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Forum Review Article

Redox regulation of tolerogenic dendritic cells and regulatory T cells in the pathogenesis and therapy of autoimmunity

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Running head: Redox regulation of DC and T cells in autoimmunity

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Abstract

Significance: Autoimmune diseases are progressively affecting westernized societies, as the proportion of individuals suffering from autoimmunity is steadily increasing over the past decades. Understanding the role of reactive oxygen species (ROS) in modulation of the immune response in the pathogenesis of autoimmune disorders is of utmost importance. The focus of this review is the regulation of ROS production within tolerogenic dendritic cells (tolDC) and regulatory T (Treg) cells that have the essential role in the prevention of autoimmune diseases and significant potency in their therapy.

Recent Advances: It is now clear that ROS are extremely important for the proper function of both DC and T cells. Antigen processing/presentation and the ability of DC to activate T cells depend upon the ROS availability. Treg differentiation, suppressive function and stability are profoundly influenced by ROS presence.

Critical Issues: Although a plethora of results on the relation between ROS and immune cells exists, it remains unclear whether ROS modulation is a productive way for skewing T cells and DC towards a tolerogenic phenotype. Also, the possibility of ROS modulation for enhancement of regulatory properties of DC and Treg during their preparation for use in cellular therapy has to be clarified.

Future Directions: Studies of DC and T cell redox regulation should allow for the improvement of the therapy of autoimmune diseases. This could be achieved through the direct therapeutic application of ROS modulators in autoimmunity or indirectly, through ROS-dependent enhancement of tolDC and Treg preparation for cell-based immunotherapy.

Introduction

Reactive oxygen species (ROS) are associated with the eradication of invading microorganisms, promotion of inflammation and tissue damage. However, their role in the immune response is far more complex. Redox signalling (158, 7, 126) is being recognized as an important player in the activation, differentiation and proliferation of immune cells. This review focuses on redox regulation in specific subsets of dendritic cells (DC) and T cells that possess immunosuppressive and/or regulatory roles during the immune response in the physiological and pathological conditions. The activity of DC with tolerogenic properties (tolDC) and regulatory T (Treg) cells is especially susceptible to ROS-mediated fine-tuning, as ROS is involved in antigen processing and presentation in DC, and T cell receptor (TCR)-mediated signalling within T cells. TolDC and Treg are extremely important for the maintenance of central and peripheral immune tolerance i.e. the ability of the adaptive immune response to distinguish between self and non-self. It is generally accepted that mechanisms of the tolerance to self-tissues can be broken due to genetic predisposition and various environmental factors, such as viral or bacterial infection, food and gut microbiota antigens, tobacco smoke, alcohol consumption, lack of vitamin D (2, 117, 29, 130), allowing for the activation of autoreactive T and B cells. Activated T and B cells induce inflammation that causes destruction of self-tissues, i.e. trigger the development of autoimmune diseases. Multiple organs and tissues are targeted by the autoimmune response in systemic autoimmune disorders, such as systemic lupus erythematosus (SLE), while specific cells and structures of the certain organs are targeted in organ-specific autoimmune diseases, such as insulin-producing β cells of the pancreas in type 1 diabetes (T1D), cartilage of the joints in rheumatoid arthritis (RA), and myelin sheaths and neurons of the brain and spinal cord in multiple sclerosis (MS).

Autoimmunity development has been also associated with inadequate redox signalling in immune cells (167). For example, ROS produced by macrophages within the pancreatic islets in T1D are detrimental for the insulin-secreting β cells since these cells are poorly equipped with the antioxidant machinery. Also, T cell-related mitochondrial abnormalities are detected in T1D, and the increase in ROS production in T cells is correlated with the major pro-inflammatory cytokine, interferon-γ (IFN-γ) secretion (24, 25, 116). The importance of redox regulation is depicted by the fact that the severity of RA is correlated with the generation of altered auto-antigens such as the oxidized form of type II collagen by ROS during the early stages of RA (147). In MS, oligodendrocytes, myelin-producing cells, are especially vulnerable to oxidative stress, as they have low antioxidant levels, high content of redox-active iron, and exposed extensive plasma membrane expansions (101).

ROS include superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$). Unlike $O_2^{\bullet-}$ or $OH^{\bullet}$, H_2O_2 does not readily mediate oxidative damage and since it is not extremely reactive, it can freely diffuse within the cell and even cross the cell membrane through aquaporins that act as membrane passages for H_2O_2 (38). They can be generated in several intracellular niches. The mere cell respiration is a source of ROS in dormant conditions. Therefore, cellular energy metabolism and ROS production are tightly related. ROS is produced during energy production (ATP) by the process of oxidative phosphorylation (OXPHOS) in the inner membrane of mitochondria. The transfer of electrons from NADH could lead to electron leakage and the generation of superoxide at Complex I and III of the electron transport chain. Complex I (NADH ubiquinone oxidoreductase) produces a large amount of superoxide in forward and reverse electron transport. When the NADH/NAD+ ratio is high, electrons generate superoxide in forward electron transfer. During reverse electron transfer, electrons derived from succinate oxidation on Complex II (succinate dehydrogenase) reversely flow to Complex I and generate superoxide. The next site in the

electron transport chain where superoxide generation occurs is Complex III (ubiquinol-cytochrome c oxidoreductase). In contrast to Complex I, it appears that Complex III produces much less superoxide under physiological conditions (110, 26). Although OXPHOS and the inner membrane are a major source of superoxide production, α-ketoglutarate dehydrogenase or other enzymes that are associated with the NADH pool, may also be a source of superoxide in mitochondria (156). The end result of superoxide production in mitochondria is the generation of H_2O_2 by superoxide dismutase or the formation of peroxynitrite (ONOO-) in reaction with nitric oxide (NO) (80).

This minute amount of ROS released during OXPHOS is usually involved in cell communication and signalling and may aid the proper function of immune cells, especially in the terms of their activation and differentiation, antigen processing, cell cycle progression and inflammatory behaviour (61). However, when excessively produced upon cellular stress, ROS could be detrimental for the cell and its surroundings. Apart from microbe eradication, ROS can provoke cellular damage, interfere with adequate immune response and may contribute to the development of autoimmunity. This ROS comes from the processes mediated by NADPH oxidase (NOX) complexes, monoamine oxidase, xanthine oxidase, lipoxygenases, cyclooxygenases, monooxygenases (165). NOX complex is formed of several subunits and it is found in the plasma membrane and in the membranes of phagosomes. NOX catalyzes the electron transfer from NADPH to molecular oxygen and thus generates superoxide ($O_2^{\bullet -}$), which is a precursor for most other ROS (76). Two membrane NOX subunits represent the catalytic core of the enzyme, $gp91^{phox}$ (often referred to as NOX2) and $p22^{phox}$, while the other three $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ are regulatory subunits (165). As ROS can be highly toxic, there are several defence mechanisms, including glutathione peroxidase, superoxide dismutase, selenoproteins and catalase enzymes that prevent oxidative damage. In addition,

molecules such as pyruvate, α-ketoglutarate, oxaloacetate, ascorbate, vitamin E, melatonin, uric acid and hydrogen sulphide are potent ROS scavengers (140, 151,172).

DC in autoimmunity

Depending on the signals provided by DC, $CD4^+$ T cells are directed towards various helper T (Th) or Treg cells (Fig 1). Thus, DC have a major role in the pathogenesis of autoimmune diseases where the overactivation or inappropriate activation of $CD4^+$ T cells is the foundation of the autoimmune reactivity (Fig 2). The improper activation comes as a consequence of inadequate inherent regulation of T cells, i.e. flaws in the central tolerance or the inefficient activity of Treg cells. But it can also be a consequence of intrinsic dysfunction of DC. Indeed, it has been shown in various animal models of autoimmunity, or even in humans affected by autoimmune diseases that their DC have distinctive functional or phenotypic properties or different abundance in comparison to healthy animals or humans (11, 33, 148, 75, 88, 111, 112). For example, intestinal DC from T1D patients were incapable of converting T cells into Treg cells (11). Diabetes-prone biobreeding rats, individuals at the preclinical phase of T1D, and first-degree relatives of T1D patients had less DC and these cells had impaired antigen-presenting function (33, 148, 75). Also, DC from patients suffering from SLE or MS, as well as the DC from the first-degree relatives of T1D patients had impaired production of IFN-α (88, 75, 12, 143. Interestingly, both immature and mature DC obtained from SLE patients had elevated expression of co-stimulatory molecules (CD40, CD86), but their ability to stimulate T cell proliferation was increased in the former and decreased in the latter, in comparison to healthy individual's DC (111). Similarly, decreased expression of CD80 and CD86 and lower ability to stimulate T cell proliferation was observed in the mature DC obtained from children with T1D (4). Also, these cells produced higher amounts of IL-10 and lower amounts of IL-12

in comparison to healthy individuals DC (5). Decreased ability of DC obtained from MS patients to express CD86, 4-1BBL, CD40 and CD83 and to stimulate IFN-γ and proliferation of T cells was observed (12, 144). Decreased expression of CC chemokine receptor 2 (CCR2) in mature DC of paediatric T1D patients was reported (112). Accordingly, genome-wide association and transcriptome studies in T1D and MS have identified autoimmunity-associated loci and specific genes that are linked to the inherent DC development and/or function and that may interfere with tolerogenic DC activities (63, 137,113). All of these data imply that there are inherent disturbances in DC number and function that are relevant for the pathogenesis of autoimmune diseases. However, the observed dysfunctions of DC in autoimmune diseases might also be the consequence of the pro-inflammatory milieu that DC are exposed to. For instance, the resistance to experimental autoimmune encephalomyelitis (EAE) in Balb/c mice can be overcome by an infection with murine cytomegalovirus, and one of the main consequences of the infection is an increased number of DC expressing co-stimulatory molecules CD86 and CD40 in the peripheral lymph organs (94). Further, NO produced by inducible NO synthase was shown to potentiate major histocompatibility complex (MHC) class II molecule expression in DC during EAE, thus increasing their inflammatory ability (142). Coagulation factor XII, which links coagulation and inflammation, was shown to act through CD87 on DC to activate T cells during neuroinflammation (51). Also, pancreatic resident phagocytes produce excessive ROS, which stimulates infiltrating macrophages and DC that can carry β cell antigens into the draining pancreatic lymph nodes to activate autoreactive T cells (19).

