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Adoptive CD8+ T-cell Therapy Possibilities

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Abstract

Adoptive T-cell immunotherapy is one of the targeted immunological methods that have received widespread use in the treatment of oncological diseases. The most used cell platform is CD8+ cytotoxic lymphocytes, in addition to CD4+ and NK cells. The method has the specificity of monoclonal antibodies and minimal side effects. Both cytotoxic T-cell (CTL) and cells containing the chimeric antigen receptor (CAR)-receptor are used. Immunotherapy cells can be obtained from both the patient (autotransplantation) and the donor (allotransplantation). Attempts are being made to create T-cells that do not require HLA-matching and are not taken from the patient so that they can be simpler and cheaper to use. Over time, the scope of application of cytotoxic T cells in the treatment of not only oncological but virus-associated diseases has been expanding. However, the methodology still does not have standard generally accepted protocols, and their development is a big task for the future.

Keywords: Adoptive T-cell Immunotherapy; Chimeric Antigen Receptor; Cancer; Lymphoma; Epstein - Barr virus; Cytomegalovirus

Abbreviations: CTL: Cytotoxic T-Cell; CAR: Chimeric Antigen Receptor; ACT: Adoptive Cell Transfer; TILs: Tumor-Infiltrating Lymphocytes; NCI: National Cancer Institute; PBMCs: Peripheral Blood Mononuclear Cells; CIK: Cytokine-Induced Killer; LAK: Lymphokine-Activated Killer; CTL: Cytotoxic T-Cell; VST: Virus-Specific T-Lymphocytes; CPT: Cell Preparation Tubes; FBS: Fetal Bovine Serum; ALL: Acute Lymphoblastic Leukemia; HD: Hodgkin Lymphoma; NPC: Nasopharyngeal Carcinoma.

Introduction

T-cell immunotherapy is one of the forms of adoptive cell transfer (ACT). It is divided into two main types according to the method of constructing cell specificity. The first approach is to use T-cells that are already specific to the disease-related Ag.

At present in cancer treatment, T-cell therapy with autologous tumor-infiltrating lymphocytes (TILs) is successfully applied [1]. The history of the T-cell immunotherapy method began from tumor-infiltrating lymphocytes of patients with metastatic melanoma, and it was the earliest trials in the National Cancer Institute (NCI) in Bethesda, Maryland, the USA in 1988 [2]. With the help of such lymphocytes, breast cancer, cholangiocarcinoma, melanoma, and others are treated [1,3,4]. However, the lymphocytes of some tumors are not active, but somewhat suppressed and do not perform their function. The treatment of epithelial cancers with

TILs remains problematic [1]. A surgically removed metastasis or the primary tumor can be used to obtain lymphocytes. In some cases before T-cells extraction from a patient, tumor Ag vaccination to enhance their specificity, as well as injection of IL-2 for cell expansion in different dosing regimen for various diseases is used [5].

Antigen-specific T cells can be isolated from a patient's blood, for example, in the case of many oncological, especially lymphoproliferative diseases or persistent viral infections. Isolation of the specific T-cells from the total number of peripheral blood mononuclear cells (PBMCs) is a separate and challenging task requiring subsequent removal of fluorescent labels. A more natural way is to isolate all PBMC cells and strengthen their specificity in a laboratory way, while the cells remain unlabeled [6]. Of these non-specific cells, the required subsets (CD8+ or CD4+) are expanded by special cell cultivation conditions. The primary therapeutic function is performed by CD8+ cells. The literature uses many similar names for such cells: cytokine-induced killer (CIK), lymphokine-activated killer (LAK), cytotoxic T-cell (CTL), virus-specific T-lymphocytes (VST) in case of viral infection treatment. To improve the long-term survival of these cells in the body *in vivo* presence of CD4+ cells among the injected cells is desirable [5]. Also, CD4+ cells play an essential role in effective antitumor immunity and contribute more durable immune-mediated tumor control than pure CD8+ T cells [7].

The second approach is engineering T-cell specificity de novo. This task can be achieved by introducing the chimeric antigen receptor (CAR) into the CD8+ lymphocytes, and this approach combines the selectivity of antibodies and the cytotoxic potential of T cells [8]. CAR structure is antigen-recognition domain derived from the sequences of monoclonal antibodies of the extracellular part and intracellular signaling module. A transmembrane module to anchor and a hinge module for preservation the molecular distance necessary for a biological reaction are located between them. CAR-T cells have a tremendous potential since it is possible to introduce several specific receptors in cell culture at the same time. It is very valuable for the treatment of cancer, whose antigenic specificity can be different in a single tumor, as well as for many viral infections that can be activated simultaneously under immunodeficiency conditions. For added safety, suicide gene system may be included into cells [9].

