Published in final edited form as:
Biochemistry. 2010 February 16; 49(6): 1059. doi:10.1021/bi902022n.

Redox Remodeling as an Immunoregulatory Strategy†

Zhonghua Yan and **Ruma Banerjee***
Department of Biological Chemistry, University of Michigan Medical Center, University of Michigan, Ann Arbor, MI 48109-5606

Abstract

Activation and proliferation of T cells require a reducing extracellular microenvironment in the immune synapse that is provided by antigen presenting cells especially dendritic cells. Stimulation of dendritic cells by T cells activates the NF-κB pathway in dendritic cells and induces an antioxidant response. It also enhances system x_c^--dependent cystine uptake, leading to increased glutathione synthesis, export and finally, degradation to cysteine outside the cell. Accumulation of extracellular cysteine supports glutathione synthesis in T cells while also leading to a more reducing redox potential that is needed for T cell proliferation. Naturally occurring regulatory T cells, a suppressor sub-population of T cells, prevent autoimmune diseases and maintain peripheral tolerance by suppressing self-reactive effector T cells. They also suppress beneficial immune responses to parasites, viruses and tumors. However, their mechanism of suppression is still not fully understood. Recently, we have found that inhibition by regulatory T cells of dendritic cell-induced extracellular redox remodeling is a component of the regulatory T cell suppression mechanism. In this review, we describe recent advances in our understanding of redox regulation and signaling in the adaptive immune system with a focus on T cell activation by dendritic cells. The role of regulatory T cells in perturbing redox remodeling by dendritic cells and its implications as a general regulatory T cell suppression mechanism are discussed.

Discriminating self from non-self is a primary function of the immune system and regulatory T cells play a cardinal role in maintaining self-tolerance and preventing autoimmunity by mechanisms that remain to be fully elucidated (1-3). T cells derive their name from the thymus, the organ in which they mature and are distinguished by the presence of T cell receptors (TCRs)[1]. The latter recognize antigens bound to the major histocompatibility complex (MHC) molecules on antigen presenting cells (MHC class II) or target cells (MHC class I). Like most immune cells, T cells are initially derived from hematopoietic stem cells in the bone marrow where progenitor T cells are formed. They migrate to the thymus via the bloodstream to mature via positive and negative selection processes (4). “Naïve” T cells released from the thymus have yet to encounter an antigen and are in the G0 stage of the cell cycle. They circulate through the vascular system to secondary lymphoid tissues such as lymph nodes where they may encounter antigen-MHC complexes and in the process, become activated. Activated T cells play important roles in cell-mediated functions in the adaptive immune system and their dysfunction is manifest in a number of immune diseases (5).

Antigen presenting cells such as dendritic cells (DCs), macrophages and B cells express MHC class II molecules and co-stimulatory molecules on their membranes, and specialize in presenting antigens to naïve $CD4^+$ T cells. DCs are the most potent professional antigen presenting cells (6) and originate from hematopoietic stem cells in the bone marrow. Precursor DCs are released from the bone marrow and circulate in the bloodstream to different tissues

†This work was supported in part by a grant from the National Institutes of Health (DK64959).

*Corresponding author. Tel: (734)-615-5238, rbanerje@umich.edu.

where they reside as immature DCs until they encounter antigens. Once internalized, antigens are processed and then displayed on MHC class II molecules and the resulting mature DCs migrate to lymph nodes where they interact with and activate antigen-specific T cells, which subsequently proliferate and differentiate into effector T cell subsets (4,7,8).

Classically, three sets of signals in the immune synapse are recognized to be essential for priming naïve T cells: (i) specific engagement of the TCR by an antigen-MHC class II complex, (ii) interactions between co-stimulatory molecules, CD28 on T cells and CD80/86 on the antigen presenting cells, and (iii) secretion of cytokines (Fig. 1A). These signals result in the activation, survival and differentiation of T cells. In addition to these signals, T cell activation and proliferation requires a reducing microenvironment that is achieved by cysteine secretion from antigen presenting cells (9,10). The physiological relevance of redox remodeling by antigen presenting cells for T cell activation is demonstrated by the hyporesponsiveness of T cells from normal gut, which results from the inability of mucosal macrophages to provide a reducing microenvironment and contrasts with the presence of this capacity in peripheral blood monocytes in the same organism (11,12). Under conditions of chronic mucosal inflammation as seen in inflammatory bowel disease, ulcerative colitis and Crohn's disease, recruitment of peripheral blood monocytes results in sustained antigen-driven responses of T cells in the gut and is believed to be important in the etiology of these diseases (12).

Regulatory T cells are critical mediators of self-tolerance and immune homeostasis (2,3). Mutations in the transcriptional regulator, Foxp3, which is preferentially expressed in regulatory T cells (13), results in multiorgan autoimmune diseases and are fatal (14). Enrichment of regulatory T cells suppresses autoimmune responses, promotes tolerance to organ grafts and feto-maternal tolerance. On the other hand, depletion of regulatory T cells augments tumor and microbial immunity while provoking autoimmunity and inflammatory bowel disease. The co-receptor, cytotoxic T lymphocyte antigen 4 (CTLA-4) (15), expressed preferentially on regulatory T cells, interacts with CD80/CD86 (Fig. 1B), i.e. the same ligand that binds CD28 expressed on naive T cells. However, CTLA-4 interacts with CD80/CD86 with much higher affinity and suppresses induction of CD80/86 expression by antigen-specific T cells, consequently limiting the capacity for activating naïve T cells (16). Given the importance of regulatory T cells in controlling autoimmunity and inflammation and their influence on tumor and microbial immunity, elucidation of the mechanisms by which these cells exert their effects have important implications for therapeutic target identification and development of intervention strategies. In this article, we describe recent insights into the significance of intercellular redox signaling during activation of naïve $CD4^+$ T cells by antigen presenting cells and the perturbation of this circuitry by regulatory T cells (10).

Redox Potentials in the Intra- and Extra-Cellular Compartments

The cytoplasmic and extracellular redox potentials are vastly different and influence the structure, stability and function of the macromolecules that reside in each compartment. Within the intracellular compartments, several redox buffers exist e.g. thioredoxin (Trx), glutathione (GSH) and cysteine and the relative concentrations of their oxidized versus reduced species sets the ambient redox poise for the system. Interestingly, the individual redox systems appear to be under kinetic control, are not in equilibrium with each other and independently regulate the redox status of their client redox partners (17,18). Quantitatively, GSH is the major intracellular redox buffer and is found at concentrations ranging from 0.5-10 mM in mammalian cells (19). The intracellular GSH/GSSG (glutathione disulfide) redox potential in dividing cells is estimated to range from -260 mV to -230 mV and is progressively more oxidized in cells undergoing differentiation/growth arrest (-220 mV to -190 mV) or apoptosis (-170 mV to -150 mV) (17) (Fig. 2). The redox potentials for Trx1 in cytoplasm and nuclei are

~-280 mV and -300 mV, respectively while the redox potential of mitochondrial Trx2 is estimated to range from -360 mV to -340 mV (18).