In addition to their ability to activate autoreactive T cells, DC can suppress the T cell response. Such tolDC can be induced in vivo in response to regulatory cytokines, i.e. IL-27 and IL-10, vitamin A and its metabolite retinoic acid, ligands of the aryl hydrocarbon receptor, such as tryptophan metabolites, or neurotransmitters, e.g. norepinephrine, as well as

in response to apoptotic cells (28, 149, 170). TolDC are of particular importance for the regulation of the immune response at the mucosal barriers, such as gut, lung and skin (Fig 3). For instance, $CD11b^{-}CCR7^{+}$MHC class $II^{high}CD103^{+}$ have been shown essential for dietary antigen tolerance (40, 28, 6). MHC class $II^{int}CD80/86^{high}CD40^{+}CD8^{-}$ cells were identified as potent Treg inducers in the lung (1) and MHC class $II^{low}CD80/86^{low}CD40^{low}$ $CD103^{+}$ tolDC were identified in the eye (146). Skin migratory dermal $CD11b^{+}CD103^{-}$ DC were shown more potent than $CD103^{+}$ DC in inducing Treg (55).

The importance of tolDC in constraining an autoimmune disease was shown in mice where depletion of DC led to aggravated MS-like disease (176). Mechanisms of tolDC immunosuppressive actions include perforin-dependent apoptosis of T cells, secretion of transforming growth factor (TGF)-β and retinoic acid, or IL-10 and IL-27 mediated generation of Treg (149). TolDC derived from human peripheral blood monocytes or from murine bone marrow cells under the influence of tolerizing agents, such as vitamin D and/or dexamethasone, are used in cellular immunotherapy of autoimmune diseases (56, 50, 144, 122,168). Their phenotype in vitro is usually as MHC class$II^{low}CD80/86^{low}CD40^{low}$. Similarly, reduced MHC class II and/or co-stimulatory molecules expression was also observed in vivo in animal models of T1D, RA and MS (123, 103, 118, 154). However, the precise phenotype of tolDC in vivo in conjunction with autoimmunity is still elusive and differs among the animal models (Fig 3). There are reports from non-obese diabetic (NOD) mice that lymphoid resident $CD11b^{+}DCIR2^{+}$ DC, corresponding to $CD11b^{+}CD1c^{+}$ DC in humans, are able to tolerize T cell response despite the ongoing autoimmune reaction against the pancreas (123). Similarly, $CD11b^{+}CD103^{-}$ and $CD11b^{high}CD103^{+}$ DC were identified as tolDC upon intravenous tolerization in EAE mice (154). However, migratory $DEC205^{+}$ or $Langerin^{+}$ DC ($CD11b^{-}CD103^{+}$ and Langerhans cells) were shown to potently induce Treg in EAE mice (67). Along the same line, $CD11c^{+}CD11b^{-}CD103^{+}$ were identified as tolDC in

multiple low dose streptozotocin-induced T1D in mice (81). Also, $CD11c^{hi}$MHC class II^{hi} perforin producing DC were identified as tolDC in EAE (179) and $CD11c^{+}CD11b^{+}IDO^{+}$ cells were recognized as tolDC in collagen-induced arthritis (118). Thus, it is possible that tolDC in autoimmune disease have different phenotypes depending on the immune context within which they perform their functions.

T cells in autoimmunity

The thymus is a central lymphoid organ where $CD4^{+}$ Th cells and $CD8^{+}$ cytotoxic lymphocytes have to pass through two checkpoints, i.e. positive and negative selection. Their T cell receptors (TCR) need to recognize the self MHC molecules in conjunction with antigenic peptides (positive selection), and then those T cells that do not bind MHC-self antigens complexes with strong avidity (negative selection) become selected to survive and exit the thymus (Fig 1). However, the evidence from healthy population indicates the presence of auto-reactive T cells specific for versatile auto-antigens (insulin, glutamic acid decarboxylase 65, melanocyte differentiation Ag tyrosinase, myelin basic protein, type II collagen, acetylcholine receptor) at the periphery (90, 121, 124, 141). Not all auto-reactive cells are pathogenic. One population of $CD4^{+}$ cells that expresses TCR with high avidity for auto-antigens normally exit the thymus and these are called the Treg cells (104, 175). Their function is to maintain self-tolerance and immune system homeostasis (Fig 2).

Although there is a variety of T cells with regulatory properties, this review will focus on natural Treg (nTreg) that develop in the thymus or induced Treg (iTreg) that are generated from naïve $CD4^{+}$ cells at the periphery. All these Treg are $CD4^{+}$ T cells that express high levels of the alpha chain of IL-2 receptor (CD25) and forkhead box p3 (FoxP3) transcription factor ($CD4^{+}CD25^{high}FoxP3^{+}$). iTreg develop from naïve $CD4^{+}$ cells (Fig 1) in the presence of

FoxP3-inducers, cytokines TGF-β, IL-2, dietary constituents (retinoic acid) in vivo, or drugs, such as glucocorticoids and rapamycin in vitro (65, 155). In contrast to nTreg that are exclusively auto-antigen-specific, iTreg can also be specific for foreign antigens (allergens, food and commensal microbiota). For example, intestinal iTreg orchestrate intestinal tolerance to harmless microbial and food antigens. The combination of retinoic acid derivatives and TGF-β derived from DC, together with IL-2 produced by innate lymphoid cells is necessary for their differentiation (52, 108).

The lack of Treg cells resulting from Foxp3 mutation is a cause of fatal autoimmunity (10). The association of the specific autoimmune disease (such as T1D, MS or RA) and the type of change in the Treg biology is quite controversial. In some cases, a defect is mirrored by the number, while in the other by the function of the Treg. Several studies in humans show that T1D and RA are associated with lower levels of Treg (129, 57, 162, 42), while others report no association (16, 105, 124). These findings largely depend upon the identification markers of Treg. In NOD mice, for example, spontaneous T1D development has been correlated with the lower diversity of TCR on Treg (43) and reduced Treg suppressive capacity (31). In brief, T1D development in individuals and susceptible mice is generally associated with reduced sensitivity to IL-2, increased Treg apoptosis, decreased stability of FoxP3 expression, increased Treg production of pro-inflammatory cytokines as previously reviewed elsewhere (66). RA individuals show decreased Treg differentiation due to aberration in specific chromatin-modifying elements (171). Also, the development and the function of Treg in MS individuals were found to be disturbed (161), while the treatment of such individuals that resulted in reduced annual relapse rate and reduced disease progression was associated with increased Treg proportions (36).

Treg promote self-tolerance by versatile mechanisms. They secrete IL-10, TGF-β and IL-35, all implicated in the down-regulation of pro-inflammatory molecules in DC, macrophages, and activated B and T lymphocytes. Contact-dependent inhibition by Treg relies on the expression of CTLA-4, a co-inhibitory molecule that competes with the co-receptor CD28 in binding to the co-stimulatory molecules (CD80 and CD86) on antigen-presenting cells. In this way, Treg cells prevent the activation of the pathogenic T cells specific for the same auto-antigen and induce tolerogenic properties in DC (177). Also, Treg can induce apoptosis of auto-reactive T cells through another co-inhibitory interaction, i.e. interaction of PD-1 with its ligand PD-1L on effector T cells (48). In addition, Treg exert contact-dependent inhibition of immune cells through the surface bound TGF-β1. This molecule is in inactive form, but when it becomes activated through the action of its linker molecule GARP, it performs its immunosuppressive functions (30, 18). Treg cells also express ectonucleotidases CD39 and CD73 that hydrolyse adenosine triphosphate and adenosine diphosphate into adenosine, which increases the intracellular concentration of immunoregulatory cyclic adenosine monophosphate in the effector T cells (131). As a shared feature with cytotoxic $CD8^+$ lymphocytes, Treg can utilize granzyme B/perforin machinery for the direct inactivation of effector cells (20). ROS production in Treg is explored as a novel mode of Treg suppressive effects.

