUNOEDITING

Dual role of the immune system in cancer development was recently named as tumor immunoediting^[15-17]. The conception describes interaction of cancer and immunocompetent cells and their humoral factors. Clones of less immunogenic cancer cells are selected during

this immunoediting, which means the proliferation and expansion of cells with lower expression of antigenic structures or with expression of these structures with low antigenicity.

The process of interaction of immune system and cancer can be divided into three phases, often named as 3E: Elimination, Equilibrium, and Escape. The first phase is in fact classical immune surveillance. It involves cells and humoral factors of both native and adaptive immune response, which control and block cancer growth and results in its elimination. The main cellular effectors are NK cells and T lymphocytes, both activated by pro-inflammatory cytokines produced by macrophages, stromal cells and malignant cells. NK cells infiltrate tumor and kill its cells either directly or via IL-12 and IFN- γ . The whole phase can be further divided into four smaller parts - immunological recognition, production of anti-cancer chemokines, production of reactive oxygen and nitrogen radicals and differentiation of cytotoxic T lymphocytes (NK-T cells). However, not all malignant cells might be destructed during this phase and the tumor moves into equilibrium.

The second phase represents the longest period of the entire immunoediting. Cancer cells escaping the destruction during the first phase, do not have immunogenic phenotype, but are genetically instable, which include common mutations and changes caused by epigenetic mechanisms. Malignant tissue is controlled by the immune system and remains in a steady, homeostatic state. Genetic and epigenetic changes result in production of less immunogenic clones. The total numbers of tumor cells are still low and usually clinically below recognition. However, the selection still occurs resulting in appearance of new variations. Some of which can be completely resistant to immune reactions and they slowly move into the last phase of the immunoediting, into Escape. As a proof of the Equilibrium phase are cases where transplanted organ from a donor who successfully survived cancer, developed new cancer in otherwise healthy recipient^[18].

Phase Escape represent situation, when a specific microenvironment is fully established in the primary tumor with subsequent uncontrolled growth of tumor. At this stage, the tumor can be clinically observed. Tumor cells constantly divide and move into additional tissue and organs. The tumor site is infiltrated by immunocompetent cells (both T and B lymphocytes), which is considered to be the action of specific adaptive anti-cancer immunity. Additional cells involved in this process are monocytes/macrophages, NK and NK-T cells, dendritic cells, neutrophils and some endothelial cells which secrete growth factors and chemokines necessary for neovascularization, as the tumor growth depends on a steady supplementation of nutrients. The immune system is oriented toward several more or less malignant cell types, but spares resistant cells selected during the equilibrium phase. In addition, several other mechanisms helping cancer cells escape reach of immune detection exist, including loss of expression of major histocompatibility complex I (HLA-A, HLA-B and HLA-C), which was found in 90% of all tumors^[19]. Tumor microenvironment is immunologically highly complex and generally immunosuppressive, but at the same time offers sophisticated protection of malignant cells. As soon as tumor reaches this phase, the only solution for final elimination of the cancer is the use of nonimmunological means and procedures, such as surgery, radiotherapy, chemotherapy, hormonal therapy or biological treatment.

BIOLOGICAL TREATMENT

The fact that immune system is involved in all three immunoediting

phases allowed the use of several novel ways to treat cancer. These procedures are commonly names biological treatment or targeted treatment. However, it is really an immunotherapy. It aims to block the division of cancer cells and their subsequent spreading into additional tissues and organs, hopefully resulting in their final liquidation. In contrary to more traditional radiation and chemotherapy which also affect healthy cells resulting in several serious side effects, biological treatment is selective and is oriented towards stimulation of the immune system resulting in stronger and more specific immune reactions.

Radiotherapy and chemotherapy can also partly affect biological treatment. It is known that in numerous patients the targeted irradiation leads to spontaneous elimination of cancerous legions even outside the irradiated tissue. It was Mole who in 1953 suggested the term “abscopal effect”, so he could distinguish between direct effects of irradiation and its secondary effects in distant tissues^[20]. Irradiation, similar to targeted chemotherapy, induce adaptive immune reaction against malignant cells in a process called “immunological cell death”. In this process, contrary to common apoptosis, cellular content containing pro-inflammatory DAMP, heat shock proteins, calreticulin and other stress factors is released^[21,22].