Extracellularly, the cysteine/cystine couple represents the major thiol/disulfide redox buffer. Plasma cystine and cysteine concentrations are reported to be 100-200 μM and 10-25 μM respectively and a redox potential of ~-80 mV for this couple has been estimated for plasma in healthy humans (20). Paralleling the changes in the intracellular GSH/GSSG redox potential, increasing extracellular cysteine/cystine potentials are associated with cells undergoing proliferation (<-80 mV), differentiation/growth arrest (~-80 mV) or apoptosis (0 mV to -80 mV) (Fig. 2). An age-dependent increase in the extracellular redox potential has been reported, which is also influenced by lifestyle choices such as smoking and by diseases such as AIDS (17). In contrast to the intracellular compartment, the GSH concentration in the extracellular space is very low (2-4 μM in human plasma). A major fate of secreted GSH is cleavage to its component amino acids, glutamate, cysteine and glycine. The cysteine thus released is a major source of extracellular cysteine and cystine, which is formed rapidly in the oxidizing milieu of this compartment (21).

Although dynamic regulation of the extracellular redox potential, which is linked to intracellular metabolism, has an important bearing on cell function, it is less well-studied and appreciated than intracellular redox control and its perturbations in pathological states. Reactive cysteines on proteins can be reversibly oxidized to sulfenic acids or form disulfide bonds, which can induce changes in their structure and function and elicit downstream effects in redox signaling pathways (22,23). Disulfide bonds on ectodomains of membrane proteins and in secreted soluble and matrix proteins form a dynamic scaffold that can be reorganized by their shuffling or by their reduction (24). It has been proposed that a general loosening of the extracellular disulfide crosslink scaffold might precede cell division (25,26). Cancer cells typically have higher membrane thiol levels in comparison to nontransformed cells, and it is speculated that this might facilitate higher proliferative rates (27). The redox status of specific membrane proteins influences their transport or receptor activity (17). For instance, CD4 (cluster of differentiation 4), a glycoprotein found on the surface of helper T cells that is used as a receptor by HIV-1 for gaining entry, has a redox sensitive disulfide bond in one of its four immunoglobulin-like domains (D2). T cell activation shifts the equilibrium from the disulfide to the dithiol state (28). Locking the dithiols in the D2 domain by chemical modification blocks HIV-1 entry indicating that a redox-linked conformational change in CD4 is critical for viral penetration into T cells (28).

T Cell Induced Extracellular Redox Remodeling by Dendritic Cells

The physiological relevance of extracellular reductive modeling during an adaptive immune response is supported by the dramatic increase in free thiols in lymphoid tissue following immunization (29). Under these conditions, enhanced nonprotein thiol staining is observed both inside cells and in the extracellular space. In contrast, Peyer's patches from the gut show virtually no staining for nonprotein thiols under these conditions, consistent with the antigenic hyporesponsiveness of this intestinal microenvironment (11,12).

The magnitude of extracellular cyteine accumulation during activation of T cells increases with time and with the DC to T cell ratio and requires sustained contact between DCs and T cells. Increased cell surface thiols on T cells is correlated with increased production of the cytokine, IL-2, in vitro and enhanced proliferation in vivo (30). Naïve T cells require cysteine for GSH synthesis. However, cysteine is the least abundant of all amino acids in circulation (31) and naïve T cells are unable to import cystine efficiently due to the absence of the cystine transporter, x_C^- (32), thus creating a metabolic dependence on antigen presenting cells to meet their cysteine needs. Antigen presenting cells possess the x_c^- antiporter that uses the glutamate

gradient to drive import of cystine, which is subsequently converted to cysteine in the reducing intracellular milieu and is ultimately secreted into the extracellular space. In addition to stimulating cysteine secretion, the interaction between antigen presenting cells and T cells results in the appearance of extracellular Trx1 (9). Trx1 is secreted by several cell types via a nonclassical leaderless secretory pathway under conditions of oxidative stress and inflammation (33). Secreted Trx1 does not appear to play a direct role in reduction of extracellular cystine leading to cysteine accumulation during T cell activation (10). Extracellular Trx1 interacts in a redox-sensitive manner with the TNF receptor superfamily member 8 (34) and exhibits proinflammatory effects by stimulating cytokine release and proliferation of lymphocytes (33,35).

The pathway for extracellular cysteine accumulation during co-culture of DCs and naïve T cells has been mapped recently (10). In principle, two metabolic routes could be considered to lead to enhanced cysteine accumulation outside the cell (Fig. 3): (i) the transsulfuration pathway (blue), which provides an avenue for conversion of methionine to cysteine, and (ii) import of cystine into the cell where it is rapidly reduced to cysteine and converted to GSH, which is subsequently secreted and degraded by the ectoenzymes γ-glutamyltranspeptidase and a dipeptidase. Expression of the catalytic subunit of the system x_c^- transporter is induced when DCs during co-cultivation with naïve T cells and is correlated with increased extracellular cystine clearance (10). Metabolic labeling and pharmacological inhibition studies have established the involvement of the convoluted metabolic pathway originating in cystine and culminating in GSH-derived cysteine as the source of extracellular cysteine provided by DCs (10). This pathway demonstrates the dynamic interplay between the intra- and extra-cellular compartments for redox homeostasis via interconnected but independent redox nodes, i.e., GSH and cysteine.

The extracellular cysteine/cystine redox potential for DCs in culture is ~-80 mV, a value that is consistent for cells experiencing growth arrest (10). Naive T cells in culture that have not received activation signals are fated to undergo apoptosis and exhibit an extracellular cysteine/cystine redox potential of ~-45 mV. In contrast, when naïve T cells receive activation signals during co-culture with DCs, a more reducing extracellular environment reflected in a redox potential of -110 mV (at 36 h), results. This redox potential change is consistent with conditions that are conducive for T cell proliferation (10).

In addition to triggering intracellular signaling pathways, engagement of DCs and T cells during activation leads to dynamic changes in the redox status of exofacial proteins in both cell types. A 30 mV potential shift is expected to lead to a 10-fold change in the ratio of reduced:oxidized cysteines in proteins. Indeed, enhanced cell surface labeling of protein thiols with the fluorescent dye, Alexa-maleimide, is seen during co-culture of DCs and T cells (by ~4 and 8-fold respectively) as visualized by confocal microscopy and quantified by FACS analysis (10).

Redox Signaling During T cell Activation

Paralleling reductive remodeling of the extracellular redox poise with consequent effects on the exofacial protein thiol status and intracellular redox metabolism, is the initiation of a flurry of redox-active signaling across the immune synapse. The timing and balance between oxidative and reductive responses to engagement of antigen presenting cells and T cells are important for modulating activation, proliferation and apoptosis of T cells. At low levels, ROS (reactive oxygen species) e.g. H_2O_2 and $O_2^{\bullet-}$, are considered to be mitogenic and their downstream effects are commonly mediated via changes in protein phosphorylation and/or activation/inhibition of transcription factors (36). Crosslinking of the TCR and the co-stimulatory molecule, CD28, results in enhanced intracellular H_2O_2 production that is needed

for NF-κB activation and IL-2 and IL-2 receptor α chain gene transcription (37) and is consistent with an important role for ROS in the immediate early events during activation. Significant sources of ROS include membrane-bound NADPH-dependent oxidase, lipoxygenase and the mitochondrial respiratory chain (Fig. 4A, red arrows). However, sustained pro-oxidant conditions inhibit T cell proliferation and promote apoptosis (38).

