Modulation of Dendritic Cells for Tolerance Induction*

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Key Words

Dendritic cells · Antigen-presenting cells · Alloimmunity

Summary

Dendritic cells (DC) play a critical role as initiators and modulators of adaptive immune responses. There is increasing evidence that DC have potent abilities to tolerize and delete T cells in an antigen-specific manner. Immature or semi-mature DC generated in the laboratory can suppress autoimmunity or alloimmunity. With emphasis on the pharmacological modulation of DC, we herewith provide an update on current strategies and concepts to promote the tolerogenic potential of DC for therapy of transplant rejection and autoimmune disease.

Schlüsselwörter

Dendritische Zellen · Antigen-präsentierende Zellen · Autoimmunität · Alloimmunität

Zusammenfassung

Dendritischen Zellen (DZ) kommt eine entscheidende Rolle als Initiatoren und Modulatoren adaptiver Immunantworten zu. Es gibt zunehmende Beweise, dass DZ ein hohes Potential besitzen, immunregulatorische T-Zellen zu induzieren. Im Labor hergestellte unreife oder semi-reife DZ sind in der Lage, Auto- und Alloimmunität zu unterdrücken. Mit Betonung der pharmakologischen Modulation von DZ geben wir hiermit einen Überblick über aktuelle Strategien und Konzepte zur Förderung des tolerogenen Potentials der DZ in der Behandlung von Abstoßungsreaktionen und Autoimmunkrankheiten.

Introduction

Dendritic cells (DC) are uniquely well-equipped antigen (Ag) presenting cells that initiate and regulate immune responses. DC play a dualistic role in the regulation of immune responses. They initiate adaptive immunity by the activating naive lymphocytes and are powerful stimulators of natural killer cells – the crucial cellular instigators of the innate immune system. Furthermore, DC induce central and peripheral tolerance by mechanisms such as deletion, anergy and induction of

regulatory lymphocytes [1]. Attention has long been focused on the exceptional ability of these professional antigen-presenting cells (APC) to stimulate T-cell and B-cell mediated responses. Recently, evidence has emerged concerning the inherent tolerogenicity of DC in the periphery [2, 3]. Mechanisms whereby DC can promote peripheral tolerance are under intensive investigation. These studies have important implications for the therapy of autoimmune disorders and allograft rejection. DC are extremely well equipped for their role in innate and adaptive immunity. They are unrivaled in their ability to capture macromolecules via macropinocytosis and mannose receptor-mediated endocytosis into intracellular MHC class II-rich compartments [4]. Following Ag uptake and activation, DC migrate from the periphery to secondary lymphoid tissue, redistribute MHC-Ag complexes to the cell

*Dedicated to Prof. Dr. Peter Hanfland, Bonn, on the occasion of his 65th birthday.

Fig. 1. Current model of DC maturation and impact on T-cell immunity. Immature DC preferentially induce T-cell anergy and are characterized by low expression of MHC class II, costimulatory molecules and no production of pro-inflammatory cytokines. In contrast to immature DC, semi-mature DC are characterized by high expression of MHC class II and costimulatory molecules and promote the generation of regulatory T cells and tolerance. Fully mature DC expressing high levels of MHC class II, costimulatory molecules and pro-inflammatory cytokines are required for the induction of T-cell immunity.

surface and upregulate surface expression of costimulatory (CD80, CD86) and other molecules that promote DC survival and DC/T-cell clustering (CD40, RANK, CD54, CD58) [1]. The process of maturation also includes the production of high amounts of the pro-inflammatory cytokines IL-12 and TNF- α converting DC into very powerful T-cell priming APC (fig. 1). In addition to the well known immunostimulatory capacity of mature DC, an increasing number of publications have described the immunoregulatory capacity of immature and semi-mature DC [5]. Immature DC induce T-cell anergy and are characterized by low surface expression of MHC class II, costimulatory molecules and the pro-inflammatory cytokines IL-12 and TNF- α [1, 6, 7]. In contrast, so-called semi-mature DC are characterized by high expression of MHC class II and costimulatory molecules and low or negative production of IL-12 and TNF- α [5]. Semi-mature DC have been suggested to have important immunoregulatory functions and induce CD4+ regulatory T cells in vitro and in vivo [5].

