

# DENDRITIC CELLS - A POTENTIAL TOOL FOR TOLERANCE INDUCTION IN ORGAN TRANSPLANTATION

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## REZUMAT

Celulele dendritice (CD) reprezintă un sistem complex de celule cu echipament unic de prezentare a antigenelor, ce controlează atât inițierea, cât și modularea răspunsurilor imune. Studii efectuate extensiv au demonstrat rolul crucial al CD nu numai ca și declansatori ai activării imune primare, dar și în inducția toleranței imune. Deși sunt necesare încă studii pentru a înțelege pe deplin biologia CD, manipularea artificială a acestora, pentru supresia față de un antigen specific a sistemului imun, oferă un potențial imens în terapia viitoare a rejecției alogene în transplantul de organe sau a bolilor autoimune. În această scurtă trecere în revistă, principiile actuale ale generării de așa-numite CD "tolerogene", precum și rezultatele diferite, obținute utilizând astfel de celule în modele experimentale, vor fi puse în evidență.

**Cuvinte cheie:** Celule dendritice, toleranță imună, imunitate înnăscută, rejecție alogenă, răspuns imunitar al celulelor T

## ABSTRACT

Dendritic cells (DCs) represent a complex system of uniquely-equipped antigen presenting cells that control both initiation and modulation of the immune responses. Extensive studies documented well the crucial role of DCs not only as triggers of the primary immune activation but also in the induction of the immunological tolerance. Although further research will be needed to fully understand the DC biology, artificial manipulation of DCs, for antigen-specific suppression the immune system, offers a tremendous potential as future therapy of allograft rejection in organ transplantation or autoimmune disease. In this brief review, the actual principles for generating the so-called "tolerogenic" DCs, as well as different outcomes reported after using such cells in experimental models, will be outlined.

**Key words:** Dendritic cells, immunological tolerance, innate immunity, allograft rejection, T-cell response.

Over the past two decades, significant progress has been made in the area of organ transplantation resulting in long lasting graft acceptance. This has been mainly possible by introducing new immunosuppressive drugs, better recipient selection, and improved postoperative management of the transplanted patient. However the persistence of chronic rejection as well as the side effects of immunosuppression (e.g. chronic infection, cancer, and atherosclerosis) are two main problems still limiting the long-term success of vascularized organ transplantation. In an effort to solve these problems, a tremendous amount of research has been undertaken during the last ten years to achieve permanent immunologic tolerance, resulting in permanent acceptance of the allograft without any immunosuppressive regimen.

## IMMUNOLOGICAL TOLERANCE – THE TRANSPLANTATION CONCEPT

In organ transplantation, tolerance is defined as a state in which absence of any efficient reaction of rejection against an antigen and the cellular structures bearing it is encountered.

This state can be only achieved by inducing the potent T cell to become *anergic* (T cell unable to respond to a second challenge), *ignorant* (T cell areactive) or to undergo *clonal deletion* (T cell undergoes apoptosis) after "facing" the foreign antigen presented by APCs (antigen presenting cells). Studying the mechanism of central and peripheral tolerance, a physiological process which acts to elude a possible activation of autoreactive T cells, numerous experimental studies have documented the central role played by dendritic cells (DCs) in this process.

In the context of transplantation, it is well documented that DCs are key initiators of the immune response leading to acute rejection. Moreover, antigen presentation by recipient DCs to T cells during indirect allorecognition, have been suggested to be partially responsible for inducing chronic rejection. Given this

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circumstances, manipulation of DCs to induce tolerance rather than immune activation has become a very attractive idea to induce permanent transplantation tolerance.

## **ORIGINS OF DENDRITIC CELLS**

Dendritic cells originate from bone marrow progenitors and have a crucial role in induction and regulation of the immune response, considered today as one of the most potent immunoregulatory cells ever discovered.