The effect of ROS on DC biology

DC utilize ROS to eliminate pathogens, as well as to process and present antigens (61, 82). Although the function of DC is not primarily related to the killing of microorganisms, these cells do activate NOX2 and subsequent ROS production after bacterial, viral, and fungal stimuli through Toll-like receptors (61, 82). These high ROS concentrations (oxidative burst)

are used for the elimination of the pathogen, as exemplified in the elimination of fungal pathogens (62). In contrast, when activated by T lymphocytes through CD40L, DC do not engage NOX2, and no oxidative burst occurs (163). Therefore, OXPHOS-related ROS production is probably involved in the intracellular signalling. Interestingly, lower OXPHOS gene expression was observed during maturation of human monocyte-derived DC (87). NO generated by inducible NO synthase was shown crucial for the maturation-related downregulation of OXPHOS in murine DC (41). On the contrary, microarray and proteome analysis showed that several genes directly related to OXPHOS were up-regulated in tolDC differentiated in vitro (44, 46). Also, tolDC derived from human monocytes in vitro had higher mitochondrial oxidative activity, production of ROS and increased spare respiratory capacity in comparison to mature DC (95). It can be, therefore, hypothesized that OXPHOS-derived ROS are associated with the suppressive phenotype of tolDC (Fig 4). However, NOX2-generated ROS at the plasma membrane affect cofilin activity in T cells, thus preventing efficient immune synapse formation, leading to T cell hypoactivation and necrotic cell death (133, 61). Thus, NOX2-derived ROS can also substantially contribute to tolerogenic activity of DC.

Generally, it is considered that ROS contribute to antigen processing and presentation in DC (61). Degradation of proteins in phagosomes occurs in a strictly controlled environment with specific pH (5.5 – 6.5) for optimal enzymatic activity. NOX2-mediated ROS are involved in maintaining alkalinisation of the phagosomal lumen as ROS inactivates the V-ATPase and subsequently increases pH (157). Therefore, ROS seem to enable proper protein degradation. Completely absent ROS in DCs lacking $gp91^{phox}$ lead to a very low pH in phagosomes, which impairs antigen presentation (134). In contrast, there was also a report on ROS-dependent inhibition of cysteine proteases cathepsins that degrade proteins for MHC II loading and impaired antigen processing in DC (132).

Maturation of DC is related to the up-regulation of co-stimulatory molecules, such as CD40, CD80, CD86 that facilitate productive DC-T cell interaction, as well as with the production of cytokines that drive the differentiation of naïve T cells. Inhibition of ROS production during lipopolysaccharide (LPS)-induced maturation in vitro suppressed CD86 expression of murine bone marrow-derived DC (100). On the other hand, it was shown that IFN-γ^+ LPS-matured DC from $p47^{phox}$ deficient mice produced more IL-12 and, consequently, potentiated the differentiation of Th1 cells (69). The negative effects of NOX2-generated endogenous ROS on IL-12 production were mediated through impairment of p38 MAPK signalling (69). Also, low concentration of H_2O_2 (0.01 μM) decreased expression of MHC class II molecules on human DC (119). The treated DC potentiated IL-4 producing T cells, i.e. Th2 cells (119). As already stated, the maturation of human monocyte-derived DC is associated with lower OXPHOS gene expression (87) and potentially lower production of mitochondrial ROS.

The effect of ROS on Treg biology

The involvement of ROS in T cell biology is still underexplored. Redox status of T cells depends upon the action of ROS producers: phagocytic NOX-2, non-phagocytic enzyme dual-substrate oxidase 1 (DUOX-1), mitochondria, and the action of antioxidant system that includes superoxide dismutase, peroxiredoxins (cysteine-dependent peroxidase enzymes), and selenoproteins that include glutaredoxins (glutathione-dependent redox enzymes) and thioredoxin reductases (enzymes that reduce thioredoxin) (27). Both intracellular and extracellular ROS can act as signalling molecules and tightly regulate the process of T cell differentiation, activation and function (47).

Intracellular ROS production in Treg probably follows the same principle as in T cells in general (27), i.e. ROS are generated upon TCR activation or during their cellular metabolism.

A cognate recognition of antigen-MHC complexes initiates TCR crosslinking in T cells and one of the consequences is the production of ROS (68, 89). The first wave of ROS production is exclusively H_2O_2 and is very rapid and transient (occurs after 2-4 mins) and is independent of NOX, but dependent upon DUOX-1. DUOX-1 activity promotes inactivation of Src homology 2 domain-containing protein tyrosine phosphatase 2. As a consequence, the phosphorylation of ZAP-70 occurs that enables its association with the Src family tyrosine kinase Lck and the CD3ζ chain of the TCR complex. Therefore, H_2O_2 generated by DUOX-1 acts in a positive feedback loop to enhance and sustain further TCR signaling (89). The second phase of ROS production after TCR activation is stable H_2O_2 generation that is NOX-dependent and provoked by FasL-Fas interaction. Superoxide anion generation, as a third event, occurs after 8–10 min and is FasL-Fas dependent and independent of NOX (68). However, TCR-initiated events also trigger OXPHOS. The efflux of calcium ions from the endoplasmic reticulum triggered by CD3 promotes calcium entry into the mitochondria and stimulates OXPHOS and concomitant ROS generation. These ROS are involved in the activation of key transcription factors like nuclear factor κB (NF-κB) that drives IL-2 production and activates T cell proliferation. The ROS-mediated stimulation of activator protein 1 and nuclear factor of activated T cells is required for further downstream signalling events that support T cell-mediated immune responses (72, 73, 135). However, the excess of ROS may negatively affect T cell differentiation and proliferation. For example, the low content of selenium (a constituent in antioxidative selenoproteins) in the food diminishes the ability of T cells to become activated upon exposure to the antigen. Also, their proliferation is decreased and can be attributed to the reduction of free thiols observed after low selenium diet (60).

In the steady state, Treg cells predominantly rely on fatty acid oxidation and OXPHOS to meet their relatively low-energy needs (59, 138, 164). However, activation shifts Treg

metabolism to the aerobic glycolysis, glutaminolytic and pentose phosphate (164). During OXPHOS in the mitochondria, the leakage of electrons occurs and ROS can be formed. To summarize, complex I produces large amounts of O2•- by two mechanisms: when the matrix NADH/NAD+ ratio is high, leading to a reduced flavin mononucleotide on Complex I, and when electron donation to the coenzyme Q pool leads to reverse electron transport (Figure 4). Although the site of O2•- production during reverse electron transport is not known, the rate of O2•- production seems to be the highest possible in mitochondria (110, 26). Therefore, although Complex III can be induced to produce O2•- with the inhibitor antimycin, its production in mitochondria under physiological conditions is far lower and is negligible compared with the maximum rates of O2•- production from Complex I. Since this is predominant energy pathway in quiescent Treg, the intracellular ROS is higher compared to other T cells.

Fine-tuning of both ROS concentrations and timing of ROS levels have a decisive role in T cell differentiation and this ROS-based control starts within the thymus and ensues at the periphery. In the thymus, intracellular redox status influences T cell fate, as it was shown that Treg precursors were predominantly in the ROS^{low} subset of thymocytes, unlike other T cell progenitors that were predominantly in the ROS^{high} subpopulation (70). Once T cells leave the thymus, Treg respiration is increased by 25% and total ROS concentration is significantly greater in comparison to other T cell subsets both in mice and humans (3, 8, 64). It was also shown that intracellular increase in ROS after Treg activation through TCR results in enhanced Treg stability. This stability is enforced by up-regulation of SUMO-specific protease 3 that controls SUMOylation and nuclear localization of BACH2 (transcription regulator protein) that represses the genes associated with $CD4^+$ T effector cell differentiation and stabilizes Treg cell-specific gene signatures (178). Intracellular ROS also affects Treg suppressive functions. Namely, it was demonstrated that thiol-bearing antioxidants and

inhibitors of NOX2 attenuated suppressive activity of Treg (37). Moreover, Treg cells with a NOX2 deletion were less efficient suppressors in vitro as a consequence of impaired TGF-β utilization (37). Treg activate multiple strategies for interfering with the extracellular redox environment during T cell activation, affecting dendritic cells as well. For example, Treg exert their suppressive activity in a sulphur redox fashion. Briefly, activated T cells need the amino acid cysteine to proliferate but lack the transporter for its oxidized form, cystine that is abundantly present in the extracellular milieu. Therefore, DC provide glutathione, which is converted to cysteine in the extracellular space for T cells to take it up (5). Treg are able to inhibit the glutathione production in DC, as well to dominantly consume cysteine (173).

Studies in mice deficient in certain antioxidant molecules show that such environment favors Treg activity. Elevated ROS levels in glutathione peroxidase-1 and neutrophil cytosolic factor-1 knockout C57BL/6 mice are associated with Treg hyperactivity. Also, hyperbaric oxygen therapy or chemicals, such as 2,3-dimethoxy-1,4-naphthoquinone and N-acetylcysteine (NAC) promote Treg activity (77). Furthermore, deficiency of peroxiredoxin (Prx) II, an intracellular antioxidant molecule stabilizes the expression of FoxO1, a transcription factor important for FoxP3 gene transcription, and leads to the increased FoxP3 expression in Treg and an increased number of Treg cells in PrxII-deficient C57BL/6 mice (169). The high selenium diet (enables high operability of selenoproteins) favors Th1 differentiation and activation leading to higher IFN-γ and CD40 ligand levels (60). Interesting observations were described in BDC-2.5 mice (that possess autoreactive T cells against beta cell autoantigen - A^{g7} mimotope) that serve as an inducible model of type 1 diabetes. When made deficient in NOX-derived superoxide, $CD4^+$ T cells from these mice skewed their phenotype towards pro-inflammatory Th1 while Treg were impaired in their activity (115). The concomitant increase in Th17 (on account on reduced Th1) was observed in NOD mice that spontaneously develop type 1 diabetes and were made deficient in NOX-2 (153, 158).