Update on Current Strategies to Promote DC Tolerogenicity

Currently, two different approaches for the enhancement of the tolerogenic properties of DC are under investigation. One involves the use of immature DC, the other the use of semi-mature DC (fig. 1). Jonuleit et al. [8] have shown that repetitive in vitro stimulation of allogeneic T cells with immature DC leads to the generation of non-proliferating, IL-10 producing T regulatory (Treg) cells. Proliferation of these T cells could not be restored by exogenous IL-2, and the proliferation of type 1 helper T (Th1) cells was inhibited in a contact-de-

pendent, but non-Ag-specific manner. In agreement with these findings, Dhodapkara et al. [9] subcutaneously injected immature autologous monocyte-derived DC pulsed with influenza matrix peptide and keyhole limpet hemocyanin into 2 human volunteers. As a result, they observed Ag-specific inhibition of CD8+ T-cell killing activity and the appearance of peptide-specific IL-10-producing T cells, accompanied by a decrease in IFN- γ -producing T cells. Two remarkable findings emerged from these studies. First, both studies associated the appearance of DC-induced Treg cells with production of IL-10, but not IL-4. Second, the loss of T-cell killing activity was not due to a decline in peptide-specific T cells, indicating immune regulation rather than deletion of effector cells.

Pharmacological Modulation of DC Maturation as an Approach to Promote Tolerance Induction

Based on the findings that modulation of DC maturation is critical for the tolerogenic or immunogenic capacity of DC, one strategy to promote the tolerogenicity of DC is the use of drugs that suppress DC maturation. This concept is reasonable, since an increasing number of publications demonstrated that DC represent emerging pharmacological targets of classical and novel immunosuppressive drugs [10]. For many years, T cells have been considered the main targets of numerous immunosuppressive/anti-inflammatory drugs. The discovery that several immunosuppressive/anti-inflammatory agents potentially inhibit DC immunogenicity has provided not only new insight into the immunopharmacology of these substances, but also offers novel strategies for the manipulation of DC for tolerance induction [10].

1,25(OH)₂D₃ – the biologically active metabolite of vitamin D₃ – or its analog 1a,25(OH)₂-16-ene-23-yne-26,27-hexafluoro-19-nor-vitamin D₃ (D3-analog) inhibit the maturation of human monocyte-derived DC [11–13] and mouse bone marrow(BM)-derived DC in vitro [14]. DC treated with 1,25(OH)₂D₃ or D3-analog are poor stimulators of allogeneic T cells, show impaired IL-12, but increased IL-10 secretion and exhibit enhanced endocytic activity [11–14]. In vitamin D receptor (VDR) knock out (KO) mice the inhibitory effects of 1,25(OH)₂D₃ and the D3-analog on DC maturation and T-cell stimulatory function were absent [14]. Interestingly, VDR KO mice show hypertrophy of subcutaneous lymph nodes, as well as increased numbers of mature DC in lymph nodes, suggesting a physiological role of the 1,25(OH)₂D₃-VDR loop in DC homeostasis and maturation in vivo [15]. Pretreatment of mice with vitamin D₃-exposed donor DC prolongs skin graft survival [15].

Two independent studies provided recent evidence that aspirin (the most commonly used analgesic and anti-inflammatory drug) profoundly suppresses the maturation and T-cell stimulatory function of human monocyte-derived and mouse BM-derived DC [16, 17]. Aspirin-treated DC failed to induce cell-mediated contact hypersensitivity reactions in vivo [17], indicating that exposure to aspirin is a highly effective and inexpensive way of manipulating the immunostimulatory potential of DC that may find clinical application. In contrast to salicylates, corticosteroids not only suppress DC maturation but also potently inhibit DC differentiation both in vitro [18, 19] and in vivo [20]. Corticosteroid-treated human monocyte-derived or mouse BM-derived DC promote the generation of IL-5- and IL-10-producing T cells while inhibiting IFN- γ secretion and therefore favor Th2/Treg immune responses [21, 22]. Future studies are necessary to determine whether ex vivo treatment of DC with corticosteroids and subsequent injection facilitate the expansion of Th2/Treg cells in vivo.