During activation, increased ROS levels launch an early pro-oxidant response that is relayed via signaling pathways in antigen presenting cells and in T cells and result in activation of protein tyrosine kinases (e.g. Fyn, Src and Lck in T cells (Fig. 4B)), oxidative inhibition of protein tyrosine phosphatases e.g. SHP1 and activation of transcription factors e.g. NF-κB (39). The NF-κB pathway regulates the expression of various inflammatory genes including cytokines, chemokines and costimulatory molecules. We speculate that as a consequence of an initial increase in ROS levels by mechanisms that are not clear, NF-κB is activated in DCs and stimulates GSH biogenesis (via activation of γ-glutamylcysteine ligase (40)) (Fig. 4A, red arrows). Increased GSH synthesis is both an autocorrective reaction to oxidizing conditions and initiates the next response phase, i.e. an antioxidant wave (Fig. 4A, blue arrows). We hypothesize that the NF-κB signaling pathway is important for stimulating extracellular cysteine accumulation (10). The combined effect of these cellular responses would be the initiation of an antioxidant response leading to a reductive milieu both in the intra- and extra-cellular space that is conducive to T cell proliferation. The importance of plasticity in redox remodeling during T cell activation is supported by the observation that deficiency of Ncf1 encoding neutrophil cytosolic factor 1 (or P47phox), the activating protein in the NADPH oxidase complex, results in a reduced capacity for ROS genesis, increased cell surface thiols and enhanced T cell autoreactivity in an arthritis model (30).

GSH serves as an important proliferative signal in T lymphocytes (41) and is required for cell cycle progression from the G1 to S phase (42). It is needed for the activity of ribonucleotide reductase and therefore, for DNA synthesis (43). Furthermore, the activities of telomerase (44) and of key transcriptional factors, e.g. NF-κB and AP1 (45), and cell cycle proteins e.g. Id2 and E2F4 (44) are redox regulated. GSH is concentrated in the nucleus during the early phase of cell proliferation and becomes more evenly distributed in confluent cells (46). GSH regulates nuclear protein function via glutathionylation and protects DNA from oxidative damage during the key stage of replication (46). GSH affects ROS levels in cells, which can either activate or inactivate specific redox sensitive targets at cell cycle checkpoints, thereby influencing cell fate (47). Interestingly, increased synthesis and nuclear sequestration of GSH and decreased sensitivity to apoptosis were observed in response to overexpression of the B cell leukemia/lymphoma 2 (Bcl-2) protein in HeLa cells (48).

Redox-sensitive signaling cascades are also elicited in T cells upon activation. For instance, a 10-30% decrease in intracellular GSH in peripheral T lymphocytes completely abrogates T cell receptor-stimulated calcium signaling (49). The adaptor protein linker for activation of T cells (LAT) (Fig. 4B), a membrane protein that plays a central role in signal transduction during T cell activation, is also influenced by the intracellular redox status (50). Marked diminution in intracellular GSH level as seen under chronic oxidative stress conditions, causes a conformational change in LAT, apparently via formation of an intramolecular disulfide bond, and results in its displacement from the membrane (50). This cytoplasmic relocalization results in failure to phosphorylate in response to T cell activation and derails the signal transduction cascade that leads eventually to expression of IL-2 and other genes. This redox-sensitive conformational displacement is associated with the hyporesponsive phenotype of synovial T cells in rheumatoid arthritis because of their depleted antioxidant capacity resulting from the chronic inflammation associated with this disease of the joints (50).

In summary, redox responsive signaling networks during T cell priming involves dynamic and spatially regulated changes in the intra- and extra-cellular compartments and comprises both small molecules (e.g. ROS and redox-active metabolites) and proteins. Redox signaling has several important implications for T cell biology (36). Hypoxic conditions as encountered in poorly oxygenated tumors might limit the efficiency of T cell priming and contribute to their anergic phenotype in this environment. Alternatively, a pro-oxidant environment resulting from ROS production by active neutrophils might facilitate priming of T cells but, if overwhelming, impair signaling via inhibitory signals such as tyrosine phosphatases or the NF-κB inhibitor, IkB. Additionally, redox signaling appears to influence T cell commitment to the Th1, Th2 and regulatory T cell phenotypes (51,52).

Regulatory T Cells Interfere with Redox Remodeling by Dendritic Cells

The immune system balances the host's needs for microbial and tumor immunity with keeping autoimmunity in check (2). To achieve self-tolerance, T cells are “educated” in the thymus and autoreactive T cells are destroyed. However, a small fraction of self-reactive T cells escape from the thymus into the periphery, and if left unchecked, can cause autoimmune diseases (53). Naturally occurring $CD4^+CD25^+Foxp3^+$ regulatory T cells, which comprise about 5%-10% of total $CD4^+$ T cell population, suppress autoreactive T cells to maintain immune tolerance (54).

Sakaguchi and coworkers made the groundbreaking discovery of this distinct T cell subpopulation in 1995 and demonstrated that depletion of the $CD25^+$ population from the $CD4^+$ T cells induced autoimmunity when T cells were transferred to the immunodeficient nude mice (1). In contrast, transfer of the $CD4^+CD25^+$ T cells together with the $CD4^+CD25^-$ T cells prevented autoimmune diseases. Besides the role of regulatory T cells in controlling autoimmunity, they also play important roles in controlling anti-microbial, anti-tumor responses and transplantation immunity (54). Regulatory T cells mature in the thymus, migrate to lymph nodes and are activated by self or nonself antigen-presenting cells. The homing receptors on regulatory T cells enable them to traffic to sites of infection to control immune responses (2). Regulatory T cells also suppress the activation and proliferation of B cells, DCs and natural killer cells by mechanisms that remain to be fully elucidated (55).

Some of the strategies used by regulatory T cells for mediating their suppressive effects (2,3) are shown in Fig. 5 and include: (i) secretion of inhibitory cytokines viz. TGFβ, IL-35 and IL-10 (56-58), (ii) cytolytic suppression by secretion of the proteases granzyme-A or granzyme-B (59,60), (iii) metabolic disruption e.g. by direct transfer of cAMP to effector T cells (61) or by secretion of pericellular adenosine (62), which inhibits effector T cell functions and enhances induced regulatory T cell generation, (iv) suppression of DC maturation and/or function (63) by induction of indoleamine 2,3-dioxygenase, which catalyzes the rate-limiting step in tryptophan catabolism and creates in turn, a shortage of this essential amino acid for effector T cells (64) and (v) by interfering with extracellular reductive redox remodeling by DCs during T cell activation (10). The panoply of suppressive strategies identified to date for regulatory T cell raises questions about their relative importance and how they are integrated in vivo. In the proposed “hierarchical” model, one or few master mechanisms govern regulatory T cell suppressive functions in various physiological settings (3). Alternatively, in the “contextual” model, the microenvironment and tissue compartment govern the suppressive strategy that is deployed resulting in the differential contribution of a given mechanism in different disease models (3).

In contrast to naïve T cells, co-culture of regulatory T cells with DCs does not affect extracellular cysteine concentration. However, regulatory T cells suppress cysteine accumulation in the extracellular compartment when added to co-cultures of DCs and naive T

cells. As a consequence, both intracellular (diminished GSH levels in T cells) and extracellular (diminished cell surface thiol labeling on T cells and on DCs) perturbations in the redox status result (10). Remarkably, although regulatory T cells are known to mediate their suppressive functions by multiple strategies, provision of a single reagent, i.e. exogenous cysteine at concentrations seen under DC-T cell co-culture conditions, alleviates inhibition of T cell proliferation (10). This observation begs the question as to whether redox regulation serves as a master switch in the multipronged suppressive action of regulatory T cells.