The discrepancy observed in potentiation of either Th1 or Th17 in the absence of superoxide is probably related to the difference in the immune response of used mouse strains.

It seems that Treg have developed ways to survive in the presence of extracellular high ROS levels. It is known that ROS are detrimental to T cells in general (53). The ablation of selenoproteins (specifically in T cells) leads to the inability to generate mature T cells and oxidant hyperproduction in T cells thereby suppressing their proliferation activated through TCR (139). Treg are the most resistant cells (compared to effector and memory T cells) to external ROS influence (106). The reason for such Treg resilience does not lie in higher production of antioxidant enzymes or scavenging molecules. It is rather a result of sustained expression of anti-apoptotic Bcl-2 under pro-oxidant treatment (106) or high levels of surface thiols (107). Extracellular ROS may favorably impact Treg function (68). The generation of functional iTreg at the periphery is dependent on ROS derived from macrophages (Fig 5). It was shown that ROS produced by NOX2 complex in macrophages induce iTreg in humans and rats in vitro, as well as in rats in vivo (86). One of the potential mechanisms of ROS-mediated stimulation of Treg differentiation is the inhibitory influence of extracellular ROS on mTORC1 complex. As mTORC1 inhibition by rapamycine is a well-known approach for enhancing Treg differentiation in vitro (23), long-term ROS exposure or high concentrations or ROS that lead to AMPK-mediated phosphorylation of Raptor (an adaptor protein in mTORC1complex that negatively regulates its activity) could result in increased Treg differentiation (91). In addition, our unpublished data suggest that the presence of H_2O_2 stimulates differentiation of Treg in vitro. Briefly, the addition of H_2O_2 (1-5 μM) 48 h after TCR stimulation of naïve $CD4^+CD25^-$ cells (in the presence of Treg growth factor IL-2 and differentiation factor TGF-β) increased the proportion of differentiated Treg. However, this effect was absent when H_2O_2 was added simultaneously with stimulation cocktail (unpublished). We could speculate that the possible reason for such resistance to extracellular

ROS is the need for prolonged Treg survival at the site of inflammation where oxidative burst occurs and where they play a role in the termination of inflammation.

Another scenario for increased Treg function upon ROS exposure is the tumour microenvironment. Namely, ROS derived from the tumour cells increase apoptosis in Treg, but induce their suppressive properties at the same time (94). This finding is corroborated by in vitro experiments where Treg treatment with either H_2O_2 or ovarian ascites (abundant in superoxide) leads to Treg apoptosis. The suppressive effect of dying Treg is mediated by increased ATP release and generation of high levels of immunosuppressive adenozin via actonucleotidases CD39 and CD73 (94). Apoptosis in Treg cells in tumour surroundings is attributed to their weak nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-associated antioxidant system compared to T conventional cells both in mice and humans (94). These results are in contrast to the ones obtained in our laboratory. The difference in Treg resistance to extracellular H_2O_2 may correspond to different Treg origins (from tumour environment or healthy mice) and also whether exposure to pro-oxidants happens during Treg differentiation from naïve cells, or when they are fully differentiated.

ROS-targeted strategies for DC manipulation

Immunotherapy based on the application of tolDC is a promising novel approach for the treatment of autoimmune diseases (152). A great number of studies in animal models of MS, T1D and RA showed the efficiency of tolDC in ameliorating autoimmunity (144). These results enabled numerous on-going clinical trials that investigate tolDC application in these diseases (Table 1).

There are several different approaches for the in vitro expansion of human tolDC for the therapy. The most common way to obtain tolDC is to culture peripheral blood monocytes in the presence of granulocyte-macrophage colony-stimulating factor and IL-4, along with tolerizing agents, such as dexamethasone or vitamin D3 (17, 159). The first clinical trial using tolDC to treat autoimmunity was performed by Giannoukakis and colleagues in T1D (clinicaltrials.gov identifier: NCT00445913) (50, 122). Since then, 10 clinical trials with tolDC application for the treatment of MS, T1D and RA have been performed (Table 1).

Having in mind that ROS have a profound influence on tolDC, ROS-targeted strategies to modify these cells for the therapeutic benefit in autoimmunity is being considered. Modulation of ROS production in conjunction with the tolerization of DC has already been documented (Fig 4). Some of the examples follow. Numerous studies have shown that glucocorticoid dexamethasone and vitamin D3 are strong tolerogenic modulators of DC (17, 159). It was reported that ROS production in dexamethasone-treated DC was higher than in the untreated human monocyte-derived DC (49). The link between ROS and tolerogenic function of DC might be found in ROS-driven increase of intracellular zinc level through the modulation of proteins involved in its cellular availability (128). The rise in zinc is usually associated with immunosuppression. For example, zinc is involved in the limitation of the production of the pro-inflammatory cytokines (IL-1β, TNF and IL-6) in myeloid cells (165). Also, the generation of DC in the presence of dexamethasone was associated with an enhanced level of $p47^{phox}$ expression proposing that ROS production in DC is regulated by NOX2 (85). Similarly, higher ROS production is observed in human monocyte-derived DC grown in the presence of vitamin D3 (45, 158).

In contrast to ROS-stimulating approaches, tolerogenic modulation of DC activity could be accomplished by antioxidants such as ascorbate (vitamin C) and α-tocopherol (vitamin E). It

was reported that eIF-2, NF-κB, protein kinase C, and p38 MAPK signalling pathways were suppressed in DC treated with vitamin C and vitamin E, alone or in combination (150). Consequently, the treated DC acquired phenotypic and functional tolerogenic properties. Importantly, reduced ROS levels are detected in the cells (150).

Pyruvate and its derivative ethyl pyruvate are well-known antioxidants and potent ROS scavengers in the cell (78). Ethyl pyruvate is a stable derivate of pyruvate that easily enters the cell and can be transformed into pyruvate. Our interest in ethyl pyruvate has arisen from its redox similarities to dimethyl fumarate, the active compound of MS drug tecfidera (136). Dimethyl fumarate acts on redox processes through Nrf2 (93, 21). Nrf2 is the master redox regulator, as it controls the expression of various genes involved in the antioxidant protection such as: glutathione-S-transferase, NAD(P)H quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1) and others (84). Both dimethyl fumarate and ethyl pyruvate have been shown to interfere with DC maturation (120), and differentiation (34). Ethyl pyruvate reduced the expression of MHC II, co-stimulators (CD40, CD86) and pro-inflammatory cytokines (IL-1β, TNF, IL-6, IL-12) in murine and human DC (34). Importantly, ethyl pyruvate tolerogenic influence was operative in the cells obtained from healthy subjects and MS patients alike (34). Furthermore, ethyl pyruvate-treated DC restrained T cell proliferation and cytokine production in the alloreaction. Ethyl pyruvate-treated DC had an elevated level of proteins involved in the cellular response to ROS, including Nrf2 and HO-1 (submitted elsewhere). These findings are in agreement with a recent report that ethyl pyruvate decreases glycolysis and mitochondrial respiration and NO production in DC (22). Therefore, ethyl pyruvate may contribute to the DC tolerogenicity due to its redox properties (Fig 6).

ROS-directed strategies for Treg manipulation

Redox regulation of Treg can be achieved both in vivo and in vitro. As Treg are resilient to high ROS levels, the substances with anti-oxidative capacity would theoretically disturb Treg function. Few studies indicate plant-derived polyphenols or polyphenol-rich extracts with high antioxidant properties as Treg reducers. For example, resveratrol was found to exert a suppressive effect on tumour-derived Treg cells in mice (173), and in healthy humans following 28-day long consumption (39). Our research on black chokeberry water extract (extremely rich in flavonoids) had shown similar results, as the proportion of Treg was significantly lower after 14 days long administration to tumour bearing mice (unpublished data). However, ample of data suggest that in vivo antioxidant application results in a decrease of autoimmunity or inflammation through the stimulation of Treg. This effect of antioxidant on Treg could be attributed to other ROS-unrelated events. As it is reasonable to assume that the addition of pro-oxidant compounds in vivo is not recommended due to various side effects, one of the possible approaches could be stimulation of Treg activity in vitro by the administration of oxidative compounds.

In vitro redox manipulation aimed at enhancing Treg function or number could be an alternative for potential use in therapy. Treg are usually obtained from peripheral blood mononuclear cells or umbilical cord. For clinical trials, the common methodology includes expansion of isolated polyclonal cells in vitro in the presence of TCR stimulators, without any further manipulation (35). Research on Treg expansion in animal models has utilized the application of various Treg stimulators such as all-trans retinoic acid (ATRA), rapamycin or IL-2 to prevent the outgrowth of contaminant effector T cells. Although it is assumed that autoantigen-specific Treg would exert more potent suppression in the specific autoimmune disease, their application has been so far limited to animal models (35). There are 8 clinical trials for the treatment of T1D, already finished or on-going, that are exploring the safety of the Treg application (Table 1).