Mycophenolate Mofetil (MMF), Cyclosporine (CsA) and Tacrolimus (FK506)

MMF is a reversible inhibitor of inosine monophosphate dehydrogenase (IMPD) which is critical for the de novo synthesis of guanosine and deoxyguanosine molecules necessary for the generation of RNA and DNA [23]. MMF is thought to selectively target the proliferation of B and T cells that depend to a greater extent on the de novo synthesis of purines than other cells [24]. Mehling et al. [25] reported that MMF directly affects DC maturation and impairs their capacity to induce a cell-mediated immune response in vivo. The findings of this study indicate a novel role of the enzyme IMPD in DC activation, which was recently confirmed by another group [26]. As

regards the calcineurin inhibitors CsA and FK 506, Lee et al. [27] suggested that CsA interfered with DC maturation via NF- κ B inhibition, while other studies have failed to demonstrate significant effects of CsA on the maturation of monocyte-derived DC [19] or on epidermal Langerhans cell function [28]. Future studies analyzing the effects of CsA on DC maturation and function in vivo are necessary to clarify this issue. Interestingly, Chen et al. [29] reported, that CsA inhibits DC migration through inhibition of CC-chemokine receptor 7 expression. With respect to the calcineurin inhibitor FK-506, 2 studies have indicated no significant effects on DC maturation [19, 30]. However, since tacrolimus suppresses TNF- α production by DC [19] and DC T-cell stimulatory capacity [31, 32], there are reasons to believe that this substance also targets functional DC maturation. However, the precise molecular mechanisms remain elusive.

Rapamycin

Rapamycin is a bacterial macrolide antibiotic with potent immunosuppressive action introduced in recent years as anti-rejection therapy in organ transplantation [33]. Rapamycin, like FK506, binds intracellularly to FK506 binding proteins. However, unlike FK506, it inhibits the function of the serine/threonine kinase target of RAPA (mammalian (m) TOR) [34]. mTOR is a common effector protein shared by many signaling pathways. Inhibition of mTOR results in suppression of cytokine-driven cell proliferation, ribosomal protein synthesis, translation initiation and cell cycle arrest in the G1 phase [34, 35].

In addition to its suppressive effect on lymphocytes, rapamycin suppresses the generation of GM-CSF-expanded human monocyte-derived DC in vitro and the generation of fms-like Flt3L(tyrosine 3 kinase ligand)-expanded DC in mice in vivo [36–38]. Moreover, we identified rapamycin as the first clinically relevant substance that inhibits DC antigen uptake in a maturation-independent manner [39]. At low concentrations, rapamycin impairs macropinocytosis and mannose receptor-mediated endocytosis of mouse BM-derived DC and human monocyte-derived DC. Furthermore, inhibition of DC antigen uptake by rapamycin was confirmed with human monocyte-derived DC and after in vivo administration of the drug [37, 39].

Sanglifehrin A (SFA)

SFAs, originally described by Sanglier et al. [40] and Fehr et al. [41], are produced by the actinomycetes strain *Streptomyces A92-308110* and belong to a novel family of immunophilin-binding ligands. Although SFA, like CsA, binds with high affinity to cyclophilin, it unlike the latter does not inhibit the activity of the calcineurin phosphatase [42]. Com-

Table 1. Treatment of allograft rejection and autoimmune diseases with DC without additional immunosuppression

DC type and modification	Route	Species	Biological effect
Semi-mature DC (IL-10, TGF- β , LPS)	i.v.	mouse	protection from graft-versus-host disease in experimental bone marrow transplantation[57]
GM-CSF cultured immature myeloid donor DC	i.v.	mouse	prolonged or indefinite heart allograft survival [46, 49]
NF- κ B ODN treated myeloid donor DC	i.v.	mouse	prolonged heart allograft survival [47]
Fas ligand transfected myeloid donor DC	i.p.	mouse	prolonged heart allograft survival [48]
Adenovirus IL-10/TGF- β transduced myeloid donor DC	p.v.	mouse	prolonged kidney allograft survival [51]
Rapamycin exposed alloantigen pulsed host-DC	i.v.	mouse	prolonged heart allograft survival [50]
			biological effect on autoimmune diseases
Adenovirus IL-4 transduced DC	i.v.	mouse	suppression of collagen-induced arthritis [62]
MBP pulsed autologous myeloid DC	s.c.	rat	prevention of MBP-induced EAE [53]
GM-CSF cultured ($\pm$ IL-4; $\pm$ islet-autoantigen pulsed) autologous myeloid DC	i.v.	mouse (NOD)	decreased incidence of type-1 diabetes [54]
Ex vivo IFN- γ stimulated autologous splenic DC	i.p.	mouse (NOD)	decreased incidence of type-1 diabetes [55]
Semi-mature DC (TNF- α)	i.v.	mouse	prevention of MOG-induced EAE [56]