We posit that the redox changes in the intra- and extra-cellular compartments influence one or more of the well known regulatory T cell suppressive mechanisms. For instance, the anti-inflammatory cytokine IL-10 has antioxidant properties (65) and TGFβ, a multifunctional cytokine is redox regulated (66). Activation of latent TGFβ requires reductive cleavage of a disulfide bond that links it to the latency-associated peptide, but over-reduction, leads to formation of inactive TGFβ monomers. Thus activation and inactivation of TGFβ are subject to redox control and dynamic changes in the extracellular redox milieu might be important for regulating TGFβ activity (67). Furthermore, granzyme A, the cytolytic T cell protease, cleaves redox factor 1 (Ref-1), which in turn enhances cell death (68). Thus, redox control may be integral to regulatory T cell mediated suppression mechanisms and could be more pervasive than previously recognized.

In Vivo Studies and Therapeutic Implications

The redox status of secondary lymphoid organs such as lymph nodes and spleens are more reduced than non-lymphoid organs (29). The nonprotein thiol content in lymphoid tissues is reported to increase in response to immunization with DCs, B cells and macrophages, contributing to the reductive remodeling (29,69). It is speculated that the reducing microenvironment might protect lymphoid organs from oxidative stress during T cell activation and antibody production (70,71). However, low levels of ROS are essential for the onset of the immune response. In vivo treatment of mouse models with catalytic antioxidants (manganese porphyrin derivatives) causes inefficient $CD4^+$ T cell activation and proliferation by inhibiting ROS generation in antigen presenting cells (72). The catalytic antioxidants inhibit DNA binding by NF-κB and subsequent production of proinflammatory cytokines (73). Redox modulation by catalytic antioxidants also suppresses $CD8^+$ T cell functions such as proliferation and lysis of target cells (74).

The x_c^- cystine transporter, which transports cystine using the glutamate gradient, plays an important role in redox-based immunoregulation. Under normal conditions, lamina propria macrophages are unable to transport cystine and secrete cysteine because they lack the x_c^- transporter (12). In inflammatory bowel disease, local recruitment of peripheral blood monocytes which exhibits high expression of the x_c^- transporter leads to extracellular cysteine accumulation and hyper-reactivity of lamina propria T cells (12). Furthermore, lymphoma cells, which cannot import cystine like naïve T cells, depend on tumor-associated somatic cells such as activated macrophages and DCs for their cysteine supply. Inhibition of the x_c^- transporter by sulfasalazine inhibits growth of lymphoma cells and tumor progression (75). Overexpression of the x_c^- transporter in lymphoma cells greatly increases intracellular and extracellular cysteine levels, protecting cells from oxidative stress induced cell death (76).

Redox modulation as a strategy for immunoregulation has been used in several diseases. HIV infects and kills $CD4^+$ T cells, leading to a significant decrease in functional $CD4^+$ T cells in AIDS. HIV infected individuals have lower cellular and plasma GSH levels compared with healthy controls, which correlates with low T cell numbers and deficient function. Administration of N-acetyl cysteine, a cysteine precursor, restores intracellular GSH levels and has shown benefits for HIV-infected individuals (77). Sulfasalazine is used in the treatment

of T cell mediated autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis. It decreases proliferation of autoreactive T cells by inhibiting the x_c^- cystine transporter on antigen presenting cells thereby perturbing the redox environment (78).

Since regulatory T cells play a central role in suppression of various immune responses, manipulation of their function is an important strategy for immune intervention. Enhancing regulatory T cell function in autoimmunity, allergy, transplantation and pregnancy disorders can diminish unwanted immune responses. On the other hand, attenuating regulatory T cell function in cancer and microbial infection may be desirable (79). The recent identification of a novel immunosuppressive strategy deployed by regulatory T cells, which impacts the intra- and extracellular redox environments during T cell activation (10), illuminates a new therapeutic target.

Concluding Remarks

Redox modulation has emerged as a key regulatory strategy in the adaptive immune system. It has long been known that T cell activation and proliferation require a reducing milieu. This reducing microenvironment is shaped primarily by the metabolic activity of antigen presenting cells, especially DCs. Interaction of DCs with naïve T cells stimulate cystine consumption and cysteine accumulation in the extracellular space, which produces an extracellular redox potential suitable for T cell proliferation (9,10). A more reducing extracellular redox potential is reflected in the increased T cell surface thiol status (10). The specific membrane targets of redox remodeling and their effects on T cell biology, i.e. activation and proliferation, remain to be elucidated. The greater availability of extracellular cysteine also influences the intracellular antioxidant capacity within T cells since cysteine limits GSH biosynthesis. Consequently, intracellular GSH levels rise and in turn, influences T cell signal transduction pathways and gene expression. The choreography of GSH localization and the GSH/GSSG redox potential changes during T cell activation, and their correlation with the onset and operation of signaling pathways and cell cycle progression await elucidation.

Modulation by regulatory T cells of the extracellular redox microenvironment during T cell activation could be mediated by one or more mechanisms. For instance, by limiting cysteine availability, regulatory T cells deprive effector T cells of a building block needed for protein and GSH synthesis. Alternatively, by perturbing the redox environment, regulatory T cells can have both indirect effects by enhancing other suppressive mechanisms used by them (as discussed above) and direct effects on T cell activation and proliferation targets, which are sensitive to the redox potential and the redox status of key signaling proteins. Many questions remain to be addressed regarding how regulatory T cells inhibit reductive remodeling by DCs. For instance, do they interfere with cystine uptake, inhibit the cysteine secretion pathway or simply compete with effector T cells for the extracellular cysteine pool? Is there a connection between the mechanism for perturbing redox remodeling and Foxp3 expression and what is the extent of cross-talk between the other suppressive mechanisms and redox remodeling? And finally, what is the physiological relevance of the redox remodeling mechanism in normal and disease states? The answers to these questions will help illuminate the biology of regulatory T cell suppressive mechanisms and identify potential therapeutic targets.

References

1. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155:1151–1164. [PubMed: 7636184]

2. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133:775–787. [PubMed: 18510923]

3. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol 2008;8:523–532. [PubMed: 18566595]
4. Tseng SY, Dustin ML. T-cell activation: a multidimensional signaling network. Curr Opin Cell Biol 2002;14:575–580. [PubMed: 12231352]
5. Abbas AK. The control of T cell activation vs. tolerance. Autoimmun Rev 2003;2:115–118. [PubMed: 12848951]
6. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–252. [PubMed: 9521319]
7. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767–811. [PubMed: 10837075]
8. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. Cell 2001;106:263–266. [PubMed: 11509174]
9. Angelini G, Gardella S, Ardy M, Ciriolo MR, Filomeni G, Di Trapani G, Clarke F, Sitia R, Rubartelli A. Antigen-presenting dendritic cells provide the reducing extracellular microenvironment required for T lymphocyte activation. Proc Natl Acad Sci U S A 2002;99:1491–1496. [PubMed: 11792859]
10. Yan Z, Garg SK, Kipnis J, Banerjee R. Extracellular redox modulation by regulatory T cells. Nat Chem Biol 2009;5:721–723. [PubMed: 19718041]
11. Sido B, Braunstein J, Breitkreutz R, Herfarth C, Meuer SC. Thiol-mediated redox regulation of intestinal lamina propria T lymphocytes. J Exp Med 2000;192:907–912. [PubMed: 10993921]
12. Sido B, Lasitschka F, Giese T, Gassler N, Funke B, Schroder-Braunstein J, Brunnemer U, Meuer SC, Autschbach F. A prominent role for mucosal cystine/cysteine metabolism in intestinal immunoregulation. Gastroenterology 2008;134:179–191. [PubMed: 18061179]
13. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299:1057–1061. [PubMed: 12522256]
14. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001;27:20–21. [PubMed: 11137993]
15. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol 2001;19:565–594. [PubMed: 11244047]
16. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008;322:271–275. [PubMed: 18845758]
17. Moriarty-Craige SE, Jones DP. Extracellular thiols and thiol/disulfide redox in metabolism. Annu Rev Nutr 2004;24:481–509. [PubMed: 15189129]
18. Kemp M, Go YM, Jones DP. Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology. Free Radic Biol Med 2008;44:921–937. [PubMed: 18155672]
19. Meister A, Anderson ME. Glutathione. Annu Rev Biochem 1983;52:711–760. [PubMed: 6137189]
20. Eck HP, Gmunder H, Hartmann M, Petzoldt D, Daniel V, Droge W. Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. Biol Chem Hoppe Seyler 1989;370:101–108. [PubMed: 2784973]
21. Karp DR, Shimooku K, Lipsky PE. Expression of gamma-glutamyl transpeptidase protects ramos B cells from oxidation-induced cell death. J Biol Chem 2001;276:3798–3804. [PubMed: 11080500]
22. Ghezzi P, Bonetto V, Fratelli M. Thiol-disulfide balance: from the concept of oxidative stress to that of redox regulation. Antioxid Redox Signal 2005;7:964–972. [PubMed: 15998251]
23. Jones DP. Radical-free biology of oxidative stress. Am J Physiol Cell Physiol 2008;295:C849–868. [PubMed: 18684987]
24. Jordan PA, Gibbins JM. Extracellular disulfide exchange and the regulation of cellular function. Antioxid Redox Signal 2006;8:312–324. [PubMed: 16677077]
25. Hynes RO, Destree A. Extensive disulfide bonding at the mammalian cell surface. Proc Natl Acad Sci U S A 1977;74:2855–2859. [PubMed: 268636]

26. Ali IU, Hynes RO. Role of disulfide bonds in the attachment and function of large, external, transformation-sensitive glycoprotein at the cell surface. Biochim Biophys Acta 1978;510:140–150. [PubMed: 667030]
27. Lawrence DA, Song R, Weber P. Surface thiols of human lymphocytes and their changes after in vitro and in vivo activation. J Leukoc Biol 1996;60:611–618. [PubMed: 8929552]
28. Matthias LJ, Yam PT, Jiang XM, Vandegraaff N, Li P, Poumbourios P, Donoghue N, Hogg PJ. Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. Nat Immunol 2002;3:727–732. [PubMed: 12089508]
29. Castellani P, Angelini G, Delfino L, Matucci A, Rubartelli A. The thiol redox state of lymphoid organs is modified by immunization: role of different immune cell populations. Eur J Immunol 2008;38:2419–2425. [PubMed: 18792398]
30. Gelderman KA, Hultqvist M, Holmberg J, Olofsson P, Holmdahl R. T cell surface redox levels determine T cell reactivity and arthritis susceptibility. Proc Natl Acad Sci U S A 2006;103:12831–12836. [PubMed: 16908843]
31. Droge W, Eck HP, Gmunder H, Mihm S. Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivatives. Am J Med 1991;91:140S–144S. [PubMed: 1928206]
32. Ishii T, Sugita Y, Bannai S. Regulation of glutathione levels in mouse spleen lymphocytes by transport of cysteine. J Cell Physiol 1987;133:330–336. [PubMed: 3680392]
33. Nakamura H, Masutani H, Yodoi J. Extracellular thioredoxin and thioredoxin-binding protein 2 in control of cancer. Semin Cancer Biol 2006;16:444–451. [PubMed: 17095246]
34. Schwertassek U, Balmer Y, Gutscher M, Weingarten L, Preuss M, Engelhard J, Winkler M, Dick TP. Selective redox regulation of cytokine receptor signaling by extracellular thioredoxin-1. EMBO J 2007;26:3086–3097. [PubMed: 17557078]
35. Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000;267:6102–6109. [PubMed: 11012661]
36. Pani G, Colavitti R, Borrello S, Galeotti T. Redox regulation of lymphocyte signaling. IUBMB Life 2000;49:381–389. [PubMed: 10902569]
37. Los M, Schenk H, Hexel K, Baeuerle PA, Droge W, Schulze-Osthoff K. IL-2 gene expression and NF-kappa B activation through CD28 requires reactive oxygen production by 5-lipoxygenase. EMBO J 1995;14:3731–3740. [PubMed: 7641692]
38. Thoren FB, Betten A, Romero AI, Hellstrand K. Cutting edge: Antioxidative properties of myeloid dendritic cells: protection of T cells and NK cells from oxygen radical-induced inactivation and apoptosis. J Immunol 2007;179:21–25. [PubMed: 17579015]
39. Secrist JP, Burns LA, Karnitz L, Koretzky GA, Abraham RT. Stimulatory effects of the protein tyrosine phosphatase inhibitor, pervanadate, on T-cell activation events. J Biol Chem 1993;268:5886–5893. [PubMed: 8383678]
40. Yang H, Magilnick N, Lee C, Kalmaz D, Ou X, Chan JY, Lu SC. Nrf1 and Nrf2 regulate rat glutamate-cysteine ligase catalytic subunit transcription indirectly via NF-kappaB and AP-1. Mol Cell Biol 2005;25:5933–5946. [PubMed: 15988009]
41. Suthanthiran M, Anderson ME, Sharma VK, Meister A. Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD3 antigens. Proc Natl Acad Sci U S A 1990;87:3343–3347. [PubMed: 1970635]
42. Messina JP, Lawrence DA. Cell cycle progression of glutathione-depleted human peripheral blood mononuclear cells is inhibited at S phase. J Immunol 1989;143:1974–1981. [PubMed: 2789253]
43. Thelander L, Reichard P. Reduction of ribonucleotides. Annu Rev Biochem 1979;48:133–158. [PubMed: 382982]
44. Borras C, Esteve JM, Vina JR, Sastre J, Vina J, Pallardo FV. Glutathione regulates telomerase activity in 3T3 fibroblasts. J Biol Chem 2004;279:34332–34335. [PubMed: 15184392]
45. Schulze-Osthoff K, Los M, Baeuerle PA. Redox signalling by transcription factors NF-kappa B and AP-1 in lymphocytes. Biochem Pharmacol 1995;50:735–741. [PubMed: 7575632]
46. Markovic J, Borras C, Ortega A, Sastre J, Vina J, Pallardo FV. Glutathione is recruited into the nucleus in early phases of cell proliferation. J Biol Chem 2007;282:20416–20424. [PubMed: 17452333]
47. Menon SG, Goswami PC. A redox cycle within the cell cycle: ring in the old with the new. Oncogene 2007;26:1101–1109. [PubMed: 16924237]

48. Voehringer DW, McConkey DJ, McDonnell TJ, Brisbay S, Meyn RE. Bcl-2 expression causes redistribution of glutathione to the nucleus. Proc Natl Acad Sci U S A 1998;95:2956–2960. [PubMed: 9501197]

