REVIEW

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CANCER IMMUNOTHERAPY: MECHANISM OF ACTION

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Summary

The prospect of effectively treating cancer patients with immunotherapy is now becoming a clinical reality. This is a consequence of clinically relevant and successful results obtained by applying monoclonal antibodies against immune checkpoint inhibitor receptors and chimeric antigen receptor (CAR) T cell therapy to patients with otherwise lethal cancers. Despite this success, only a limited number of cancer types and a subset of cancer patients currently respond to these therapies. Efforts are now made to increase the number of cancer types and patients that can be treated successfully. This is an overview of the various approaches taken to this end.

KEY WORDS: *cancer immunotherapy, immune checkpoint inhibitor, cancer vaccine, chimeric antigen receptor (CAR), adoptive cell therapy.*

IMUNOTERAPIJA TUMORA: MEHANIZAM DJELOVANJA

Sažetak

Klinička imunoterapija onkoloških bolesnika je postala klinička realnost. To je posljedica klinički relevantnih i uspješnih rezultata dobivenih primjenom monoklonskih protutijela protiv imunoloških kontrolnih molekula funkcije inhibitornih receptora te T-limfocita s kimeričnim antigenskim receptorima. Usprkos tim uspješnim i obećavajućim rezultatima, rezultati su postignuti samo protiv dijela tumora i opet u samo dijela bolesnika se postiže terapijski odgovor. U prikazanom radu dan je kratak pregled tih raznih imunoterapijskih pristupa.

KLJUČNE RIJEČI: *imunoterapija raka, inhibicija molekula kontrolnih točaka, tumorske vaksine, kimerični antigenski receptor (CAR), adoptivna stanična imunost.*

INTRODUCTION

Until recently, standard cancer treatment of oncological patients comprised surgery, chemotherapy, hormonal therapy, targeted therapy and radiotherapy but not immunotherapy. Immunotherapeutic approaches were based on the assumption that tumor cells can be antigenically distinct from normal cells and that the host's immunological system can recognize this antigenic difference and consequently should mount an anti-tumor immune response against autologous tumor cells. In clinical testing and applications these various immunological approaches were usually ineffective or, when they were effective, which was rare, were not easily and broadly applicable and therefore not in routine use (1-8). Some of these tumor antigens which can be recognized on autologous tumor cells can be unique for particular tumor cells, i. e. tumor specific. They might be produced as a consequence of somatic gene mutations in tumor cells in the course of their malignant cell transformation or by *new* gene formations in the places of cromosomal translocations.

According to their generation process these antigens are labeled *neoantigens*. In some cases over or aberrantly expressed normal molecules from non-mutated genes on tumor cells can act as tumor antigens. They then form the so-called group of tumor-associated antigens (TAAs). Examples of these is HER-2 molecule or cancer/testis antigens, respectively (9,10).