Similarly to tolDC, Treg cells can be affected by the action of antioxidants (Fig 5). ROS are important for Treg activity, but also excessive ROS production may be detrimental for Treg function. Therefore, the effect of ROS deprivation has conflicting effects on Treg biology. Apart from ROS reduction, antioxidants may also affect the cellular signalling pathways that are directly or indirectly important for the Treg activity.

In line with the data that ROS are mandatory for the proper function of Treg, down-regulation of ROS with vitamin C treatment goes hand in hand with the reduced ability of nTreg to suppress effector T cell proliferation in vitro. However, when vitamin C is administered in vivo, it enhances TGFβ-induced $Foxp3^{+}$ iTreg differentiation (114). A similar effect is exerted by vitamin A metabolite, ATRA (150). Vitamin C also facilitates induction of a $FOXP3^{high}$ iTreg population in human naïve T cells. Both vitamin C and A promote Treg stability even in the presence of Th17-polarizing conditions in vitro and enhance their suppressive properties (109, 114). Vitamin D3 has been shown to change memory Th cells isolated from healthy or RA individuals into regulatory cells that produced anti-inflammatory factors, including IL-10 and CTLA4 and exhibited strong suppressive activity (32).

Superoxide is known to support promotor hypermethylation generally through recruitment of DNA methyltransferase (DNMT), an enzyme that transfers methyl groups to DNA and inhibits gene expression (58). It is shown that vitamin C stabilizes Foxp3 expression by promoting demethylation of specific regions of conserved non-coding DNA sequence 2 (CNS2) in FoxP3 locus. Also, vitamin C can enhance demethylation and subsequent expression of CTLA-4 and Eos in antigen-specific iTreg, preventing their conversion into inflammatory ex-Foxp3 iTreg (74). By promoting hypomethylation of the Treg-specific demethylated region in the FoxP3 gene, phospho-vitamin C can support conversion of γδ T cells (potent cytotoxic effector cells) into Treg (83). This effect was accompanied by

increased suppressive activity as determined by up-regulated inhibitory action on Teff proliferation. Phospho-vitamin C also provoked better stability of converted Treg as they still kept their phenotype on day 14 after conversion (83). Another antioxidant, vitamin E, is a natural inhibitor of DNMT and therefore may be involved in the promotion of FoxP3 expression as well. These vitamins may have a dual role in Treg biology, through ROS scavenging or through the influence on the Treg signalling pathways.

Intracellular metabolites that have scavenger activity are also related to Treg status. α-ketoglutarate is a product of glutamine catabolism and a well known intracellular scavenger. It has been found that treatment of naïve cells with glutamine prevents differentiation to Treg and rather favours Th1 (79).

A completely different scenario occurs when naïve $CD4^+$ T cells are exposed to ethyl pyruvate. Our results indicate that ethyl pyruvate stimulates proliferation of healthy murine Treg in vitro and in vivo (Fig 6). The addition of ethyl pyruvate (125 μM) to purified $CD4^+$ T cell cultures that have been already exposed to Treg differentiation cocktail (anti-CD3 and anti-CD28 antibody, IL-2 and TGF-β) for 48 h, stimulates Treg proliferation. This effect is mediated through enhanced glycolysis and correlates with the increased proportion of ROS^+ cells. Although ethyl pyruvate is ROS scavenger, the observed up-regulation in ROS^+ cells is a reflection of the increased number of activated Treg (unpublished data). The stimulatory effect of ethyl pyruvate (100 mg/kg body weight) on Treg proliferation was also evident after in vivo administration to healthy (unpublished data) or diabetic C57BL/6 mice. In addition, both suppressive activity and Treg migration were enhanced after intraperitoneal application of ethyl pyruvate during multiple low-dose streptozotocin-induced T1D pathogenesis in mice (compared to control group of mice that received equal volumes of Hartmann solution) (81).

Conclusion and future perspectives

ROS appear to be crucial for the function of T cells and DC acting as signalling molecules involved in the activation and differentiation of these cells. Overall, the literature suggests that ROS produced by NOX-dependent reactions are generally involved in the elimination of infectious agents by phagocytes, while those produced at enhanced energy demands (activation, proliferation, differentiation) are involved in the maintenance of DC and Treg function and metabolism. Although tolDC and Treg are extremely resistant to ROS-induced damage, in the circumstances of uncontrolled ROS production from the mitochondria, the initiation and progression of inflammatory and autoimmune diseases can occur. For instance, T1D etiopathogenesis is highly associated with oxidative stress in the pancreas (115). Thus, it does not come as a surprise that antioxidants, e.g. NAC or dimethyl sulfoxide (DMSO) prevent or alleviate T1D and promote survival of transplanted pancreatic islets in NOD mice (9, 92). Pathogenesis of MS is also linked to the enhanced systemic oxidation levels and antioxidants, such as vitamin E, C, D, A that have been suggested to prevent or counteract oxidative damage in the disease (54, 105, 127). While their potency to be used as supplementary therapeutic agents is yet to be determined, another redox-active compound dimethyl fumarate proved to be a disease-modifying drug for MS (136). It is important to test whether the observed beneficial effects of antioxidants are associated with the increased fitness of Treg and tolDC, as already suggested for DMSO or retinoic acid and Treg (92, 145). Also, dimethyl fumarate and its redox analogue ethyl pyruvate have already been investigated as tolerizing agents for DC (22, 34, 120). Further studies on the tolerizing potential of other antioxidants are certainly of interest. Also, an open question remains: Why some antioxidants and scavengers promote, while others inhibit Treg? This seems to largely depend upon the Treg subtype, microenvironment and the concentration of extracellular ROS. Finally, there is no evidence what artificial pro-oxidants could do for the fitness of Treg. Further studies

devoted to unrevealing the complex interplay between anti- and pro-oxidant regulation of Treg and DC are warranted.

Abbreviations

ATRA – All-trans retinoic acid; CNS2 – conserved non-coding DNA sequence 2; DC – dendritic cells; Dex – dexamethasone; DMSO – dimethylsulfoxide; DNMT – DNA methyltransferase; FoxP3 – forkhead box p3; HO-1 – heme oxygenase-1 ; IFN – interferon; iTreg – peripheral (induced) regulatory T cells; LPS – lipopolysaccharide; MHC – major histocompatibility complex; MS – multiple sclerosis; NAC – N-Acetylcysteine; NF-κB – nuclear factor κB ; NO – nitric oxide; NOD – non-obese diabetic; NOX – NADPH oxidase; NQO1 – NAD(P)H quinone oxidoreductase 1 ; Nrf2 – nuclear factor (erythroid-derived 2)-like 2; nTreg – natural regulatory T cells; OXPHOS – oxidative phosphorylation; Prx – peroxiredoxin ; RA – rheumatoid arthritis; ROS – reactive oxygen species; T1D – type 1 diabetes; TCR – T cell receptor; TGF – transforming growth factor; Th – T helper ; tolDC – tolerogenic dendritic cells; Treg – regulatory T cells

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Figure legends

Figure 1 CD4$^+$ T cell activation by DC. Naïve CD4$^+$ T cells, including natural Treg (nTreg) leave the thymus. Dendritic cells are professional antigen-presenting cells, *i.e.* they have the ability to activate naïve T cells. Dendritic cells present antigens to CD4$^+$ T cells on their MHC class II molecules. Depending on the co-stimulatory signal provided by DC and cytokines (produced by DC and other immune cells) present in the extracellular environment, CD4$^+$ T cells are directed to differentiate into various T helper (Th) subpopulations. Also, they can differentiate into induced Treg (iTreg). These subpopulations express specific transcription factors, produce specific set of cytokines and express specific surface receptors.

Figure 2 DC and T cells in autoimmunity. The activation of auto-reactive cells takes place in the lymph nodes. DC activate T cells, while Treg and tolDC actively suppress this process. In the case of ineffective suppression, activated auto-reactive T cells migrate to the target organs where they mediate tissue destruction in interaction with the local immune cells, such as macrophages (Mf) and microglia (Mg). Treg are also attracted to and accumulate in the affected tissue in an attempt to suppress the autoimmune response.

Figure 3 TolDC in humans and experimental models. TolDC are present at mucous barriers where they control tolerance to the non-self antigens. Animal and human studies have provided insight into the possible phenotypes of tolDC in the eye, the skin, the lung, and the gut. Also, murine experimental models of human autoimmune diseases have provided data on the phenotype of tolDC in autoimmunity (EAE, T1D, RA).