49. Staal FJ, Anderson MT, Staal GE, Herzenberg LA, Gitler C. Redox regulation of signal transduction: tyrosine phosphorylation and calcium influx. Proc Natl Acad Sci U S A 1994;91:3619–3622. [PubMed: 7513425]

50. Gringhuis SI, Papendrecht-van der Voort EA, Leow A, Nivine Levarht EW, Breedveld FC, Verweij CL. Effect of redox balance alterations on cellular localization of LAT and downstream T-cell receptor signaling pathways. Mol Cell Biol 2002;22:400–411. [PubMed: 11756537]

51. Hasan AA, Ghaemmaghami AM, Fairclough L, Robins A, Sewell HF, Shakib F. Allergen-driven suppression of thiol production by human dendritic cells and the effect of thiols on T cell function. Immunobiology 2009;214:2–16. [PubMed: 19159822]

52. Peterson JD, Herzenberg LA, Vasquez K, Waltenbaugh C. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. Proc Natl Acad Sci U S A 1998;95:3071–3076. [PubMed: 9501217]

53. Fehervari Z, Sakaguchi S. Peacekeepers of the immune system. Sci Am 2006;295:56–63. [PubMed: 16989481]

54. Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004;22:531–562. [PubMed: 15032588]

55. Sakaguchi S, Powrie F. Emerging challenges in regulatory T cell function and biology. Science 2007;317:627–629. [PubMed: 17673654]

56. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 2001;194:629–644. [PubMed: 11535631]

57. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 1999;190:995–1004. [PubMed: 10510089]

58. Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 2007;450:566–569. [PubMed: 18033300]

59. Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol 2005;174:1783–1786. [PubMed: 15699103]

60. Cao X, Cai SF, Fehniger TA, Song J, Collins LI, Piwnica-Worms DR, Ley TJ. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity 2007;27:635–646. [PubMed: 17919943]

61. Bopp T, Becker C, Klein M, Klein-Hessling S, Palmetshofer A, Serfling E, Heib V, Becker M, Kubach J, Schmitt S, Stoll S, Schild H, Staege MS, Stassen M, Jonuleit H, Schmitt E. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. J Exp Med 2007;204:1303–1310. [PubMed: 17502663]

62. Kobie JJ, Shah PR, Yang L, Rebhahn JA, Fowell DJ, Mosmann TR. T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5′-adenosine monophosphate to adenosine. J Immunol 2006;177:6780–6786. [PubMed: 17082591]

63. Tadokoro CE, Shakhar G, Shen S, Ding Y, Lino AC, Maraver A, Lafaille JJ, Dustin ML. Regulatory T cells inhibit stable contacts between CD4+ T cells and dendritic cells in vivo. J Exp Med 2006;203:505–511. [PubMed: 16533880]

64. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P. Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 2003;4:1206–1212. [PubMed: 14578884]

65. Haddad JJ, Fahlman CS. Redox- and oxidant-mediated regulation of interleukin-10: an anti-inflammatory, antioxidant cytokine? Biochem Biophys Res Commun 2002;297:163–176. [PubMed: 12237098]

66. Barcellos-Hoff MH, Dix TA. Redox-mediated activation of latent transforming growth factor-beta 1. Mol Endocrinol 1996;10:1077–1083. [PubMed: 8885242]

67. Blakytny R, Erkell LJ, Brunner G. Inactivation of active and latent transforming growth factor beta by free thiols: potential redox regulation of biological action. Int J Biochem Cell Biol 2006;38:1363–1373. [PubMed: 16531095]
68. Fan Z, Beresford PJ, Zhang D, Xu Z, Novina CD, Yoshida A, Pommier Y, Lieberman J. Cleaving the oxidative repair protein Ape1 enhances cell death mediated by granzyme A. Nat Immunol 2003;4:145–153. [PubMed: 12524539]
69. Carta S, Castellani P, Delfino L, Tassi S, Vene R, Rubartelli A. DAMPs and inflammatory processes: the role of redox in the different outcomes. J Leukoc Biol 2009;86:549–555. [PubMed: 19564570]
70. Matsue H, Edelbaum D, Shalhevet D, Mizumoto N, Yang C, Mummert ME, Oeda J, Masayasu H, Takashima A. Generation and function of reactive oxygen species in dendritic cells during antigen presentation. J Immunol 2003;171:3010–3018. [PubMed: 12960326]
71. Masciarelli S, Sitia R. Building and operating an antibody factory: redox control during B to plasma cell terminal differentiation. Biochim Biophys Acta 2008;1783:578–588. [PubMed: 18241675]
72. Tse HM, Milton MJ, Schreiner S, Profozich JL, Trucco M, Piganelli JD. Disruption of innate-mediated proinflammatory cytokine and reactive oxygen species third signal leads to antigen-specific hyporesponsiveness. J Immunol 2007;178:908–917. [PubMed: 17202352]
73. Tse HM, Milton MJ, Piganelli JD. Mechanistic analysis of the immunomodulatory effects of a catalytic antioxidant on antigen-presenting cells: implication for their use in targeting oxidation-reduction reactions in innate immunity. Free Radic Biol Med 2004;36:233–247. [PubMed: 14744635]
74. Sklavos MM, Tse HM, Piganelli JD. Redox modulation inhibits CD8 T cell effector function. Free Radic Biol Med 2008;45:1477–1486. [PubMed: 18805480]
75. Gout PW, Simms CR, Robertson MC. In vitro studies on the lymphoma growth-inhibitory activity of sulfasalazine. Anticancer Drugs 2003;14:21–29. [PubMed: 12544255]
76. Banjac A, Perisic T, Sato H, Seiler A, Bannai S, Weiss N, Kolle P, Tschoep K, Issels RD, Daniel PT, Conrad M, Bornkamm GW. The cystine/cysteine cycle: a redox cycle regulating susceptibility versus resistance to cell death. Oncogene 2008;27:1618–1628. [PubMed: 17828297]
77. Herzenberg LA, De Rosa SC, Dubs JG, Roederer M, Anderson MT, Ela SW, Deresinski SC. Glutathione deficiency is associated with impaired survival in HIV disease. Proc Natl Acad Sci U S A 1997;94:1967–1972. [PubMed: 9050888]
78. Edinger AL, Thompson CB. Antigen-presenting cells control T cell proliferation by regulating amino acid availability. Proc Natl Acad Sci U S A 2002;99:1107–1109. [PubMed: 11830651]
79. Becker C, Stoll S, Bopp T, Schmitt E, Jonuleit H. Regulatory T cells: present facts and future hopes. Med Microbiol Immunol 2006;195:113–124. [PubMed: 16715254]

Abbreviations

[1]TCR	T cell receptor
MHC	major histocompatibility complex
DC	dendritic cell
Foxp3	forkhead box P3
CTLA-4	cytotoxic T lymphocyte antigen 4
Trx	thioredoxin
GSH	glutathione
GSSG	glutathione disulfide
AIDS	acquired immunodeficiency syndrome
HIV	human immunodeficiency virus
TNF	tumor necrosis factor
FACS	fluorescence-activated cell sorting

