

# From bone marrow transplantation to complex biological therapy

Do transplante de medula à terapia biológica complexa

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## ABSTRACT

The major complications after bone marrow transplant are related to opportunistic infections or to graft-versus-host disease. Today, there is a wealth of information associated with bone marrow transplantation and new treatment approaches have been proposed to overcome these complications. Behind these new therapies, such as adoptive transfer of T cells or mesenchymal stem cell infusions, there is significant basic research to support these clinical advances. Most of this knowledge has derived from the development of animal models and intense laboratory work to test and confirm hypotheses. There is no doubt that basic research is still necessary to better understand the basis for clinical outcome improvements.

**Keywords:** Bone marrow transplantation; Biological therapy

## RESUMO

As principais complicações após o transplante de medula óssea estão relacionadas a infecções oportunistas e doença do enxerto contra hospedeiro. Atualmente, existe muito conhecimento sendo adicionado ao campo de transplante de medula óssea e novas terapias foram propostas no sentido de superar essas complicações. Por trás dessas novas terapias, como a transferência adotiva de células T ou a infusão com células-tronco mesenquimais, existe um desenvolvimento significativo de pesquisa na área básica que corroborou esses avanços clínicos. Muito desse conhecimento foi derivado do desenvolvimento de modelos animais e trabalho intenso em laboratório, que possibilitou testar e confirmar tais hipóteses. Por isso, não existem dúvidas de que a pesquisa básica é necessária como suporte para o melhor desempenho da clínica.

**Descritores:** Transplante de medula óssea; Terapia biológica

## INTRODUCTION

Several new approaches to decrease infection after bone marrow ablation and subsequent bone marrow transplantation, as well as graft versus host disease have been proposed with excellent clinical outcomes<sup>(1,2)</sup>. On

the frontline fighting infections we have adoptive T-cell transfer therapy, which is based on the isolation and expansion of primed T-cells against specific viruses or a broad T-cell pool expansion that has been proposed with varying degrees of success<sup>(3,4)</sup>.

Nonetheless, there are several details associated with establishing such treatment protocols. First, we need to understand the biology of the virus of interest and the proteins that should be used for the development of virus-derived immunogenic peptides. It is common to use a peptide library and select peptides from the entire sequence of the chosen protein. Subsequently, one must investigate which one(s) will be able to trigger T-cell responses.

Most of these peptides, also known as epitopes, should be shared among the different virus subspecies, implying that T cells with such specificities will recognize and protect against multiple virus serotypes, but not disregarding HLA phenotypes. In addition, it is important to identify both CD8 and CD4 T-cell epitopes, meaning HLA class I as well class II-restricted epitopes that address the variety of alleles in the population<sup>(5)</sup>.

There is also important information that must be gathered about T cell priming, specifically about antigen presenting cells (APC). The balance between immunity and tolerance is largely determined by the conditions of antigen presentation. Antigen distribution, localization, dose, persistence and the activation status of the APC are all believed to have an important function in the immunity<sup>(6)</sup>.

Usually, systemic and persistent exposure of T cells to antigens in the absence of co-stimulation leads to T cell tolerization. While, some lymphocytic choriomeningitis virus (LCMV) strains can cause rapid and overwhelming infections in T lymphoid organs leading to exhaustion of the antiviral CTL responses, the slow replication LCMV strains can induce long lasting immunity<sup>(7)</sup>.

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However, the type and level of co-stimulation triggered during the first encounter between antigen and T cells are determining factors for the immune response outcome. This depends mostly on the activation status of the APC that will present the antigenic peptide to the T cells. The co-stimulatory state of APC is promoted by CD4 Th cells, in particular by interactions between CD40L on Th cells and CD40 on the APC<sup>(8)</sup>.

Besides CD40/CD40L, the interaction between DCs and T cells results in up-regulation of receptor-ligand pairs of the TNF superfamily, including CD137L, CD95L, CD27, CD30, RANK-L or TNF-related activation-induced cytokine (TRANCE), lymphotoxin, TNF-related apoptosis-inducing ligand, and members of the TNFR superfamily including CD137 and RANK<sup>(9,10)</sup>.

The interaction of these receptor-ligand pairs induces downstream signaling via TNFR-associated adapter molecules. This signaling upregulates adhesion and co-stimulatory molecules, enhances cellular interactions between DCs and T cells, regulates survival of either the APC or the T cell, and leads to the production of T cell stimulatory cytokines such as IL-12, IL-1, and IL-6 and/or down-regulation of T cell inhibitory cytokines such as IL-10. The result is the intensification and maintenance of the subsequent immune response<sup>(11-14)</sup>.

Understanding the role of CD4 and CD8 T cells during the viral infection pertaining to antiviral studies, the requirements for CD4 differ according to virus type, but frequently, defense against intracellular pathogens involves neutralizing antibodies and CTL responses, both dependent on CD4+ T cell help<sup>(15)</sup>.

On the other hand, mesenchymal stem cell (MSC) infusions aim at modulating the exacerbated immune responses such as alloresponse. The alloresponse can be divided into two components. The first is the allorecognition, which refers to the recognition of antigens expressed on the surface of non-self origin cells, by the lymphocytes. The second part is the immune effector mechanisms generated by this recognition process. T-cells recognize these antigens either directly or after being processed like conventional antigens by APC, in what has been termed indirect presentation<sup>(16)</sup>.

CD4+ T cells play a central role in coordinating the immune response to alloantigens by secreting cytokines to, amongst other things, attract effector cells such as CD8+ T cells and being able to interact with B cells that will secrete highly specific alloreactive antibodies.

Also important are MSC which apparently act on the immune cells in a variety of ways; some authors have demonstrated their impact on T cells<sup>(17-21)</sup>, and others the impact on dendritic cells<sup>(22-25)</sup>. Most studies describe human MSC as MHC class I positive and MHC class II negative; however, the evidence is controversial. The data conflicting with these findings may represent

different stem cell lineages or maybe this differential expression refers to biological differences among the donors, but we are not ruling out the possibility of it being a result of the process of cell-cell transfer<sup>(26)</sup>. The expression of MHC class I by MSC is important because this expression protects MSC from NK cell depletion mechanisms. For instance, a major function of NK and NK-like cells is to kill tumor cells that have down-regulated class I<sup>(27)</sup>.

HLA-G is an MHC-like protein that is known to protect the fetal allograft against NK-mediated rejection. This protein has been shown to bind to the two major inhibitory NK receptors: KIR1 and KIR2, and to inhibit NK killing<sup>(28, 29)</sup>. However, no studies of HLA-G expression by MSC have been published to date.

Recently, controversial data has been published regarding the immune modulation capacity of MSC, and Waterman et al. have suggested the existence of two MSC phenotypes as an explanation for it, a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. These phenotypes would be generated after stimulation of specific toll-like receptors (TLRs), meaning that TLR4-primed MSC, or MSC1, mostly create pro-inflammatory mediators, while TLR3-primed MSC, or MSC2, express mostly immunosuppressive mediators<sup>(30)</sup>.

Complex biological therapy is still an open field for research and the precise mechanism of action of these therapies on the immune system is not fully understood. The combined use of cell-based therapies and other biological products such as soluble cytokines, cytokine receptors, and humanized monoclonal antibodies - although highly efficient in some patients - need to be further investigated in humans and in animal models to help the development of customized treatment with both medical and economic gains.

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