Figure 4 Effects of ROS and antioxidants on DC. DC produce ROS by NOX2 in response to pathogen-associated molecular patterns (PAMP), such as LPS. PAMP bind to their receptor, such as TLR. ROS produced by NOX2 in the phagosomes has a dual role in the process of antigen processing that provides antigens for presentation in the complex with

MHC molecules. They regulate pH within the phagosomes, thus allowing for efficient antigen processing. On the contrary, they inhibit cathepsin (Cts), thus inhibiting antigen processing. NOX2 produced in the plasma membrane interferes with T cell cofilin, inducing T cell hyporesponsiveness and death. OXPHOS ROS generation is inhibited during the process of DC maturation, NO generated by iNOS playing the major role in the inhibition. Well known tolerizing agents, dexamethasone and vitamin D3, increase ROS generation in DC. Antioxidants vitamin C and E down-regulate ROS in DC, although at the same time they have tolerizing effects on DC.

Figure 5 Effects of ROS and anti-oxidants on T cells. ROS generated by NOX2 in Treg, or by other cells in the surroundings (predominantly macrophages), stimulates expression of CTLA4, the release of TGF-□ and FoxP3 gene expression. TCR crosslinking leads to Ca_2^+ influx from the endoplasmic reticulum and consequent activation of OXPHOS in the mitochondria. ROS generated in this way contributes to the activation of NFkB and subsequent expression of IL-2, which is essential for T cell proliferation. Intracellular ROS inhibit IFN-□ in activated T cells, thus shifting Th cells from Th1 to other Th populations. Vitamin A and C stimulate differentiation of iTreg and contribute to their stability through up-regulation of CTLA-4 and Eos (a zinc finger transcription factor). Vitamin C contributes to demethylation of the FoxP3 CNS2 promoter region, and vitamin E inhibits DNMT, both effects promoting expression of FoxP3 and its stabilization. □-ketoglutarate inhibits differentiation of naïve T cells into iTreg. Vitamin C and resveratrol inhibit, while 2,3-dimethoxy-1,4-naphthoquinone and N-acetylcysteine potentiate nTreg suppressive activity.

Figure 6 Effects of EP on T cells and DC. *In vivo* ethyl pyruvate promotes the migration of tolDC into the target tissue and induces $CD4^+CD25^{high}$ Treg proliferation ($Ki67^+$), activation

($CD44^+$), and increases the proportion of Treg ($Tbet^+$) that actively suppress Th1. Also, ethyl pyruvate enhances Treg migration through up-regulation of CD103 (a ligand for E-cadherin), CD11a (a part of adhesion molecule leukocyte function-associated antigen 1), CXCR3 (a chemokine receptor for migration into the pancreas). Ethyl pyruvate increases Treg suppressive properties through stimulation of CTLA-4 inhibitory molecule expression and IL-10 and TGF-β production. Although ethyl pyruvate is not affecting ROS production *in vivo*, it specifically decreased OXPHOS and nitric oxide (NO) in DC *in vitro*. Further, ethyl pyruvate induces tolerogenic profile of DC *in vitro* through down-regulation of pro-inflammatory transcription factor NF-κB, serine/threonine-protein kinase AKT and extracellular signal-regulated kinases (ERK) and subsequent production of pro-inflammatory cytokines IL-6, IL-1β and TNF. Concomitantly, ethyl pyruvate increases Nrf2 (a transcription factor that regulates the expression of anti-oxidant enzymes) and anti-inflammatory cytokine IL-10. Similarly, ethyl pyruvate stimulates differentiation of Treg *in vitro*, increases their suppressive properties through up-regulation of CTLA-4, programmed cell death protein 1 (PD-1), glucocorticoid-induced TNFR-related protein (GITR) and IL-10. Ethyl pyruvate down-regulates pyruvate dehydrogenase kinase (PDK) and subsequently enhances pyruvate dehydrogenase (PDH) activity, thus supporting Treg stability in inflammatory conditions.

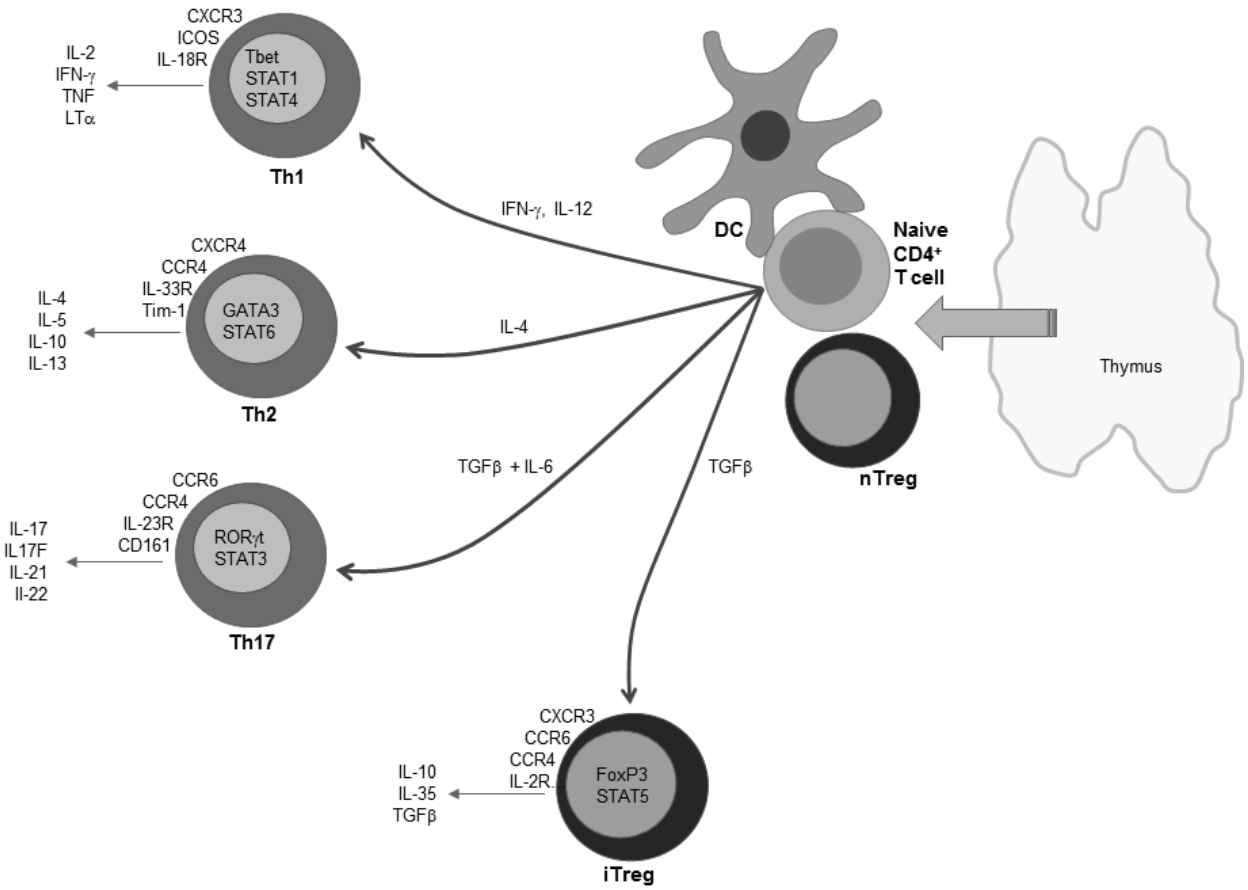

CXCR3
ICOS
IL-18R
Tbet
STAT1
STAT4
IL-2
IFN-γ
TNF
LTα
Th1
IFN-γ, IL-12
DC
Naive
CD4+
T cell
Thymus
CXCR4
CCR4
IL-33R
Tim-1
GATA3
STAT6
IL-4
IL-5
IL-10
IL-13
Th2
IL-4
nTreg
TGFβ + IL-6
TGFβ
CCR6
CCR4
IL-23R
CD161
RORγt
STAT3
IL-17
IL17F
IL-21
Il-22
Th17
CXCR3
CCR6
CCR4
IL-2R
FoxP3
STAT5
IL-10
IL-35
TGFβ
iTreg