ROS	reactive oxygen species
NF-κB	nuclear factor-κB
NADPH	nicotinamide adenine dinucleotide phosphate
Ncf1	neutrophil cytosolic factor 1
AP1	activator protein 1
Bcl-2	B cell leukemia/lymphoma 2
LAT	linker for activation of T cells
TGFβ	transforming growth factor beta
Ref-1	redox factor 1, LCK, leukocyte-specific protein tyrosine kinase
ZAP-70	zeta-chain-associated protein kinase 70
GRB2	growth factor receptor bound protein 2
PLCγ1	phospholipase C γ1

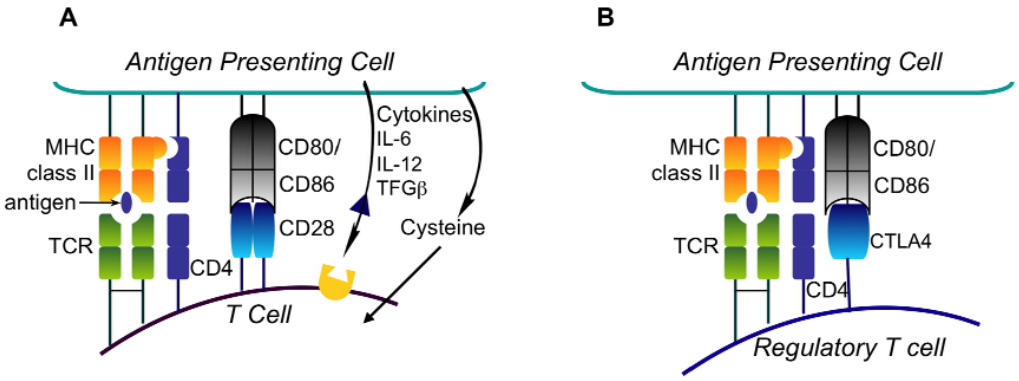

Figure 1.

Molecular interactions in the immune synapse. (A) Signals required for $CD4^+$ T cell activation and proliferation include: (i) TCR-antigen•MHC complex interaction, (ii) interaction of CD28 on T cells and CD80/CD86 on antigen presenting cells, (iii) secreted cytokines such as IL-6, IL-12 and TGFβ and (iv) a reducing microenvironment shaped mainly by extracellular cysteine accumulation. (B) Interaction of regulatory T cells with antigen presenting cells. Regulatory T cells constitutively express high levels of CTLA-4, which interact with CD80/CD86 on antigen presenting cells, thus inhibiting their presentation capacity for interactions with naive T cells.

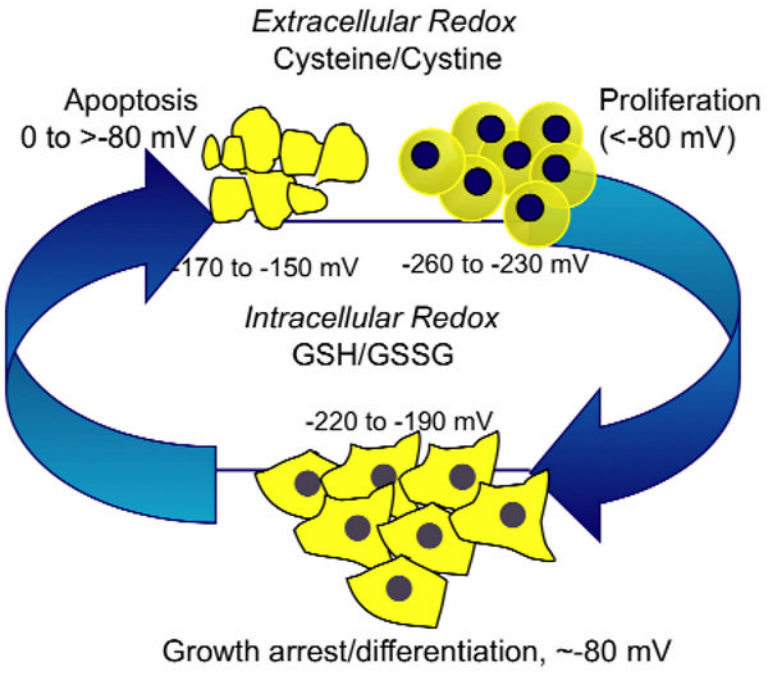

Figure 2.

Correlated changes between cell cycle progression and the extra- and intra-cellular redox potentials. The GSH/GSSG couple represents the major intracellular redox buffer. The redox potential of the intracellular GSH/GSSG couple becomes more oxidized when cells progress from proliferation (-260 mV to -230 mV) to differentiation/growth arrest (-220 mV to -190 mV) to apoptosis (-170 mV to -150 mV). The cysteine/cystine couple is the main extracellular thiol/disulfide pool. Changes in the extracellular cysteine/cystine redox potential follow the same pattern, i.e. it is most reduced during proliferation (<-80 mV) and becomes increasingly oxidized during differentiation/growth arrest (~-80 mV) and apoptosis (0 mV to -80 mV). This figure is adapted from reference (17).