Immunotherapy cells can be obtained from both the patient (autotransplantation) and the donor (allotransplantation). Auto T-cells are preferable since their introduction a priori implies minimization of side effects, and there is no need to waste time on the selection of the HLA-matched donor. In order to facilitate the search for donors for cellular immunotherapy, in some scientific centers, lists of advance healthy donors, willing to blood donation, with known HLA have been created.

The next logical step was the creation of cell banks, where ready-to-use specific T cells from healthy donor cells are stored frozen. To date, there are a small number of cell banks exist for cells specific for different cancer and viral antigens like EBV, CMV, adenovirus infections [10]. At the same time, work is underway to create universal, less personalized cytotoxic T cells with the desired specificity that can be mass produced and used as easy as biopharmaceutical drug products [11].

General Principles of Preparing T-Cells for Adoptive Immunotherapy

First of all, it is necessary to isolate peripheral blood mononuclear cells (PBMC), from the blood of a healthy donor or the patient in the case of autotransplantation [12]. First of all, it is necessary to isolate PBMC from the blood of the donor or the patient. The volume of the required number of cells, and, accordingly, the withdrawn blood varies from study to study. For example, for the treatment of nasopharyngeal carcinoma $5x10^6$ PBMCs isolated from whole blood were used [13]. In such cases, enough venipuncture. PBMCs from the blood can be isolated by traditional methods such as Ficoll or Percoll gradients or newer cell preparation tubes (CPT) and SepMate [14]. If treatment requires multiple infusions and large volumes of injected cells, mononuclear cells are isolated by the method of apheresis, followed by counter flow elutriation on Elutra, where they are distributed by density [15].

Further cultivation conditions can be lead to expanding mainly CD4+ or CD8+ cells. Fetal bovine serum (FBS) has been previously used for ex vivo culture of T cells; currently, serum-free culture media X-VIVO15 and AIM-V are preferred [12]. For T-cell expansion, co-activation of CD3 and CD28 receptor is necessary, in combination with the addition of cytokines (mainly IL-2) [16]. Coactivation is achieved by various methods, including monoclonal antibodies to OKT3 (the component of CD3), anti-CD3 and anti-CD28-coated magnetic beads, dendritic cells, feeder cells in the form of γ-irradiated allogeneic PBMC or allogeneic EBV-transformed LCL, and so on [12].

The third step is enhanced or constructed de novo cell specificity. For cytotoxic lymphocytes, without CAR

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receptor an antigen must be added to the cell culture for a few days or weeks. The antigen can be added in the form of a generic pool of several dozen peptides that represent distinct dominant T-cell epitopes with various HLA restrictions and can be purchased ready to use [17]. A culturing method for several weeks with cells of the required specificity (certain cell lines, autologous specific dendritic cells) is also used. For the introduction of the CAR receptor in a T-cell, it is required to add it to the medium too. Viral vectors are widely used, of which lentiviral, with the RNA interference integration mechanism, are preferred. After several days the vector is washed out [18]. Some researchers have used the method of naked plasmid DNA and electroporation, which is effective regarding a safe, reproducible and affordability, but takes much time [12]. In recent years, the Sleeping Beauty system with transposon/transposase elements received development [19].

The last step before the injection of cells to the patient is their activation and enhancement of cytotoxic properties. The main activators of T-cells are cytokines IL-2,7,12,15,21 [12], of which IL-2 is the most important, it is applied in all studies on this topic. IL-15 is second in importance, and there are suggestions about his superiority over the IL-2, whose function it partially duplicates [20]. Doses of cytokines for cell activation are not standardized, each laboratory acts peculiarly, and at this stage, active development of protocols for T-cell activation *in vitro* is underway.

Possibilities of Method in the Treatment of Oncological Diseases

The adoptive CD8+ T-cell therapy method showed good clinical results in lymphomas treatment. Anti-B19 CAR-T cells or CD20, CD22 are often used in the treatment of B-cell lymphomas, depending on the phenotype of the tumor [21]. Some researches show that the results of the CAR-T cell trials in B malignancies, including NHL and acute lymphoblastic leukemia (ALL) are impressive with about 90% remission rates in cases with ALL [22,23]. In another study, seven patients with mantle cell lymphomas received CD20-specific CAR-modified T cell infusion, where only two patients had continued complete response and one had a partial response [24].

There are some studies concerning the Hodgkin disease [25,26]. Some of them has shown that EBV-CTLs, especially those expressing both native and chimeric receptor, persist in EBV+ Hodgkin lymphoma (HD) to produce complete tumor response [26]. Based on the success of treating PTLD, several studies have investigated the potential of activated EBV-specific CTLs for immunotherapy to cause regression of advanced nasopharyngeal carcinoma (NPC) where patients achieved the encouraging outcomes that the most of the metastatic lesions disappeared [27-29].