Fig. 1

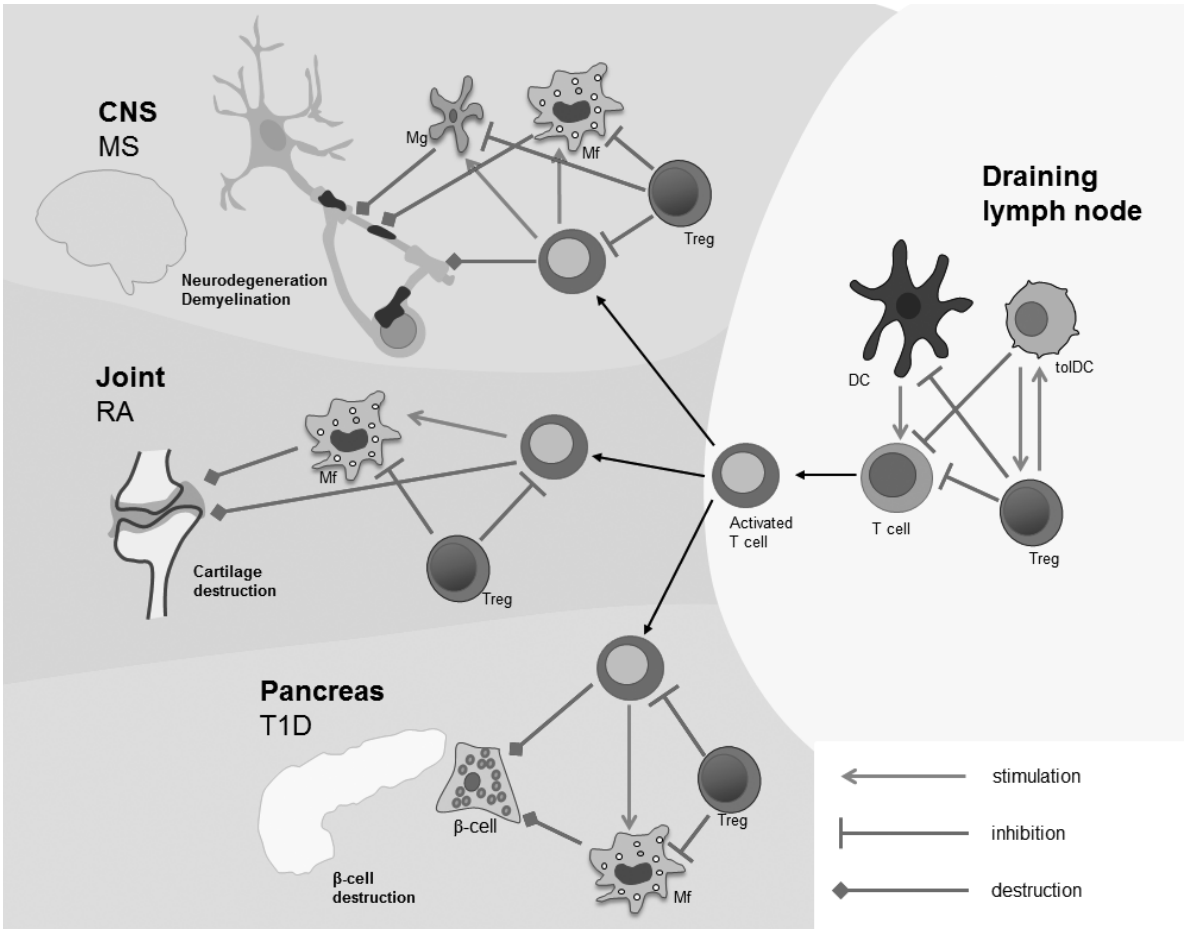
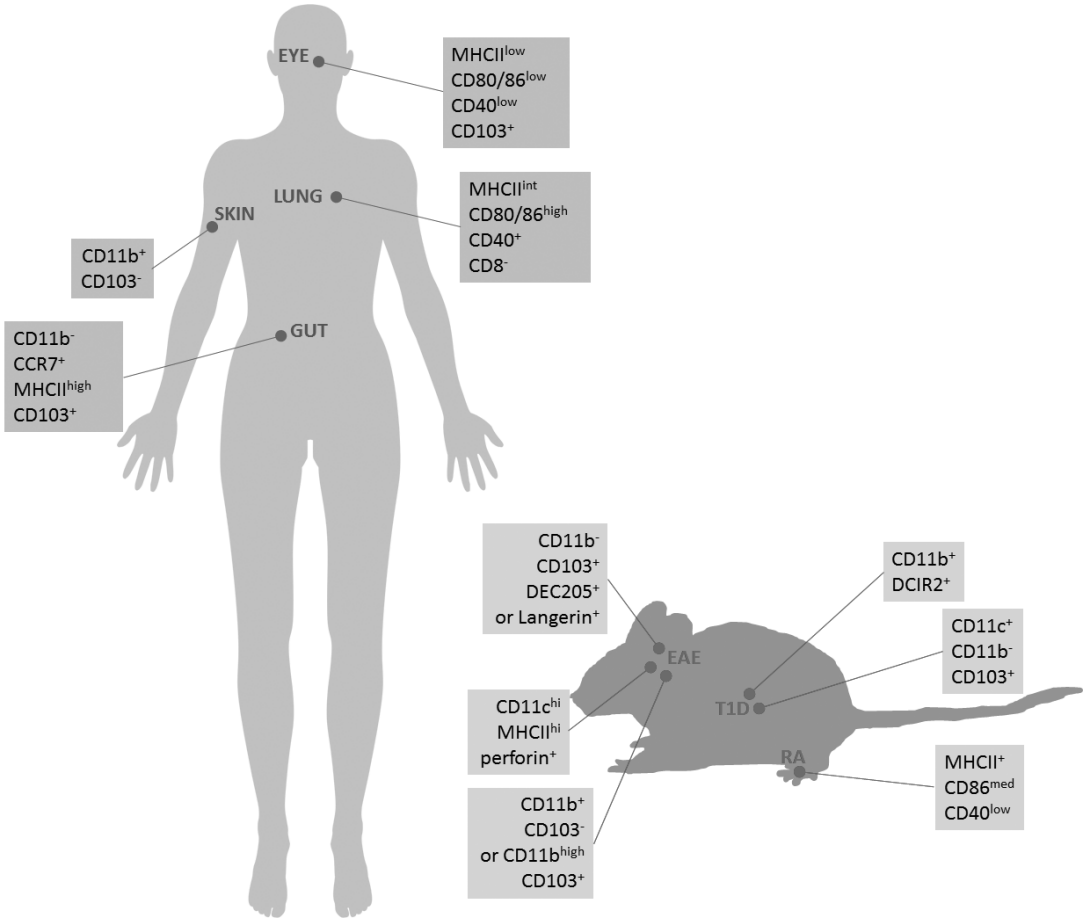

CNS
MS
Mg
Mf
Treg
Neurodegeneration
Demyelination
Draining
lymph node
DC
tolDC
Joint
RA
Mf
Cartilage
destruction
Treg
Activated
T cell
T cell
Treg
Pancreas
T1D
β-cell
Treg
β-cell
destruction
Mf
stimulation
inhibition
destruction

Fig. 2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CD80/86^{low}
CD40^{low}
CD103^{+}
LUNG
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CD80/86^{high}
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CD8^{-}
SKIN
CD11b^{+}
CD103^{-}
GUT
CD11b^{-}
CCR7^{+}
MHCII^{high}
CD103^{+}
CD11b^{-}
CD103^{+}
DEC205^{+}
or Langerin^{+}
CD11b^{+}
DCIR2^{+}
CD11c^{+}
CD11b^{-}
CD103^{+}
EAE
CD11c^{hi}
MHCII^{hi}
perforin^{+}
T1D
RA
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CD86^{med}
CD40^{low}
CD11b^{+}
CD103^{-}
or CD11b^{high}
CD103^{+}

Fig. 3

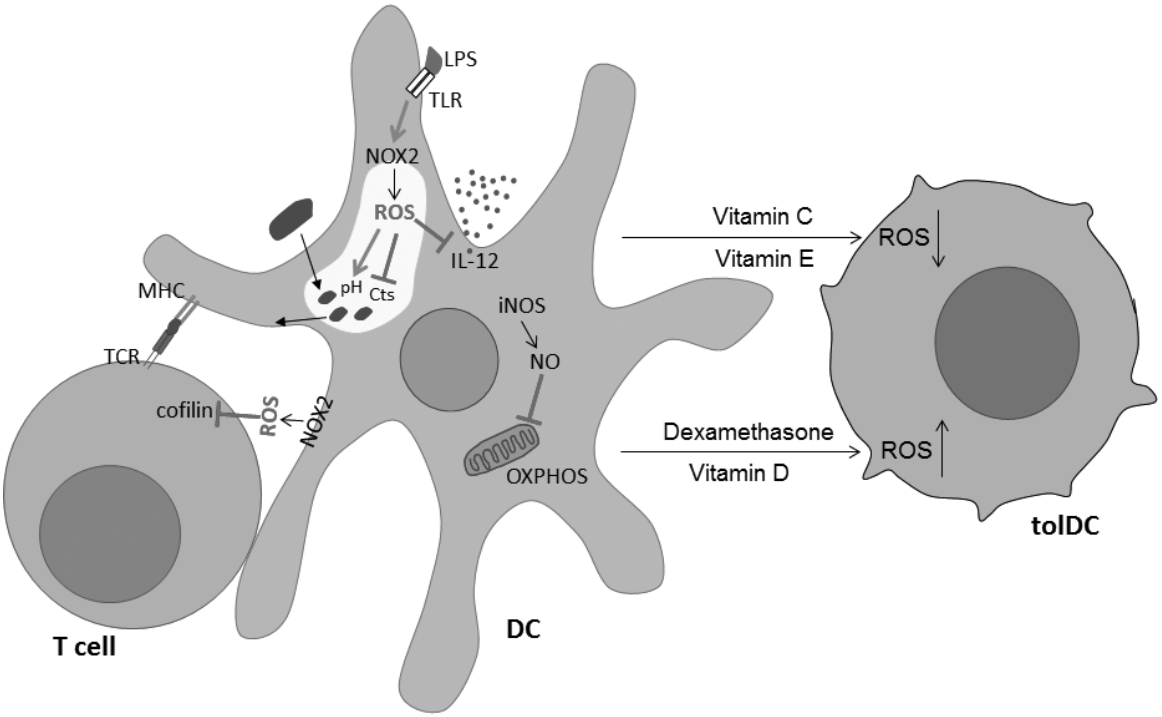
LPS
TLR
NOX2
ROS
IL-12
pH
Cts
MHC
TCR
iNOS
NO
cofilin
ROS
NOX2
OXPHOS
T cell
DC
Vitamin C
Vitamin E
ROS
Dexamethasone
Vitamin D
ROS
tolDC

