

SELECTIVE T CELL CO-STIMULATION MODULATORS, AN IMPROVED THERAPEUTIC APPROACH OVER CALCINEURIN INHIBITORS IN IMMUNOSUPPRESSIVE DRUG THERAPY

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Abstract

Transplant tolerance is an important issue in the clinical practice. Successful transplant depends on the ability to block the rejection of the transplanted organ. Currently, improving transplant tolerance is accomplished using immunosuppressive agents, but having also many toxic effects on the host body. So far, the conventional immunosuppressive therapy with calcineurin inhibitors such as cyclosporine or the newer tacrolimus, is the most common way to treat and prevent rejection; but, since the appearance of the fusion proteins, it became clear that the transplanted patients might have a chance for life-on-therapy but without the many toxic effects of the conventional immunosuppressive therapy.

This new drug category - co-stimulation blockers - have the potential to eventually replace current therapies to become the new standard therapy of immunosuppression after organ transplantation.

Rezumat

Toleranța transplantului reprezintă o problemă importantă în practica medicală. Succesul operației de transplant depinde de capacitatea de a bloca rejectarea organului transplantat. În prezent îmbunătățirea toleranței transplantului se realizează prin folosirea agenților imunosupresivi care însă prezintă și numeroase efecte toxice pentru organismul gazdă.

Până acum, terapia imunosupresivă convențională cu inhibitori de calcineurină cum ar fi ciclosporina sau mai noul tacrolimus este cel mai comun mod pentru tratarea și prevenirea rejectiei; însă, de la apariția proteinelor de fuziune, a devenit clar că pacienții cu transplant ar putea avea o șansă de terapie pe viață, dar fără atât de multe efecte toxice ale terapiei convenționale imunosupresive. Această nouă categorie de medicamente - blocante ale costimulării - promit să devină noul tratament standard în imunosupresia după transplantul de organe.

Keywords: T cells, rejection, immunosuppressants, calcineurin inhibitors, belatacept, abatacept.

Introduction

Advances in transplant surgery depend on the progress in immunosuppressive therapy. Currently, the most widely used immunosuppressive

drugs used to prevent acute or chronic rejection of the transplanted organ are cyclosporine A and tacrolimus.

The price paid for blocking immune reaction against the transplanted organ are serious infections, occurrence of cancers which are normally prevented by a competent immune system and serious reactions such as renal failure. Various combinations of immunosuppressive drugs have been shown to be just as toxic without offering additional benefits. In these conditions the pharmaceutical industry is looking for new immunosuppressive agents [1].

As T cells play an important role in the occurrence of rejection, studies in recent years have offered a better understanding of T cell activation. New drugs targeted to these cells have been developed – generic named “co-stimulation modulators” of T cells. These cells are used in some types of transplant and autoimmune diseases whose mechanism is known, such as rheumatoid arthritis (RA) or psoriasis.

T cell response – main target of new immunomodulators

Now there are available a series of drugs that target the actions of T cells in the immune response generation. T cell response involves multiple levels of activation, some of them being potential targets for a new immunomodulators drugs generation. Thus, T cell activation requires a first signal from the antigen-presenting cell (APC) - macrophages, dendritic cells, B lymphocyte B - by antigen binding to a receptor on the surface of antigen-specific T cells. The second signal, non-specific, is represented by the connection established between the receptors on the surface of T cells, cluster of differentiation 28 (CD28), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), and various ligands, such as B7-1 B7-2 proteins, from the APC. Molecules B7-1 and B7-2 are also known as the CD80 and CD86 respectively. Without achieving these connections - T cells generate an inappropriate immune response – anergy state. This second stimulus is called co-stimulatory and is targeting a new category of drugs called immunomodulators co-stimulatory activators.

A third stimulus in activating T cells is considered to be the cytokine, interleukin 1 (IL-1), tumor necrosis factor alfa (TNFalfa), which amplify the T helper cell response [2].

Activated CD4⁺ T cells release interferon and interleukin-17, which stimulate the activity of other immune cells leading to the release of chemical mediators of inflammation like IL-1 and IL-6, tumor necrosis factor (TNF-alpha). TNF-alpha and IL-1, play an important role in joint injury in rheumatoid arthritis [1].

At T cell surface there are other B7 receptors - such as the CTLA-4 cell-surface molecules (also known as CD152). CTLA-4 appears only after T cell is activated, competes for B7 (both CD80 and CD86) having a higher receptor affinity than CD28 and has an inhibitory effect on T cell proliferation. CD28 is constitutively expressed on the surface of T cells whereas CTLA-4 is expressed only at 24 hours after activation with a peak at approximately 48-96 hours. As the

capacity of CTLA-4 to bind to CD80 and CD86 is higher than of CD28 the activation of the T cell is stopped [1, 2].