These cells are characterized by their characteristic “dendritic” morphology, having extensive cytoplasmic extensions, low density and the ability to present antigens to T cells. Although they were first described in peripheral lymphoid organs of mice,<sup>1</sup> cells with the same features were isolated from many non-lymphoid tissues – skin, lung, intestinal tract - and peripheral blood of both rodents and man.<sup>2-4</sup> Thus, the DC family includes lymphoid organ DCs (thymus, spleen), epidermal DCs – also referred to as Langerhans cells, veiled DCs (afferent thoracic duct and lymphatic) and interstitial DCs (connective tissues of non-lymphoid organs). Newly, there is increasing evidence that DCs may originate from both a myeloid (DC1 type) and a lymphoid precursor (DC2 type), the latter being typically resident in the thymus, where they appear to have immunoregulatory functions.<sup>5</sup>

## **DENDRITIC CELLS AS INITIATORS OF TRANSPLANT REJECTION**

After a solid organ has been transplanted, the recipient’s body will recognize the vascularized graft as an “intruder” and therefore will mount a strong adaptive immune response against it.

The main players in this process are the recipient T lymphocytes which, recognizing the donor MHC complex antigens (Ags) and donor derived Ags presented on self MHC molecules will activate and clonally expand, finally migrating into the allograft and destroying it.

As already mentioned, T cells recognize the antigens through their interaction with antigen presenting cells (APCs). The APCs are able to (i) efficiently take up Ags of both endogenous or exogenous origins (ii) process them in 10 to 20 amino acid peptides and finally (iii) present them on peptide - MHC complexes to antigen-specific T cells.

Dendritic cells (DCs) are considered to be one of the most potent professional APCs, holding a dual role in both initiating and silencing acquired immune

responses. Thus, they are key effectors, mainly responsible for triggering the allospecific immune reaction after transplantation. DCs exhibit the ability to efficiently stimulate naive CD4<sup>+</sup> and CD8<sup>+</sup> T cells, being able to transport the internalised Ags from the periphery into the lymph node areas where they will be presented to allospecific T cells in a MHC-restricted fashion.

In the normal “steady” state, DCs reside in the peripheral tissues including solid organs, where they continuously sample antigens. In the context of inflammation triggered by transplant surgery and necrosis due to ischaemia/reperfusion injury, a series of mediators such as proinflammatory cytokines (IL-1b, TNF-alpha, IFN-g) and cyclooxygenase metabolites (PGE2) are released locally within the DC microenvironment. This triggers the maturation of DCs with subsequent upregulation of chemokines - CCR7, CCR3, CCL21, CCL19 - and adhesion molecules - ICAM-1, ICAM-3 - both having a crucial role in DC migration. Furthermore, inflammation-induced oligomerization of TNFR family molecules (CD40, RANK) and ligation of Toll-like receptors (TLR-4, TLR-9) on the DC surface induce transcription of important nuclear factors (NFkB) required for DC activation, and T - cell priming in the lymph nodes. Additionally, DCs through maturation, upregulate on their surface costimulatory molecules - CD80, CD86, ICOS-L - all necessary for proper interaction with T cells and the establishment of the DC : T - cell “immune synapse”.<sup>6,7</sup>

In the context of organ transplantation, DCs present donor-derived Ags in both direct and indirect manners. After the graft has been revascularized, donor-derived DCs habituating the transplanted organ migrate into the recipient’s circulation as “passenger leukocytes”. After reaching the T - cell areas of peripheral lymphoid organs they will present allogeneic MHC molecules to resident T cells, through a mechanism known as the *direct pathway of allorecognition*. Alternatively, recipient DCs migrate into the allograft together with the initial inflammatory infiltrate and acquire donor antigens, derived from donor necrotic or apoptotic cells, by phagocytosis. These alloantigens will be processed and presented to T cells as donor MHC-derived peptides bound to self-MHC molecules, a phenomenon referred to as the *indirect pathway of allorecognition*. Nevertheless, the percentage of T cells recognizing donor-derived peptides presented through the indirect pathway is reduced compared with the direct pathway. Thus, the direct pathway of allorecognition is considered today to be the most important triggering mechanism of

rejection. This concept is supported by animal experiments showing that kidney, heart or pancreas allografts are tolerated longer in allogeneic recipients when purged from “passenger leucocytes”.

Through antigen presentation by DCs, the recipient’s T cells clonally expand and acquire helper functions. This will lead to generation of an allospecific immune response, having as a consequence the migration of immune cells into the graft tissues with subsequent destruction of the transplanted organ.