Immunological approaches and interventions against tumors are conceptually diverse and technically-technologically of various degrees of complexity. These approaches are undergoing dynamic change because new scientific knowledge about the components of the immune system and how they function at the cellular and molecular levels is continuously accumulating. They aim to evoke by the autologous tumor cells or tumor antigens in various forms the antigenic activation of the cells of the immune system, predominantly of the adaptive immunity, and to consequently achieve autologous tumor cell destruction. The advantage of such an evoked immune reaction is specificity, a relatively low level of possible side-effects and the formation of the immunological memory which enables a fast reactivation of the immune response in the case of reappearance of the same tumor cells or tumor antigens (1-8). Since these immunological approaches are diverse, there are also several possibilities for their classification (11). One option is to position them in the patients' disease course. Thus they can be positioned as adjuvant therapy, with preventive intent, or as curative therapy in the case of patients with a metastatic disease. Another option is to divide them with respect to active and passive immunity. In active immunity procedures the aim is to activate the patients' immune system, in the past most frequently by using various vaccines. These vaccination approaches were based on the successful results obtained with experimental animals and their tumors. The majority of these approaches, sometimes technologically relatively simple, were usually therapeutically effective on experimental animals. When tried on cancer patients, they were, unfortunately, not effective. As a source of possible tumor antigens, killed autologous or alogeneic tumor cells were used or can be used (for example vaccine canvaxin) (12). Also frequently used are synthesised tumor antigens usually in the form of peptides with various adjuvans and DNA molecules encoding tumor antigens. More recent vaccination approaches also include autologous dendritic cells which are professional antigen presenting cells. An approach frequently adopted in the case of these cells is to first generate such cells *in vitro* from the patients' peripheral blood cells, then to incubate or stimulate them with potential tumor antigens, and finally to reinfuse them back into the patients (for example vaccine sipuleucel-T) (13). Also, in tumor cells or dendritic cells various gene encoding potential tumor antigens or immunostimulatory cytokines can be inserted. More recent approaches use genetically modified oncolytic viruses (for example talimogene laherparepvec) (14) or humanized monoclonal antibodies against cell surface regulatory molecules involved in regulatory feedback circuits (check-point molecules) (15,16). From the functional point of view these regulatory molecules on lymphocytes can be conceived as either stimulatory or inhibitory receptors (1-8,11,15,16).

CHECKPOINT BLOCKADE

Antigenic activation of adaptive immunity cells (T- or B- lymphocytes) after antigen recognition through the antigen-specific receptor (the so-called *first signal*) includes, besides cytokine participation, cell-cell regulatory interactions with cell membrane bound costimulatory or coinhibitory molecules on other cells (the so-called *secod signal*). Through their antigen-specific receptor T-lymphocytes recognize antigens in the form of peptides as a molecular complex with the molecules of the major histocompatibility complex on other cells. These other cells can be antigen-presenting cells (such as dendritic cells) or target cells which, after their recognition, are going to be lysed by effector T-cells. Cell-cell interactions of the *second signal* are also molecular specific in terms of receptor-ligand interactions. In these interactions the molecules present on lymphocytes function as receptors, while the ligand molecules are on other cells (antigen presenting or target cells). The net-effect of this second signal on antigen stimulated lymphocytes can be their additional activation or inhibition of the initiated activation. These inhibitory interactions or signals have a physiological regulatory function. They form a negative feedback mechanism that aims to prevent a too strong immune activation or reactions, since this can cause, as a side effect, damage to the

body's normal cells (autoimmune reactions). Because of this regulatory function, these membrane bound regulatory molecules are also called checkpoint molecules and they in fact function on lymphocytes, as has already been mentioned, as stimulatory or inhibitory receptors. The antibodies against inhibitory receptors can block these negative feedback signals with the net-effect of lymphocyte (re)activation. Examples of second-signal molecules include the stimulatory CD28 receptor molecule on T-lymphocytes and ligand molecules CD80 (B7-1) / CD86 (B7-2) on dendritic cells. Upon T-lymphocyte activation through these molecular interactions, what comes to be later physiologically expressed on these T-lymphocytes is the molecule CTLA-4 (cytotoxic T-lymphocyte antigen 4; CD152), which is a molecular homolog of the molecule CD28. The function of this subsequently expressed molecule CTLA-4 is to initiate the negative feedback mechanisms and thus prevent a too strong immune activation and reaction. The molecules CTLA-4 and CD28 are then in mutual competition for the molecules B7, but the molecules CTLA-4 have a higher affinity than the molecules CD28 for interaction with the molecules B7. Consequently, what follows after the initial T-lymphocyte activation is the physiological braking of their activation. It should be noted that there exist other molecules on T-lymphocytes and their ligands on other cells which also form second activatory signals and are currently being investigated. For example, the molecule OX40 (CD134) on T-lymphocytes and the ligand OX40L (CD252). The first phase 3 clinical results in studies that used blocking monoclonal antibodies against inhibitory receptors on T-lymphocytes appeared in 2010 and such studies continue to be performed. The first successful results were obtained in patients with metastatic melanoma in which the humanized monoclonal antibody ipilimumab against the inhibitory receptor molecule CTLA-4 was used. Later results that were even more successful and that pertained to several additional cancer types were obtained with the use of monoclonal antibodies against the inhibitory receptor PD-1 (programmed cell death protein 1; CD279) on T-lymphocytes or against the ligand molecule for this receptor on tumor cells (molecule PD-L1) (1-8,15-17).