MBP = Myelin basic protein; MOG = myelin oligodendrocyte glycoprotein; NF- κ B ODN = oligodeoxyribonucleotides encoding for NF- κ B binding sites; p.v. = portal vein; TCR = T-cell receptor; NOD = non-obese diabetic.

petitive experiments with a non-immunosuppressive cyclophilin-binding derivative of CsA have suggested that the immunosuppressive activity of SFA is not dependent on cyclophilin binding [42]. In addition, SFA does not bind to FK506 binding protein 12 (FKBP12) and does not inhibit enzymatic activity of p70^{s6k} kinase, a major downstream target of mTOR [42, 43]. These results suggest that SFA represents a novel class of immunophilin-binding immunosuppressants with a new, yet undefined mode of action. Studies of the immunosuppressive effects of SFA have been focused on T and B lymphocytes. SFA has been reported to exhibit a lower immunosuppressive activity in the mixed leukocyte reaction (MLR) than CsA [40]. Recently, we discovered that SFA rapidly blocks bioactive IL-12 production by human DC. In direct comparison to the related agents CsA and rapamycin, we found that SFA acts uniquely within 1h inhibiting 80–95% of DC IL-12p70 production [44]. Additionally, Woltman et al. [45] reported that SFA potently inhibits DC antigen uptake receptor expression and DC endocytosis.

Prevention and Treatment of Experimental Transplant Rejection and Autoimmune Diseases with DC

Evidence for the tolerogenic ability of DC, either as immature cells or semi-mature cells, was provided by studies in which DC were administered to non-immunosuppressed patients prior to transplantation or tested in models of autoimmune disease (table 1). These studies have demonstrated that DC of donor or recipient origin can prolong heart, kidney or pancreatic islet allograft survival [46–52]. Autologous DC – with or without autoantigen pulsing – inhibit the development of experimental allergic encephalomyelitis (EAE) or type-1 diabetes [53–55] (table 1).

A growing number of reports have described the immunoregulatory capacity of semi-mature DC. Menges et al. [56] demonstrated that repeated injections of DC matured with TNF- α induced IL-10-producing peptide-specific T cells in vivo and antigen-specific protection from experimental autoimmune encephalomyelitis, which was not the case with immature DC or DC matured with lipopolysaccharide (LPS) and CD40L. The tolerogenic DC were characterized as MHC II^{high} and costimulatory^{high}, but were found to be weak producers of proinflammatory IL-12p70 compared to LPS/CD40L matured DC. A similar approach was successfully employed by Sato et al. [57, 58] who expanded DC in the presence of IL-10 and transforming growth factor beta (TGF- β) and matured these APC with either LPS or TNF- α . These semi-mature DC expressed high levels of MHC II^{high} and low levels of costimulatory molecules, and it is conceivable (although not having been directly investigated) that these DC were low producers of proinflammatory cytokines. By using a murine model for graft-versus-host disease (GVHD) and leukemia relapse, it was demonstrated that host-matched semi-mature DC protected the mice in an antigen-specific manner from GVHD lethality and induced expansion of IL-10-producing CD4+ CD25+ suppressor T cells [57]. Treatment with semi-mature DC retained the graft-versus-leukemia (GVL) effect in recipients of allogeneic bone marrow and spleen mononuclear cells. However, without abrogating the GVL effect, the underlying mechanisms of GVDH protection are yet to be resolved. Several investigators have used DC together with immunosuppressive treatment of the recipient. In particular, the blockade of the CD40-CD154 signaling pathway using anti-CD154 monoclonal antibodies is highly synergistic with DC therapy [59]. The value of blocking the CD40 pathway is highlighted by the fact that this strategy promotes skin allograft survival even in combination with Flt3L expanded donor DC

[60]. Interestingly, with respect to chronic rejection of murine heart transplants, we found that the blockade of the CD40-CD40L pathway ameliorated transplant vasculopathy, while preservation of near-normal vessel architecture was achieved by simultaneous administration of donor DC [61].

Conclusion

In recent years, many groups worldwide have generated important insights into the specific effects of classical and novel

immunosuppressive agents on key aspects of DC function and activation. Further experimental and pre-clinical studies are now necessary in order to identify the most promising agents and protocols suitable for the generation of tolerogenic DC.

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