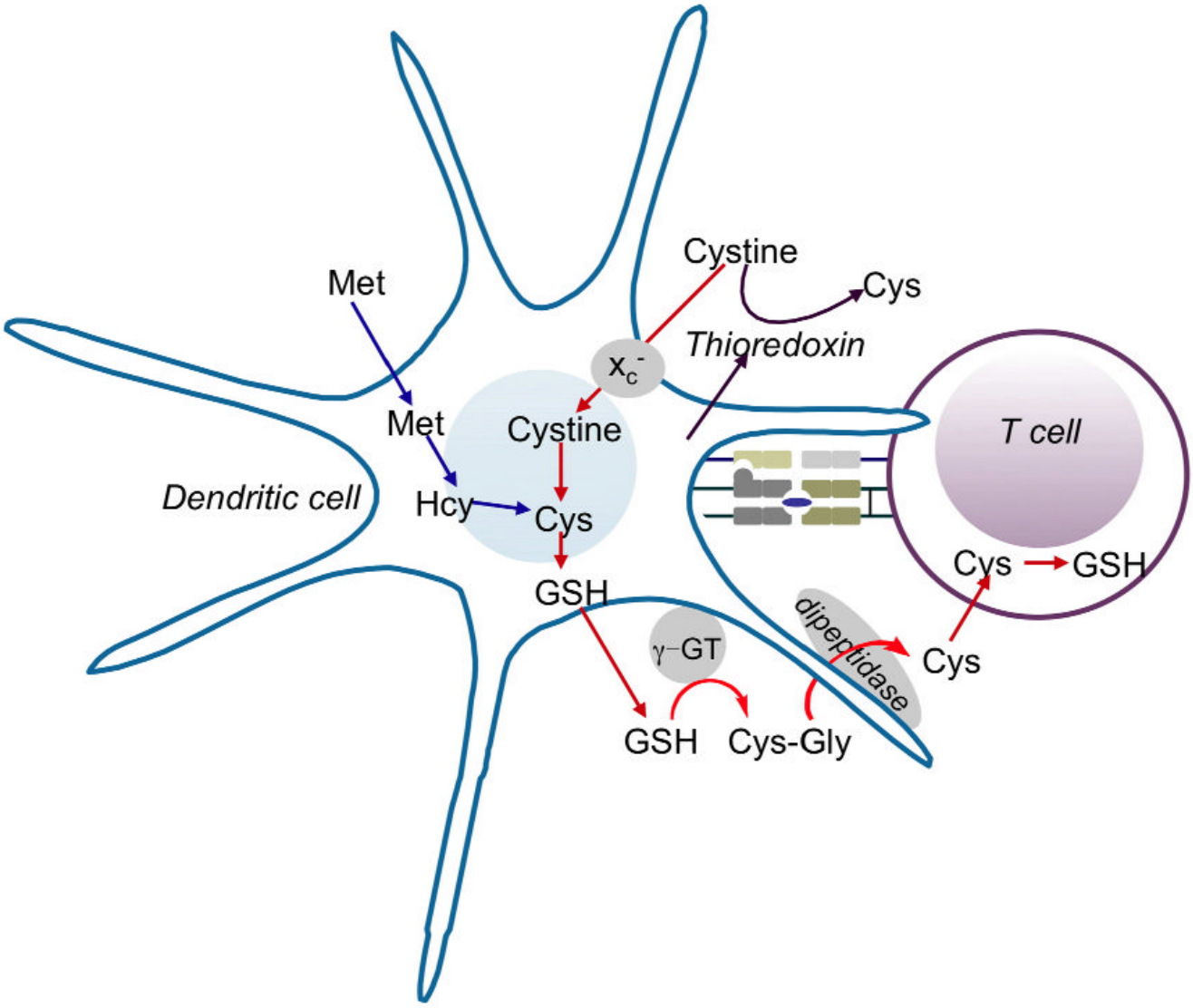

Figure 3.

Mechanism of redox remodeling by DCs. The possible sources of extracellular cysteine that accumulates during DC and T cell co-culture include: (i) increased flux through the transsulfuration pathway leading to enhanced synthesis of cysteine from methionine, (ii) direct reduction from cystine catalyzed by extracellular thioredoxin. (iii) x_c^--dependent import of cystine, its subsequent intracellular conversion to GSH, which is exported and degraded by the ectoenzymes, γ-glutamyltranspeptidase and a dipeptidase, to furnish cysteine. The extracellular accumulation of cysteine results in a reducing microenvironment for T cell activation and proliferation and also provides T cells with cysteine needed for the synthesis of GSH.

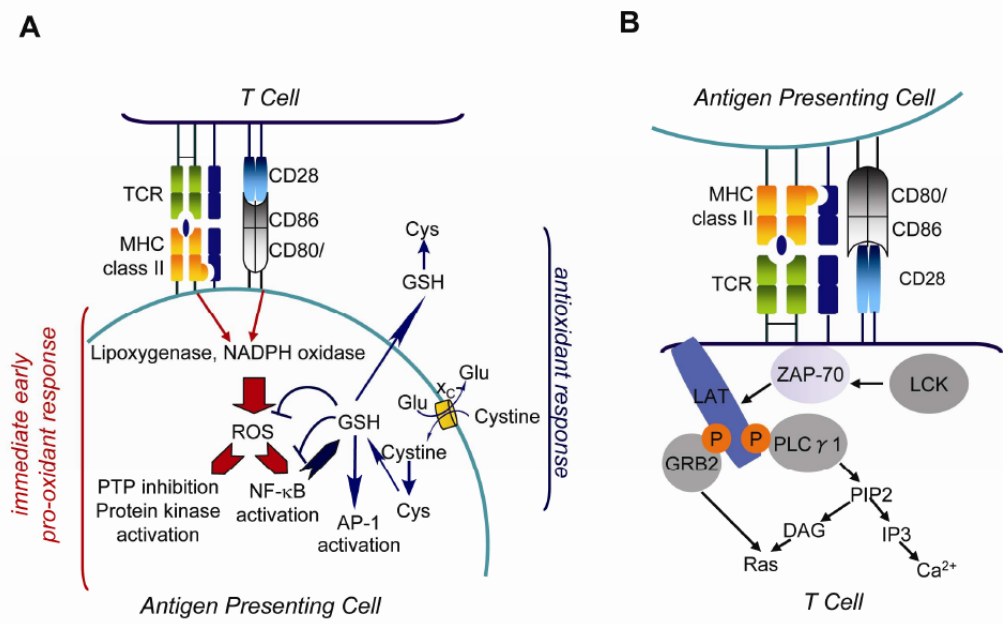

Figure 4.

Redox signaling responses in DCs and T cells during T cell activation. (A) Redox signaling in DCs. The TCR-antigen•MHC complex interaction and the co-stimulatory signal results in an immediate early pro-oxidant response in DCs with ROS production e.g. by lipoxygenase and NADPH oxidase. Low levels of ROS act as signaling molecules to inhibit protein tyrosine phosphatases (PTPs) and activate protein kinases. ROS also activates the NF-κB pathway, which stimulates the expression of γ-glutamylcysteine ligase, thus increasing GSH synthesis. GSH activates the AP1 signaling pathway and initiates an antioxidant response. We postulate that system x_c^--dependent cystine uptake, GSH export and degradation into extracellular cysteine are stimulated as part of this response. (B) TCR signaling in T cells. Stimulation of T cells by DCs via the TCR results in phosphorylation and activation of ZAP-70 by leukocyte-specific protein tyrosine kinase (LCK). ZAP-70 directly phosphorylates the adaptor protein LAT and causes the assembly of multiprotein signaling complexes. Recruitment of the growth factor receptor bound protein 2 (GRB2) and phospholipase C γ1 (PLCγ1) to LAT leads to activation of downstream Ras and calcium signaling pathways.

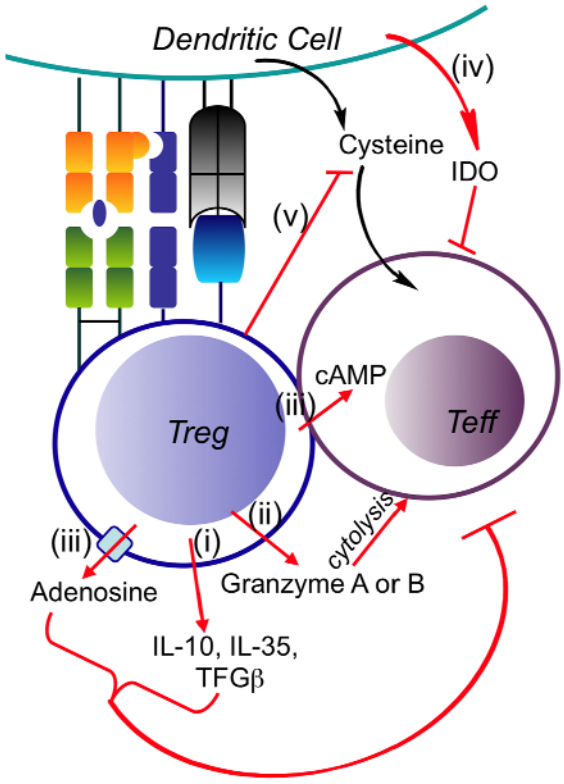

Figure 5.

Mechanisms used by regulatory T cells for suppressing autoreactive effector T cells. Regulatory T cells suppress the function of effector T cells via the following mechanisms: (i) secretion of inhibitory cytokines such as TGFβ, IL-10 and IL-35, (ii) cytolysis by granzyme A or granzyme B, (iii) metabolite disruption e.g. cAMP and adenosine, (iv) inhibition of DC function via the CTLA4-dependent induction of indoleamine 2,3 dioxygenase (IDO) and (v) modulation of the extracellular redox microenvironment. The red arrows denote the actions of regulatory T cells.