### **TOLEROGENIC DENDRITIC CELLS AS A TOOL TO TARGET ALLOGRAFT REJECTION**

During the last decade, a major step forward has been made by understanding the development, differentiation and function of dendritic cells (DCs). DCs are organized in distinct subpopulations that differ in their lineage affiliation, surface molecule expression and biological function.

As described above, dendritic cells have also been shown to hold the key role in initiating a vigorous immune response against transplanted allografts, which ultimately leads to rejection. In addition, extensive research is currently being performed on how to generate DCs which induce tolerance instead of immunity.

### **PHARMACOLOGIC MANIPULATION FOR GENERATION OF TOLEROGENIC DENDRITIC CELLS**

There is a variety of pharmacologic agents which have been demonstrated to inhibit DC maturation, thus making them acquire tolerogenic properties.

*Vitamin D3* has been shown to inhibit DC maturation, its T-cell stimulatory function and its capacity to secrete IL-12. This compound exerts its action by binding to a specific receptor (VDR – vitamin D receptor) expressed on immature DCs. VDR signaling induces the maintenance of the immature phenotype in monocyte-derived DCs in-vitro. In addition, inhibition of DC maturation by Vitamin D3 in mice lacking the VDR receptor was absent. The VDR-dependent mechanism is associated with inhibition of upregulation of CD86, CD80, CD40, impaired capacity of DCs to stimulate allogeneic T cells and inhibition of IL-12 leading to T-cell unresponsiveness. When Vitamin D3-conditioned donor-derived DCs are injected in-vivo into allogeneic recipients, they induce prolonged survival of skin grafts.

The immunosuppressive *15-deoxyspergualine*,

was reported to prevent graft rejection and induce tolerance in murine models of allotransplantation. Lately, it has been demonstrated that, when used in its more potent and non-toxic form – LF15-0195 (LF) – it has a tolerogenic effect upon immature DCs. When DCs are exposed in-vitro with LF before maturation with TNF-alpha or LPS, they fail to upregulate their maturation markers (MHC II, CD86, CD80, CD40) and show an impaired IL-12 production. *Calcineurin inhibitors (Cis)* represent another class of compounds, which by inhibiting the IL-2 production of allospecific T cells after transplantation, block their activation and induce immunosuppression. Recently, it has been reported that during activation, DCs also transiently produce IL-2 and that this property seems to be crucial to their full maturation and ability to induce immune responses. When Cyclosporine A is added in-vitro, following maturation of DCs, impairment of both IL-2 and IL-12 secretion is observed at DCs level, but with no influence upon the DC final differentiation. Yet, mature DCs with impaired IL-12 and IL-2 production have been shown to selectively polarize T cells to become Th2 cells favouring tolerance rather than immunity.

When cultured in-vitro in the presence of Mitomycin C, rat mature monocyte-derived DCs, seem to lose their allostimulatory capacities by drug-induced downregulation of important costimulatory and adhesion molecules on the cell surface (CD80, CD86 and ICAM-1). Mitomycin C-treated DCs are not able anymore to induce allogeneic T-cell activation in-vitro. Moreover, when donor derived DCs treated with Mitomycin C are administered into the portal vein of allogeneic recipients, a significant prolongation of cardiac allograft survival is noted.<sup>11</sup>

### **BIOLOGIC MANIPULATION FOR GENERATION OF TOLEROGENIC DENDRITIC CELLS**

#### **1. Blockade of costimulation**

It has been documented that CD80 and CD86-B7 family of receptors - which are both expressed on mature DCs, can deliver either stimulatory or inhibitory signals to T cells by binding to CD28 or CTLA4 (CD152) respectively. The CD80/CD86:CD28 signal induces IL-2 production, T-cell proliferation and differentiation and expression of anti-apoptosis genes. On the other hand, the binding of CD152 to the B7 receptors, renders the T cells unresponsive by inducing either strong inhibitory signals or sequestration of B7 costimulation. In the same time, the ability of CD152 to stop T-cell activation varies with the grade of TCR

engagement and the maturation state of DCs.