However, other researchers noted that adoptive immune cell therapy with tumor-infiltrating lymphocytes (TILs) needs a further investigation in advanced melanoma cases [30]. Therapeutic responses to treatment of advanced stage melanoma of nine women and one man included a complete remission, a partial remission, 2 stabilization, and 6 progressions [4]. According to some studies concerning the breast cancer, the TILs can outstandingly improve clinical outcomes in all subtypes of breast cancer [3]. The number of TILs infused vary from study to study, and even for one disease there is no common standard.

Possibilities of Method in the Treatment of Virus-Associated Diseases

Although cellular immunotherapy has a great potential in the treatment of chronic non-oncological viral diseases, it exists mainly at the stage of study and is not entirely clinically implemented.

In EBV-associated diseases treatment, it is desirable to target T-cells simultaneously to main latent (EBNA-1, LMP-2A,B) and lytic (BZLF-1, pp65) antigens. There are ready-to-use peptide pools to enhance the specificity of cytotoxic T cells, for example MACS GMP EBV (Miltenyi Biotec), or vector for CAR-T AdE1-LMPpoly. There are a couple of reports on the use of adoptive immunotherapy in the treatment of EBV-associated chronic diseases in patients with hemophagocytic lymphohistiocytosis, active EBV infection, post-transplant lymphoproliferative disease. In early reports from 2002, 8 of 8 patients with moderately severe chronic active EBV infection showed the distinct improvement of the clinical presentation [31]. Since then, the method has become more widespread. For instance, in the treatment of 49 patients with the post-transplant lymphoproliferative disease after bone marrow transplantation, 19 of them were administered EBV-specific T-killers, and 68% were finally able to achieve complete or partial remission [32]. Moreover, other authors have noted the positive experience of using this method in post-transplant lymphoproliferative disease [33].

In a study conducted on patients with immunosuppression after transplantation, the treatment was carried out using T-cell lines specific for five viruses

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simultaneously (EBV, CMV, herpes virus type 6, adenovirus, BK virus). Reproduction of the injected cells occurred *in vivo* in this case. Only in 14 of 48 patients, the cells retained the response to all five antigens. Despite this, as a result, a significant clinical result was achieved, the viral load was reduced in the majority of patients; as well as no side effects were noted [34]. A similar approach to the multidirectional action of specific T-lymphocytes can be successfully applied in the case of chronic fatigue syndrome, associated simultaneously with EBV and human herpes viruses 6 and 7 types.

Antiviral T-cell therapy (including EBV-specific) was carried out in 26 patients with primary immunodeficiencies of different etiologies, here partial or complete response to therapy was observed in 81% of cases [35]. An unambiguously positive effect from the introduction of EBV-specific T-lymphocytes, accompanied by minimal side effects, was demonstrated in a large study conducted on 114 patients [36]. L.P. McLaughlin and co-workers in their review on cellular immunotherapy for EBV infection, notes some problems related to the practical side of the implementation of such method, the technical complexity of its application, the lack of a standardized technique, as well as low accessibility for the public [37].

The problem of CMV infection resistant to ganciclovir therapy is very important. The use of peptide mixes derived from full length pp65 and IE-1 for cytotoxic T lymphocytes is possible. CAR-T can be directed against the CMV encoded glycoprotein B (gB), but there is no unambiguous agreement with regard to the antigens used for the therapy. In patients after transplantation of hematopoietic cells with persistent, resistant to antiviral therapy of CMV infection, T-cells showed an excellent clinical result. The use of cellular immunotherapy can lead to the partial restoration of specific immunity (immune reconstitution) [38,39]. However, in some cases, infected cells are resistant to CAR-T therapy. Degranulation of cytotoxic cells occurs, but it is ineffective [40]. This is probably due to the strong immunosuppressive effect of the virus on the microenvironment [41], and CMV properties to inhibit suicide of infected host cells [42].

Conclusion

The cellular immunotherapy can be considered as a feeble experimental method for the treatment of numerous cancer diseases, virus-associated diseases, especially for patients with immunodeficiency. The method has great promise, including due to the ability to achieve multitargeting from a cytotoxic cell, as well as due to minor side effects associated mainly with additional cytokine injections to the patient for stimulation of T-cells *in vivo*. To speed up further development of adoptive immunotherapy, it is necessary to develop standard protocols for cell selection, cell activation and dosing to achieve a therapeutic effect. Given the excellent clinical effect, overcoming these challenges is a matter of time.

Conflict of Interest: The authors declare no conflict of interest.

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