Fig. 4

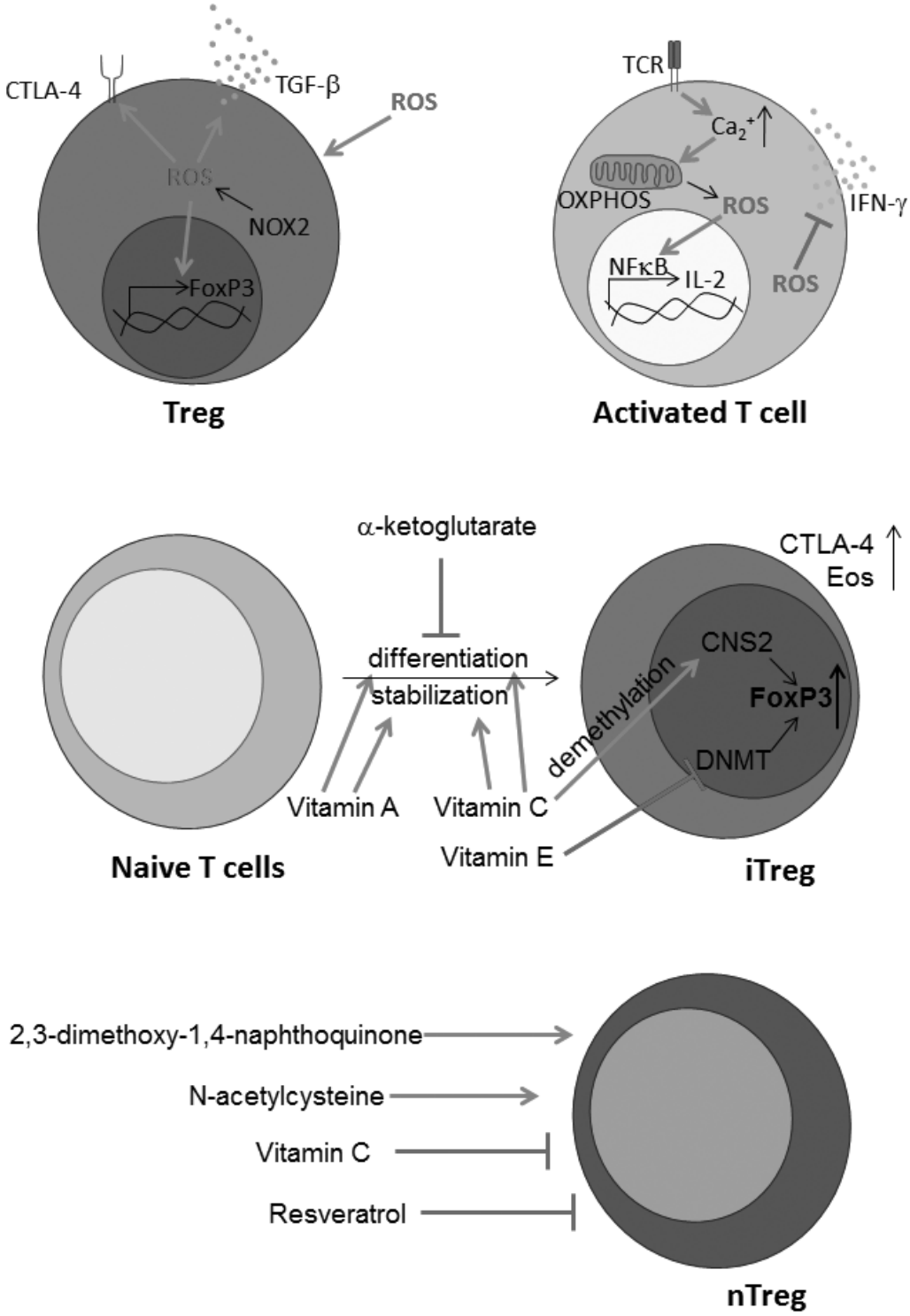
CTLA-4
TGF-β
ROS
ROS
NOX2
FoxP3
Treg
TCR
Ca2+
OXPHOS
ROS
IFN-γ
NFκB
IL-2
ROS
Activated T cell
α-ketoglutarate
differentiation
stabilization
Vitamin A
Vitamin C
Vitamin E
Naive T cells
CTLA-4
Eos
CNS2
FoxP3
DNMT
demethylation
iTreg
2,3-dimethoxy-1,4-naphthoquinone
N-acetylcysteine
Vitamin C
Resveratrol
nTreg

Fig. 5

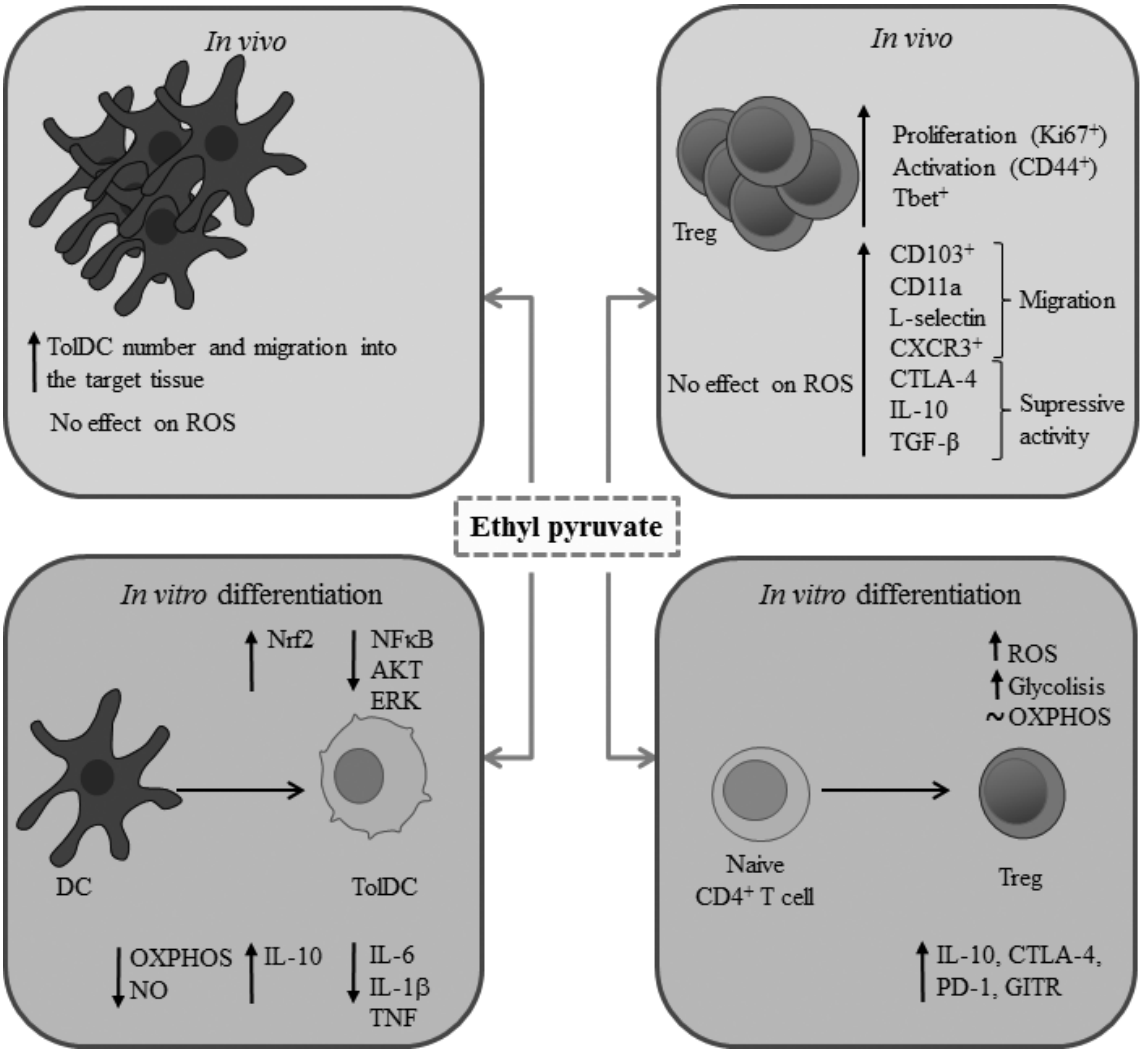
In vivo
TolDC number and migration into the target tissue
No effect on ROS
In vivo
Treg
Proliferation (Ki67+)
Activation (CD44+)
Tbet+
CD103+
CD11a
L-selectin
CXCR3+
Migration
No effect on ROS
CTLA-4
IL-10
TGF-β
Supressive activity
Ethyl pyruvate
In vitro differentiation
Nrf2
NFκB
AKT
ERK
DC
TolDC
OXPHOS
NO
IL-10
IL-6
IL-1β
TNF
In vitro differentiation
ROS
Glycolisis
~OXPHOS
Naive CD4+ T cell
Treg
IL-10, CTLA-4, PD-1, GITR

Fig. 6