Biological treatment is considered to be the most recent way how to treat not only cancer, but also several additional disease including autoimmune diseases. It is, however, important to note that biological treatment of tumors by use of infection was documented already in ancient Egyptian papyrus app. 2 600 years BC. Based on so called Ebers papyrus^[23], the tumors were treated by surgery followed by compresses resulting in development of infection and inflammation followed by tumor regression. During the following millennia, the physicians forget these procedures completely. It was Virchow who around 1860 introduced this hypothesis about combination of immunity with cancer growth into modern medicine, which was based on Virchow’s observation of infiltration of tumor sites with leukocytes. Targeted infection of tumors in cancer treatment has been used in the second half of the 19. century, when German physicians W. Bush and F. Feldman independently observed that streptococcus-mediated acute erysipiel of the skin leads in some patient to elimination of tumors^[24,25]. Later, W.B. Coley treated some types of cancer by a mixture of bacterial species^[26]. The content of so called Coley’s toxin was formed by produces of *Streptococcus pyogenes* and *Serratia marcescens*^[27]. Coley, who probably did not know the work of Bush, can be considered to be a father of immunotherapy^[28,29]. His extract is known as Coley’s vaccine or Coley’s toxin. Coley used his material with mixed results in treatment of inoperable sarcomas and metastases to long bones^[30,31]. Despite the risks of using two dangerous pathogens, the attempts to revive this option are still alive^[32].

Since the beginning of 20th century was described the observation showing the lower occurrence of tumors in patients with tuberculosis^[33]. Animal models later confirmed this hypothesis by findings of anti-cancer effects of the BCG vaccine and in 1972 was this vaccine successfully used in treatment of a bladder carcinoma^[34]. Since then, this treatment became standard. Intravesical application of BCG vaccine is now one of the most successful immunotherapeutic treatments of tumors^[35].

The strategy of biological treatment includes wide variety of possible treatments, including use of specific population of effector cells (macrophages, NK cells and T lymphocytes), application of specific humoral factors such as cytokines and interleukins, and numerous specific and humanized monoclonal antibodies, oriented towards specific tumor antigens^[36]. Additional possibilities include

the application of bioactive molecules isolated from plants such as polyphenols, terpenoids, carotenoids, phytosterols and flavonoids^[37,38]. Additional option is the use of nonspecific biologically active substances such as enzymes or oligonucleotides^[39-41] and biologically active nanoparticles^[42] including liposomes, PLGA nanoparticles, Au nanoparticles and polymeric particles, and dendrimers, all used noninvasively by microinjections^[43].

This list is by no means complete. Immunotherapy is currently one the most studied ways for cancer treatment and new and new possible molecules and new protocols are being developed. It is important to note that it is important to find specific type of molecules where their molecular structure allows strong binding to the tumor antigens, but also consider immunological status of every patient, which is based on the MHC antigenic spectrum. It means that the characterization of the cancer growth in the same organ or the same tissue is different among individual patients.

The situation is further complicated by the fact that very little is known about the possible involvement of microbiome, which was until recently rather overlooked question. Microbiome, its composition and activity is different in each individual. In addition, it keeps changing based on numerous factors such as age, health conditions and nutritional habits. This all means that each and every patient might and most probably will react differently on any given version of the biological treatment.

Observations of the novel cancer treatment via stimulation of immune system by blocking surface protein CTLA-4^[44] are extremely interesting, as this protein is expressed on the membrane of activated T lymphocytes and acts as inhibitor of their activation. This team developed an antibody blocking CTLA-4, which later resulted in development of ipilimumab, currently used in treatment of late stages of melanoma. Similar action using PD-1 protein is using different mechanism^[45], but antibodies based on this observation are currently used in cancer treatment. Both options represent new, revolutionary approach, because they do not aim on cancer cells, but on effector cells. Glucan with known interaction with PD-1 immediately become an important addition.

CONCLUSION

This short review was aimed to describe the novel, often unknown possibilities of biological treatment of diseases including cancer. It is possible to conclude that our current knowledge of the immunotherapy of cancer still remains at the level we used to have several decades ago of hormones and vitamins. We believe that it is a time to radically change the procedures offered by immunotherapy. The use of various PAMPs, particularly glucan known for activation of trained immunity, might represent one of the novel approaches for using immunotherapy.

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