T cells activation via nuclear factors of activated T cells (NFAT) - calcineurin - Interleukin 2 (IL 2) pathway

Another pathway involved in the T cells activation is *via* interleukin-2. The molecular events involved are: secretion of IL-2, expression of IL-2 receptor, IL-2 binding to IL-2 receptor. [3] The nuclear factors of activated T cells (NFAT) are part of a family of transcription factors very well controlled at the level of nuclear import. In fact, their discovery came from the finding that nuclear extracts of antigen-stimulated cells contained factors that recognize the antigen response element of interleukin 2 promoter. Though, NFAT located in the cytoplasm, functional inactive and hyperphosphorylated in resting cells whereas translocating and nuclear NFATs are dephosphorylated. The mobilization of NFAT to the nucleus is strictly dependent on the elevation of intracellular calcium concentration following T-cell antigen receptor (TCR) stimulation. Upon the termination of calcium signaling, NFATs are rapidly rephosphorylated and exported from the nucleus. [4] A key to understanding how elevated calcium levels could promote the nuclear import of NFATs came with the analysis of the major immunosuppressant drugs cyclosporine A and tacrolimus (FK506). Both drugs were known to block calcium-dependent signaling in T cells by binding through their intracellular receptors called immunophyllins to calcineurin, a calcium-calmodulin-dependent-phosphatase [4]. Thus, came the idea that calcineurin could be of interest in NFAT activation and nuclear shuttling [5]. Calcineurin is a calcium calmodulin dependent phosphatase (called protein phosphatase 3, known also as PPP3C). This phosphatase, calcineurin, is activated by calcium-bound calmodulin. The association of calmodulin with calcineurin induces a structural change allowing the access of the substrate at the phosphatase active site. Thus, it is induced the dephosphorylation of NFAT and a conformational switch of NFAT that allows the nuclear import of NFAT through importin receptors [6]. In this way, NFAT becomes activated, is imported by the nuclear importins and activates the transcription of IL-2 which goes to T-cells activation. It is worth to note that in children with acute lymphoblast leukemia at the onset it might occur a defect in the signal transduction pathway of the T-cell-receptor/CD3 complex, resulting in inefficient expression of interleukin-2 (IL-2) receptor α -chain (CD25) [7].

Calcineurin inhibitors (CNI): mechanism of action and nephrotoxicity

The current immunosuppressive therapy in renal transplantation is mainly based on cyclosporine and tacrolimus. These compounds also known as calcineurin inhibitors (CNI) have immunosuppressive properties by inhibiting the calcium-calmodulin-dependent phosphatase, calcineurin. In order to bind and inhibit calcineurin phosphatase function, they need to bind to specific intracellular receptors: cyclophilin for cyclosporine and FKBP12 for tacrolimus [8, 9]. By inhibiting calcineurin function, they impair the nuclear translocation of NFAT, disrupt the IL-2 expression and further T cells activation [10].

Despite their mechanism of action that revolutionized the transplantation, a major concern is raised by their toxic effects, especially by their nephrotoxicity which hamper their benefit in long-term kidney graft and patient survival. Myers et al [11] were the first who recognized the damaging effects of cyclosporine on renal function such as: reversible decrease of glomerular filtration rate (GFR) and irreversible renal functional damage. The effects of tacrolimus on renal function were similar to those of cyclosporine, they are also confirmed and they are indistinguishable on renal allograft biopsy [12, 13, 14].

The CNI nephrotoxicity can be classified into two types: acute CNI nephrotoxicity which is reversible and chronic CNI nephrotoxicity which is irreversible. Several factors are the cause for the acute nephrotoxicity induced by calcineurin inhibitors: increased release of vasoconstrictors such as endothelin, decrease of vasodilators (such as nitric oxide (NO), by inhibiting NO synthesis), activation of the renin-angiotensin system, augmentation of the vasoconstrictor effect of angiotensin II, attenuation of cyclooxygenase 2 (COX-2) expression, tubular injury. The chronic nephrotoxicity is the major disadvantage of the current immunosuppressants being the cause for the late allograft kidney dysfunction and failure and it is a combination between the hemodynamic changes and the toxic effects on the tubular cells [15]. De Mattos *et al* [16] also note that nephrotoxicity is among the most important side effects of calcineurin inhibitors and that is a dose-limiting significant effect. They comment that both cyclosporine and FK506 induce reversible acute nephrotoxicity caused by the vasoconstriction of the afferent arteriole and also a chronic nephrotoxicity which is an irreversible interstitial fibrosis that develops in some patients after approximately 6 to 12 months of drug therapy. They raise also the problem of differential diagnosis between the chronic graft rejection and the induced nephrotoxicity. Though it may be impossible to differentiate if they occur simultaneously, there are some findings that must be used after performing a kidney biopsy for the final diagnosis. These findings are arterial lesions such as intravascular fibrinoid, necrosis, cellular infiltration, proliferation, and sclerosis in the case of chronic graft rejection and afferent arteriolar lesions with nodular focal or circular protein deposits in the tunica media, tubular vacuolization and new onset or progressive arteriolar hyalinosis in the case of drug induced nephrotoxicity [16].