Interference with B7/CD28 costimulation by using anti CD80 or anti CD86 monoclonal antibodies has been shown to disable allogeneic T-cell proliferation in-vitro and prolong allograft survival in-vivo.<sup>12</sup> Nevertheless, according to recent findings in-vivo, the survival of allotransplants in recipients treated with recombinant CD152 (CTLA4Ig – fusion protein produced from the human CTLA4 with the Fc portion of IgG<sub>1</sub>) or anti B7 antibodies, seems to finally depend on the level of CD80/CD86 expression on DCs and on the CD152 concentration reached in the DC: T cell microenvironment. In this regard, models of islet transplantation in large animals showed that, although systemic administration of CTLA4-Ig resulted in prolongation of allograft survival, the protein's "tolerogenic" concentration was transient and additional therapy was necessary to maintain tolerance.<sup>13</sup> Thus, the CD80/CD86:CTLA4 pathway is essential for induction of tolerance but not enough to maintain it.

The engagement of CD40 expressed on DCs with CD40L (CD154) expressed on T cells is also considered to be one of the critical costimulation pathways required for T-cell activation and differentiation during alloimmune responses. Antigen recognition through TCR: MHCII complex induces expression of CD40L on T cells, which in turn, by binding to CD40 will up-regulate the expression of CD80 and CD86 on DCs. Thus, CD154:CD40 interaction plays a dual role in T-cell activation, by providing itself a primary positive signal and additionally inducing DCs to express B7 receptors, which by binding to CD28, will generate a secondary T-cell activation impulse. Nevertheless, T-cell activation in-vitro through B7:CD28 signalling is not dependent on CD154:CD40L interaction, the latter one having only a synergistic effect.<sup>14</sup>

In-vivo experiments have shown that preconditioning graft recipients with the monoclonal anti-CD154 antibody prevented allograft rejection and development of alloantibodies.<sup>15</sup>

## 2. Genetic engineering to obtain tolerogenic dendritic cells

In-vitro generated DCs can be genetically engineered to express transgenes which encode pro – apoptosis molecules (CD95), immunoregulatory proteins (indoleamine 2,3-dioxygenase), Th2 response driving cytokines (IL-10, TGF-b) or DC : T-cell interaction inhibitors (sCTLA4Ig). Based on results obtained in experimental models it can be concluded that genetically engineered DCs are a promising approach to induce allograft acceptance.

Among all transfection methods available for gene delivery into dendritic cells, replication-defective adenoviruses have been the most commonly used vectors. Both murine and human DCs derived in-vitro from different progenitors were adenovirally transduced with CTLA4Ig, TGF-b or IL-10 transgenes, and then further cultured in the presence of allogeneic T cells. DCs expressing all transgenes at a time have shown the capacity to induce T-cell unresponsiveness in-vitro. In murine transplantation models, transgenic DCs expressing CTLA4Ig prolonged allograft survival and showed a marked downregulation of CD86 ligand.<sup>16</sup>

Dendritic cells transduced with "killer proteins" such as CD95L (FasL), by engaging the CD95 on the T –cells, can induce allospecific tolerance by driving the activated T-cell clones to apoptosis. Supportive theories for this mechanism as a physiologic counterpart exist in immune privileged sites (e.g. eye, testis) where Fas-mediated apoptosis is responsible for defence against T-cell mediated attack. When administered in-vivo, transgenic DCs expressing CD95L are inducing a slight prolongation of allograft survival and inhibit alloreactive T-cell proliferation in-vitro.<sup>17,18</sup>

Lately, transgenic DCs expressing indoleamine 2,3-dioxygenase (IDO), an enzyme involved in the tryptophan catabolism, have been obtained and shown to induce suppression of allogeneic T-cell proliferation in-vitro.<sup>19</sup>

In a transplantation scenario, using IDO – expressing donor DCs, tolerance may be induced by selective downregulation of the immune response, silencing only the allospecific T-cell clones. Nevertheless, although the results obtained with transgenic IDO-DCs have been promising, opening a new possibility to induce specific transplantation tolerance, the in-vivo effects of IDO expressing DCs upon allospecific T cells in-vivo, remain to be clarified.

## CONCLUSION

At present, the crucial role of DCs as key regulators of the entire immune system is fully acknowledged. The actual challenge of the contemporary applied DC biology is to ascertain whether DCs can be in the future effectively utilized for shutting down undesired immune responses, such as the one occurring in allograft rejection and autoimmune disease.

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