Through this blockade of the negative feedback mechanisms, the activation of T-lymphocytes (anti-CTLA-4 blockade) or the reactivation of anergized T-lymphocytes (anti-PD-1 or anti-PD-L1 blockade) can be obtained, which in clinical settings can result in tumor disease control, tumor regression and even a cure for patients suffering from certain types of cancer (melanoma, non-small cell lung cancer, renal cancer, Merkel cell carcinoma, urothelial carcinoma, head and neck carcinoma) (15-17).

PASSIVE IMMUNITY APPROACHES

Examples of immunotherapeutic approaches that are based on passive immunity involve injections of monoclonal antibodies against various molecules in cancer patients. Some target molecules for these monoclonal antibodies can be cell membrane receptor molecules for growth factors (anti-EGFR, anti-HER2) where the applied monoclonal antibodies prevent or inhibit cancer cell stimulation. In cases where the applied monoclonal antibodies are expected to evoke complement activation, facilitate tumor cell phagocytosis or activate antibody-dependent cell-mediated cytotoxicity (ADCC), some of these target molecules can be tumor antigens. Moreover, active cytotoxic drugs or isotopes can be linked to monoclonal antibodies. Such conjugation with monoclonal antibodies can lead to a higher specificity of linked molecules against tumor cells and less side-effects. Examples include trastuzumab-emtansin and 131 I-tositumomab combinations. Patients having metastatic melanoma or renal cell carcinoma can also be treated with immunostimulatory cytokines, such as interferon-alpha (IFN-α) and interleukin-2 (IL-2). Patients suffering from metastatic melanoma have also the adoptive cell therapy (ACT) treatment option, which is a highly personalized cell cancer therapy involving the administration to the cancer-bearing patient autologous immune cells with direct anticancer activity. These immune cells (T-lymphocytes) were previously isolated from the patients' peripheral blood or their tumors, cultured *in vitro*, checked for reactivity, expanded *in vitro*, and reinfused back to the patients. In some patients with melanoma this approach can lead to a durable, complete regression. Owing to the technical complexity of this approach and the period of time required to obtain the required number of cytoxic T-cells *in vitro*,

which is a minimum of several weeks, this method is used in no more than a few institutions in the world. Currently also widely conducted are clinical studies involving the use of similarly produced and *in vitro* grown T-lymphocytes with inserted genes as chimeric antigen receptors (CARs, Chimeric Antigen Receptors). These CARs lymphocytes are predominatly used in patients with hematological neoplasms and in which lymphocytes with CARs are directed against the CD19 molecules (1,3,6,7,11,17-19).

CONCLUSION

In conclusion, it can be expected that as a result of the application of monoclonal antibodies that block molecular interaction in immunological negative feedback mechanisms and their relatively simple applicability, immunotherapy (immunooncology) will become a part of the standard everyday therapeutic arsenal in the treatment of oncological patients with certain tumor types. Underway are intensive investigations into potential predictive parameters for the application of these monoclonal antibodies since not all patients with „responsive“ tumor types are going to benefit. Account should also be taken of the possible side-effects of these monoclonal antibodies, which are usually autoimmune in nature. Medical personnel should have knowledge of the possible side-effects, their recognition and treatment. It can further be expected that these monoclonal antibodies will be tested in combination with anti-tumor vaccines, which were not proven to be effective, in order to improve these vaccines' chances of inducing effective anti-tumor immunity (1,3,15-25).

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