Naesens et al confirm that tacrolimus has a lower potential of nephrotoxicity than cyclosporine and that tacrolimus has a higher benefit than cyclosporine regarding the preservation of renal function [15]. Morales et al bring the results from a long-term European multicentric study which shows that tacrolimus patients tended to have lower serum creatinine levels, had better creatinine clearance and exhibited an excellent graft function [17]. Though, the results of the comparison between tacrolimus and cyclosporine benefits with regard to renal function remain controversial. Mayer et al show that there was no significant difference in renal function when comparing trials of patients on tacrolimus and cyclosporine, though after transplantation the use of tacrolimus was associated with higher levels of serum creatinine than cyclosporine [18]. Stoves et

al studied the effect of immunosuppressants conversion on the development of chronic allograft nephropathy. It was shown that the reduced dose of cyclosporine added to mycophenolate mophetil was superior to that of tacrolimus to cyclosporine or to standard immunotherapy [19].

Abatacept and belatacept - new drugs that cause co-stimulation blockade.

In recent years, two drugs involved in the co-stimulation blockade have passed Phase III trials: the first one, abatacept, is already marketed under the trade name Orencia[®], having as major indication rheumatoid arthritis treatment. The second one, belatacept, is waiting the approval to be marketed and is reserved for the renal transplantation.

Abatacept (CTLA-4-Ig) is a fusion protein consisting of the extra cellular domain of the cytotoxic T-lymphotropic associated antigen 4 and the crystallizable fragment (Fc) of immunoglobulin 1 (IgG1) [2]. CTLA-4 binds to CD80 (B7-1) and CD86 (B7-2) of the APC thus blocking activation of T cells through CD28. CTLA4-Ig is 100 fold less potent at inhibiting CD86-dependent co-stimulating than CD80 [20]. Abatacept binds to CD80 and CD86 on APCs, leaving CD28 without stimulus. preventing positive co-stimulation signals required for optimal T-cell activation. As a result, in RA is suppressed the proliferation of auto reactive T cells and restored the balance between self-tolerance and autoimmunity [1]. CTLA4-Ig was proven to induce long-lasting tolerance only in few models of heart and kidney transplantation. CTLA4-Ig is not capable to ensure an indefinite donor-specific graft survival after a single administration at the time of transplant [2]. Today it is considered that selective modulation with CTLA4-Ig achieved a selective modulation of B7/CD28/CTLA-4 co-stimulation, leaving other immune co-stimulatory way open. It is assumed that is achieved only a modulation of T cells activity and not their immunosuppression. Used in psoriasis, CTLA4-Ig showed that although there is an inhibition of T cells is not prevented immune response to other antigens [21,22]. Clinical improvement of T-cell mediated autoimmune condition after abatacept administration recommends the use of this drug in psoriasis and rheumatoid arthritis but not in transplants. Insufficient CD28/B7 interaction blockade seems to be responsible for the insufficient immunosuppression in a transplant.

Belatacept is a second-generation variant of cytotoxic T-lymphocyte antigen (CTLA)-4 immunoglobulin (Ig), in which 2 amino acid substitutions (L104E and A29Y) reduced the dissociation rate of the compound from its receptors, both CD86 and CD80. LEA29Y binds 4 fold more avidly to CD86 and 2 fold more to CD80 compared to CTLA4-Ig. As a result, the inhibition of T-cell is 10 times higher [20].

In 2005, Vincenti et al published the data from a phase II trial in which it was compared the daily cyclosporine administration in kidney transplant recipients with two different dosing schedules of belatacept infusions. They showed that belatacept maintained low rates of acute rejection, and preserved renal function as indicated by higher GFRs among patients receiving the intensive and less-intensive belatacept regimens than among those receiving cyclosporine. A larger percentage of patients in both the intensive and the less-intensive belatacept therapy groups were free from pathologic changes of chronic allograft nephropathy after 12 months than in the

cyclosporine-treated group (29% and 20% vs 44%, respectively). Also, the safety reports show that the frequency of the adverse events was at least 5% points higher in the cyclosporine group than in either belatacept group and included leucopenia, anemia, edema, hypertension, urinary tract infection, hypokalemia, hypomagnesemia, acidosis, tremor, hypertrichosis, and diabetes mellitus; the frequency of infection was similar among the groups: 73 % in both belatacept groups and 75 % in the cyclosporine group [22,23].

The results of the phase III BENEFIT study were published recently, indicating that after 12 months, both belatacept regimens, the less-intensive one and the more-intensive one had similar patient/graft survival *versus* cyclosporine and were associated with superior renal function. Though, a problem appeared in belatacept-patients; these patients experienced a higher incidence of acute graft rejection [24].

In his editorial, published in 2010, Kaplan recognizes the promise offered by the new protein based immunosuppressant belatacept because of its precision of action and mechanism, lack of toxic effects other than those of immunosuppression itself. He reminds that the main basic results from the two studies named BENEFIT and BENEFIT-EXT, following almost the same design, were similar: belatacept-treated patients had a better renal function after 12 months after transplantation than CNI-treated patients and also presented fewer atrophy and fibrosis phenomena [6].

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